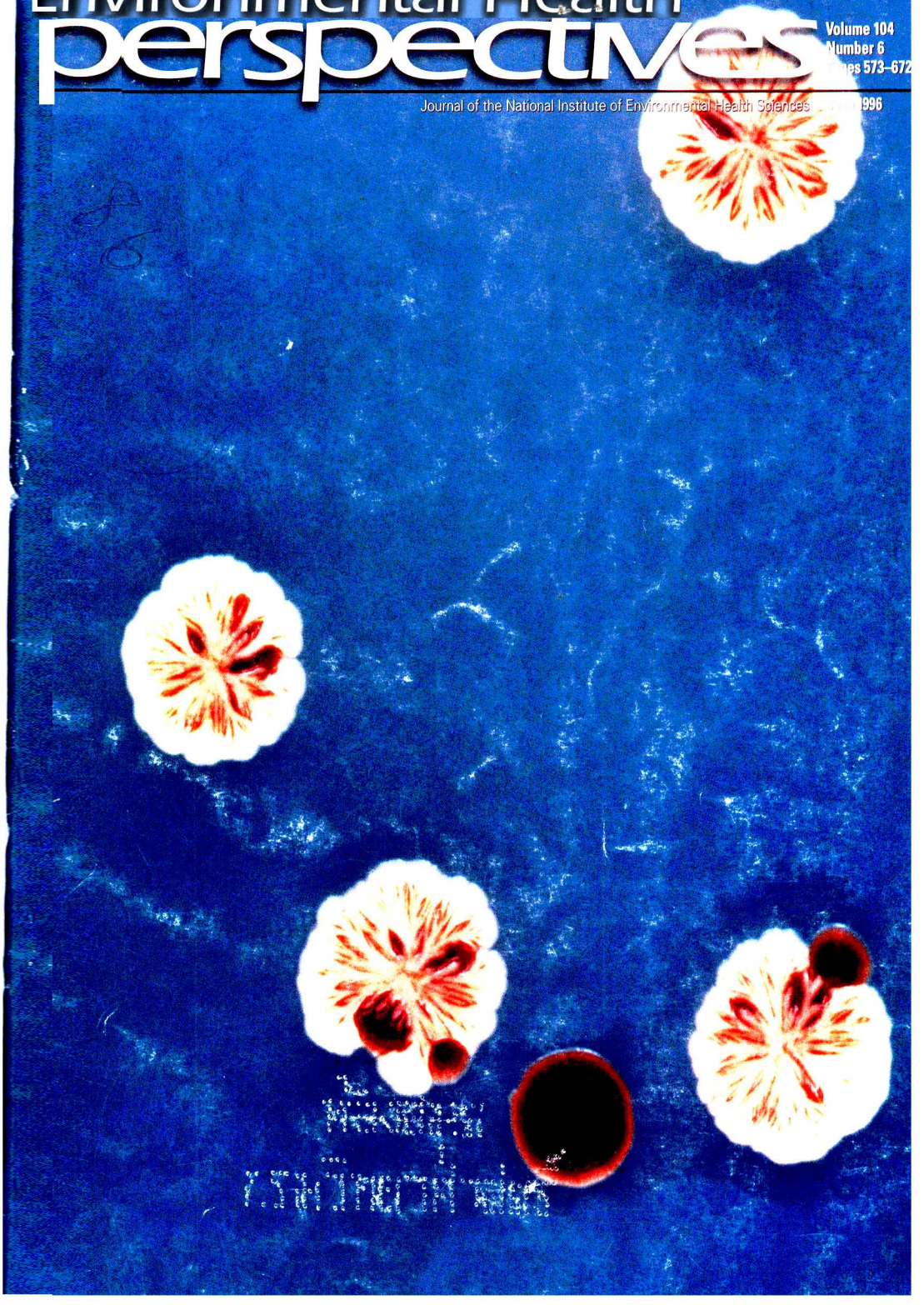
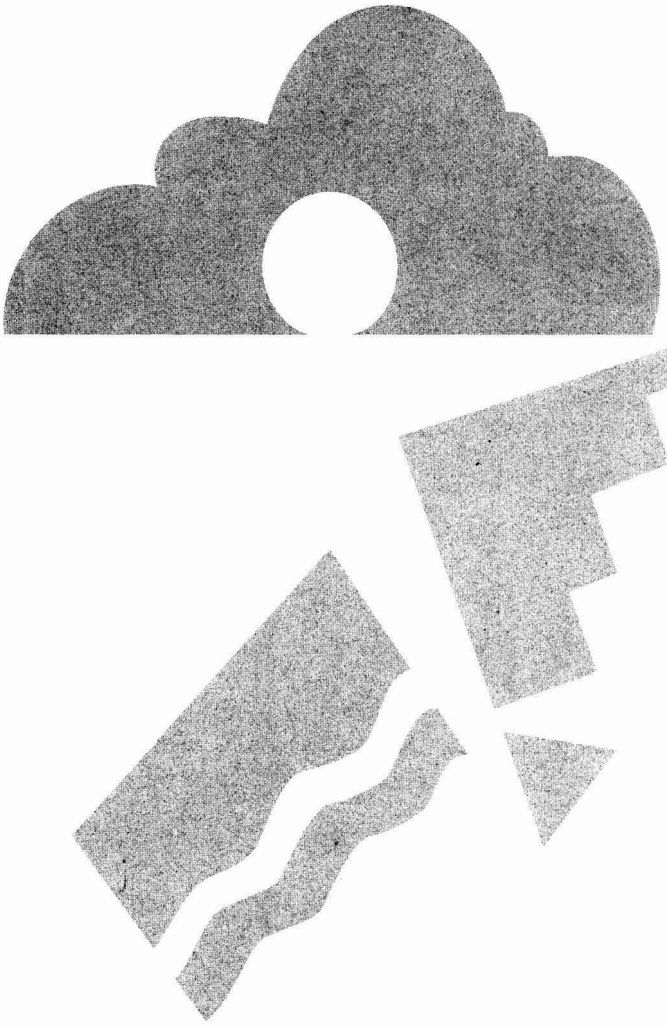
Environmental Health
perspectives
Volume 104
Number 6
Pages 573–672
Journal of the National Institute of Environmental Health Sciences 1996





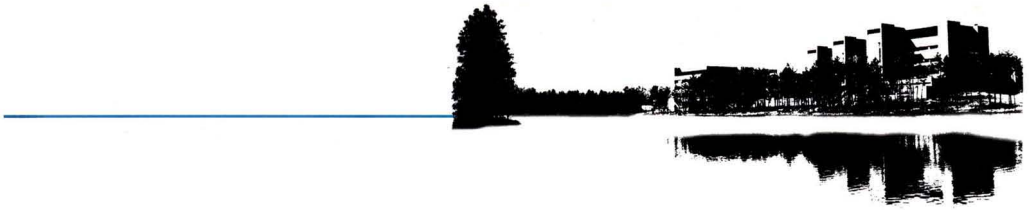
The National Institute of Environmental Health Sciences
National Institutes of Health

DISCRIMINATION PROHIBITED–Under the provisions of applicable public laws enacted by Congress since 1964, no person in the United States shall, on the grounds of race, color, national origin, handicap, or age, be excluded from participation in, be denied the benefits of, or be subjected to discrimination under any program or activity (or, on the basis of sex, with respect to any educational program or activity) receiving Federal financial assistance. In addition, Executive Order 11141 prohibits discrimination on the basis of age by contractors and subcontractors in the performance of Federal contracts, and Executive Order 11246 states that no federally funded contractor may discriminate against any employee or applicant for employment because of race, color, religion, sex, or national origin. Therefore, the National Institute of Environmental Health Sciences must be operated in compliance with these laws and Executive Orders.

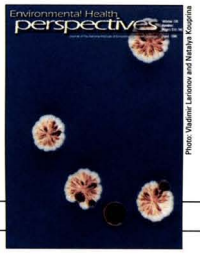
Environmental Health perspectives
Journal of the National Institute of Environmental Health Sciences
Volume 104
Number 6
June 1996

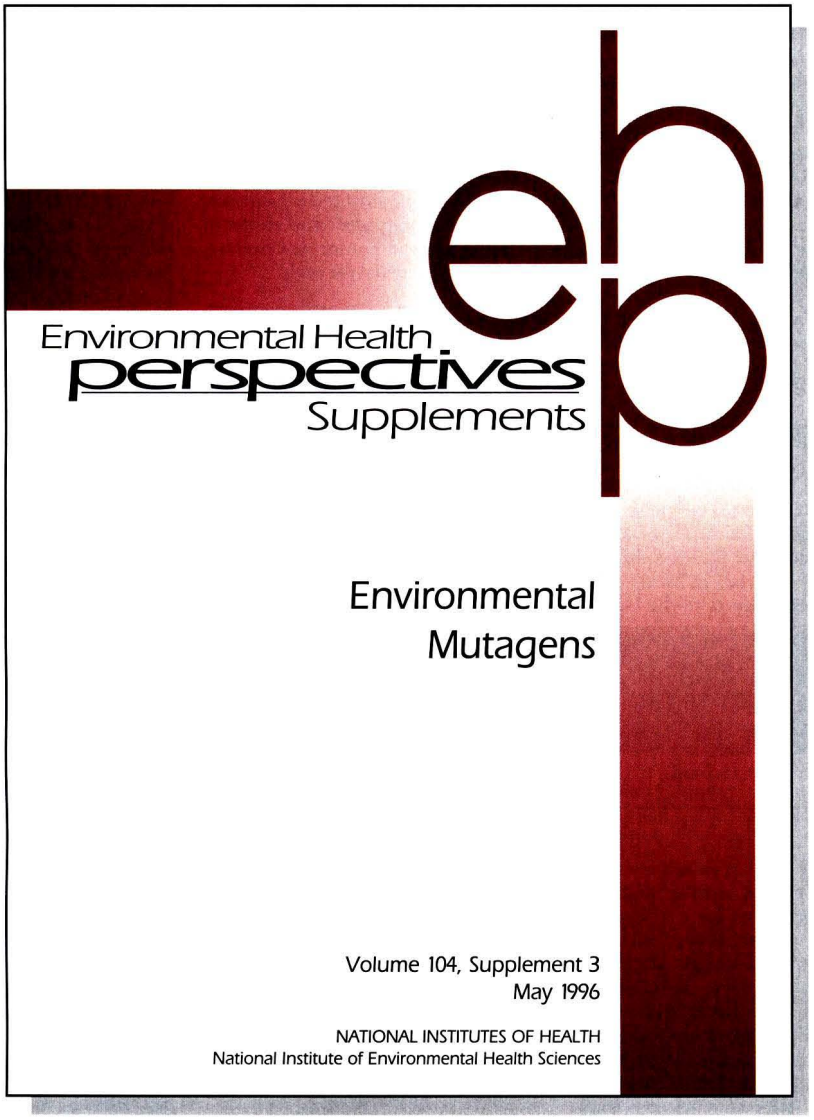
EHP online: http://ehpnet1.niehs.nih.gov

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



On The Cover: Visualization of genetic instability in yeast using color markers has aided the development of systems (**Innovations, p. 616**) for the transformation-associated recombination (TAR) cloning of human DNA as stable artificial chromosomes in yeast.





A Tradition of Progress

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

For subscription information, see p. 664.
For a listing of published volumes of *EHP Supplements*, see p. 669.

In This Issue

DDT Use in Mexico

A review of DDT production and consumption in Mexico indicates that the prevalence of this insectide is greater there than in any of the other Latin American countries. Published studies suggest that DDT/DDE levels in blood, adipose tissue, and breast milk from Mexican women in tropical and agricultural areas greatly exceed levels in U.S. women. In a **Commentary** on p. 584, López-Carillo et. al. argue that pesticide exposure in Mexico may be due to consumption of contaminated foods, although definitive data on this relationship, as well as the relationship between DDT levels in adipose tissue and breast cancer incidence, are lacking.

Waste on the Border

The North American Free Trade Agreement has encouraged the proliferation of *maquiladoras* in Mexico. These are foreign-owned industrial facilities that use imported raw materials in Mexico and return finished products to the United States. There is potential for environmental abuse because hazardous materials that are imported or generated as by-products should be returned to the states for proper disposal. In a **Commentary** on p. 590, Carter et al. discuss the considerable scientific and political issues involved.

Tissues as Tools

Environmental health scientists are often faced with the research quandary of having either adequate data on human exposures to toxicants or tissue samples indicating the presence of disease, but rarely both from the same subjects. Added to the situation is the increasingly controversial question of consent. The **Focus** article on p. 606 discusses how new attempts to design tissue monitoring and collection systems with future research studies in mind are helping to ensure that the tools for valid and useful environmental health research are available.

Passports for Alternative Tests

Around the world scientists are looking for alternatives to animals for testing of toxic chemicals. Once found, however, new methods may be "stopped at the border" of new countries due to different standards for validation and acceptance. The **Spheres of Influence** on p. 612 discusses recent efforts by international organizations to harmonize such standards to prevent duplication of research efforts and provide new methods that can be used around the world.

YAC, YAC, YAC

For scientists trying to unravel the mysteries of the human genetic code, isolating and cloning human DNA material has been a tedious, somewhat hit-or-miss, and vexing problem. A new method developed through a collaboration between researchers at the NIEHS and Russian scientists, discussed in the **Innovations** on p. 616, allows human DNA to be quickly and cleanly cloned into yeast, producing stable yeast artificial chromosomes, or YACs.

Arsenic in Chilean Water

A biomarker study by Hopenhayn-Rich et al. (p. 620) assessed the distribution of arsenic metabolites in urine because methylation is considered the detoxification process for inorganic arsenic. A high exposure group with 580 µg/l urinary arsenic was compared to a low exposure group with 60 µg/l. The ratio of monomethylarsonate to dimethylarsinate was over 1.5 times greater in the high compared to the low exposure group. Urinary speciation analyses indicated no evidence for a threshold for methylation capacity. Further research was recommended to clarify the interindividual metabolic differences that may influence health risks from arsenic exposure.

Metals in Lungs of Mexico City Residents

Fortoul et al. (p. 630) measured concentrations of cadmium, copper, cobalt, nickel, and lead in lungs from autopsy samples of Mexico City residents. Sharp increases were noted between the 1950s and the 1980s, possibly due to extensive increases in air pollution in crowded metropolitan areas during the same interval. Analyses indicated that metal concentrations in lungs were in the same range or higher for women compared to men, and that cadmium increased with age during both time frames.

PCBs and Liver Toxicity

Brown et al. (p. 634) used a rat model to evaluate the the mode of action of PCB toxicity in the liver. Minimally toxic doses of lipopolysaccharide were administered to rats along with Aroclor 1248 to determine the extent of promotion of liver injury by the PCB. An increase in liver toxicity occurred in rats treated with both chemicals, but not in rats treated with PCB alone. These data and *in vitro* studies with rat liver cells suggest that PCBs may augment toxicity in the liver by activating cytotoxic neutrophils in a manner analagous to inflammatory reponses.

Kidney Cancer in Petrochemical Workers

Gamble et al. (p. 642) examined risk factors for kidney cancer in workers at a refinery/petrochemical plant because there was nearly a two-fold excess of tumors associated with exposure to aromatic chemicals. The authors found no significant association with cumulative exposure, but increased body weight, tenure, and blood pressure appeared to be important risk factors. Risk of kidney cancer for workers that were 25% overweight increased about 2.6-fold, while risk for workers with high blood pressure was increased about 4.5-fold.

Editorial

Pura Vida

. . . Costa Rica is not alone in linking economic development and biodiversity preservation . . .

Would you find it enticing to live in a country that has temperatures of 15 to 30°C year round? Would you consider living in a country situated between two continents, with coastlines so close to each other that easy access is available to both the Pacific Ocean and the Caribbean Sea? What if there was such ecological diversity that recreational possibilities included fishing, hiking, jungle exploring, white water rafting, and bird watching? Suppose there were added bonuses: the inhabitants were warm and friendly; primary education for both sexes was obligatory and free; neutrality had been declared in perpetuity; and no armed forces existed. Is this paradise? Is it a place of *pura vida* (pure life)? Does such a country exist and, if so, can something so good last?

Yes, this country of good living actually exists and can be found in Central America. The country is Costa Rica, a relatively small country with a population of just over 3 million people and an area of around 51,000 square kilometers. Scenic natural beauty is everywhere from the extensive coastlines to the strength of the central mountain ranges. Nearly every type of ecosystem can be experienced there; volcanoes, rain forests, jungles, white water, dry forest lands, and beaches are all found in Costa Rica. There is beauty here, no doubt about that, but the wealth the country possesses goes even deeper. Untold wealth lies in the biological diversity that can be found there. Within its narrow borders are an estimated 4% of the terrestrial biodiversity of the world.

The difficult question is whether a place so captivating can last after human intrusions. Is it possible to preserve the natural beauty, maintain the current level of biodiversity, and still provide for the desirable and inevitable economic growth? Fortunately, Costa Ricans (Ticos as the inhabitants prefer to be called) have come to appreciate their wealth and have sought ways to preserve it.

The country, in fact, holds one of the world's best conservation records, boasting that one-quarter of the country is under some form of official protection in the form of national parks or biological reserves. Awards have included the Cantico a Todas Las Criaturas —"Song to all Creatures"— award given by the Rome-based Franciscan Center for Environmental Studies and the first environmental award presented by the American Society of Travel Agents. In 1992 Costa Rica was named the most environmentally conscious country in the world by the San Francisco-based News Travel Network, and the National Biodiversity Institute was awarded the Peter Scott Award by the International Union for the Conservation of Nature (*1*).

These reserves and parks will continuously be faced with the pressures of development, so simply setting aside areas for preservation cannot be the total solution. Costa Rica has pondered this dilemma and come up with a solution based on the assumption made by their Instituto National de Biodiversidad (INBio): "a tropical society will conserve a major portion of its wild biodiversity only if protected areas can generate enough intellectual and economic income for its own upkeep."

The INBio was founded in 1989 as a private, nonprofit organization for the public good. The Institute's strategies are to develop an inventory of fauna and flora and to publicize and promote the nondestructive use of biodiversity by the commercial world. The INBio views biodiversity as "a potentially powerful engine for intellectual and economic development." Biological prospecting is fostered by the Institute with the anticipated financial rewards that will make the program self-supporting and provide funds for maintaining the natural diversity of the country. User fees are charged and royalties are negotiated from the sale of any products that result from the biological materials collected in Costa Rica. Principles, potential rewards, and problems of bioprospecting have been discussed by Reid et al. (*2*).

While biodiversity can be a powerful engine for intellectual and economic development, current laws frequently fail to ensure that indigenous people receive any benefits when companies develop products that use the biological researches of another country. These concerns were raised in a two-day conference on Biodiversity and Human Health held last April in Washington, DC (*3*). During the conference, the United States National Cancer Institute reported that it had developed legal agreements to guarantee that countries would receive financial rewards and scientific assistance for their contributions to new drug discoveries. It was also emphasized that a holistic approach is needed to preserve biodiversity as a source for future discoveries. Entire ecosystems and cultures must be preserved, not just a single species. So Costa Rica is not alone in linking economic development and biodiversity preservation; but because of the unique attributes of the country, including its size, political stability, and ecological awareness of its population, Costa Rica may represent the best chance for success.

Ah, but all is not perfect in this paradise as deforestation of lands outside of the national parks and reserves continues. The demand for land remains high because of activities such as a growing cattle industry and the migration of expanding populations into virgin land areas previously considered unsafe because of war. On top of these added stresses, the Government is trying to cope with the problem of sometimes poorly managed and chronically underfunded biological reserves and refuges, the conflict between the agricultural expansionists and environmentalists, and potential reversion of land currently in the national park system back to private use because of lack of funds to honor the previous purchase agreements.

Will Costa Rica succeed? I expect they will. My confidence is supported by the observation that there is widespread appreciation of nature among the citizens of Costa Rica. People talk about the birds and the monkeys. They can identify trees and plants. They talk of the mountains and beaches with respect. Besides appreciating the beauty of nature, many truly understand that a diminution of biodiversity would deal a devastating blow to their economic future. There is a very common and mellifluous expression used by Costa Ricans that says it all — from taxi drivers to the ecotour guides one

hears the expression "pura vida!" Costa Ricans are a people who recognize the important facets of life. For the good of our planet, we all need to support their efforts in preserving biodiversity.

Thomas J. Goehl, Ph.D.

References

1. Baker C. Costa Rican handbook. Chico, California: Moon Publications, 1994.
2. Reid WV, Laird SA, Meyer CA, Gamez R, Sitterfled A, Janzen DH, Gollin MA, Juma C. Biodiversity prospecting: using genetic resources for sustainable development. San Jose, Costa Rica:World Resources Institute, 1993.
3. Forum: Natures medicine cabinet. Environ Health Perspect 103:543–544 (1995).

Commentary

Is DDT Use a Public Health Problem in Mexico?

Lizbeth López-Carrillo,[1] Laura Torres-Arreola,[1] Luisa Torres-Sánchez,[1] Felipe Espinosa-Torres,[1] Carlos Jiménez,[1] Mariano Cebrián,[2] Stephan Waliszewski,[3] and Ofelia Saldate[4]

[1]National Institute of Public Health, 62508 Cuernavaca, Morelos, Mexico; [2]Department of Toxicology, Center of Research and Advanced Studies, National Polytechnic Institute, Mexico City; [3]Institute of Forensic Medicine, Veracruz University, Veracruz, Mexico; [4]Laboratory of Public Health, Secretary of Health, Mexico City, Mexico

We review the potential impact of DDT on public health in Mexico. DDT production and consumption patterns in Mexico during the last 20 years are described and compared with those in the United States. In spite of the restrictions on DDT use in antimalaria campaigns in Mexico, use of DDT is still higher than in other Latin American countries. We analyzed information from published studies to determine accumulated levels of this insecticide in blood, adipose tissue, and breast milk samples from Mexican women. Current lipid-adjusted DDE levels from women living in Mexico City are 6.66 ppb in mammary adipose tissue and 0.594 ppm in total breast milk. Finally, the methodological limitations of existing epidemiological studies on DDT exposure and breast cancer are discussed. We conclude that DDT use in Mexico is a public health problem, and suggest two solutions: identification of alternatives for the control of malaria and educational intervention to reduce DDT exposure. We also recommend strengthening epidemiological studies to evaluate the association between accumulated DDT levels in adipose tissue and breast cancer incidence among Mexican women. *Key words*: adipose tissue, breast cancer, DDT, epidemiological studies, human milk, Mexico, pesticide exposures, serum. *Environ Health Perspect* 104:584–588 (1996)

In the last 5 years, there has been a resurgence of international interest in research on exposure to DDT (dichlorodiphenyl tricholorethane) as a possible avoidable cause of breast cancer. Due to the estrogenic activity of DDT (*1*), it has recently been proposed that this compound acts as a xenoestrogen that increases the risk of breast cancer in women who are not necessarily exposed in an occupational environment. Given the tremendous public interest in breast cancer, developing effective research strategies in this area is a real challenge for environmental health researchers. Current evidence suggests that exposure to DDT may elevate the risk of developing breast cancer. So far that evidence remains tantalizingly incomplete (*2–5*).

Based on research carried out in populations occupationally exposed to DDT, it has also been suggested that DDT could play an important role in the etiology of pancreatic cancer (*6*) and leukemias (*7–9*), as well as producing alterations in reproductive function, such as decreases in sperm count (*10*), increases in the frequencies of preterm births (*11,12*) and congenital malformations (*13*), and decreases in the duration of lactation (*14*).

Since the 1940s, DDT has been widely used throughout the world to combat agricultural pests, indoor insects, and in sanitation campaigns against malaria. At present its use has been totally banned in developed countries due to its persistence (low biodegradability), accumulation, and bioconcentration in lipid systems, including subcutaneous fat, breast tissue, brain, and adrenal glands (*15,16*). In Mexico, DDT application in sanitation campaigns against malaria began intermittently in 1956 and has continued systematically since 1960 (*17*). Currently, the World Health Organization recommends the use of DDT for malarial outbreaks, although public health experts do not uniformly endorse this use. DDT targets adult insects and cannot kill larvae. Resistance of insects to DDT has occurred worldwide (*18*).

Devastating and obvious effects of DDT on wildlife, such as endangerment of the American bald eagle and the peregrine falcon, were the grounds for the banning of DDT in the United States in the 1970s. As ecological levels of DDT have dropped in the United States, these previously endangered species have recovered (*19*). More recently, a spill of the pesticide dicofol (which contained 10% DDT as an active ingredient) into Lake Apopka, Florida, has been tied to alterations in the sex ratio of alligators and increased defects in male alligators (*20*).

Breast cancer is the second cause of death among Mexican women, with a rate of 2.8 per 100,000 women in 1994 (*21*). Diagnosis of this malignant neoplasia generally occurs when the disease is at stage II or greater, as is shown in the information of the Mexico Cancer Registry (MCR) for 1989. During 1989, 1521 new cases were reported to the MCR. Only in 2% of these patients was the tumor discovered when it was still *in situ* (30 cases) (*22*).

Exposure to endogenous estrogens is the risk factor that links most known causes of breast cancer. Early age at menarche, late age at menopause, nuliparousness, and absence of breastfeeding increase lifetime estrogen exposures and are all associated with an elevated risk of breast cancer (*23*). Estrogen replacement therapy in postmenopausal women and alcohol and saturated fat consumption are factors that have been inconsistently linked to an increase in the risk of breast cancer. In spite of the progress made in knowledge of the etiology of breast cancer, it is estimated that only 30% of breast tumors can be explained by these factors (*24,25*).

A number of epidemiological studies have been carried out to evaluate the association between exposure to DDT and breast cancer. These studies have been conducted in populations in which DDT use has been banned for over 27 years and are subject to a number of methodological limitations (*2–5,26–28*).

In this article, we first present comparative information about the production and consumption of DDT in Mexico. Second, we document the levels of accumulated DDT in blood, adipose tissue, and breast milk samples from women who reside in Mexico City, including data about the levels of DDT in foods. Finally, we discuss the methodological limitations of epidemiological studies on breast cancer and DDT, exploring how these studies may have been biased toward a certain conclusion about this possible association. We propose that given the unusual agricultural exposures among populations living in urban areas of Mexico, a number of research studies should be conducted to clarify whether DDT is an avoidable cause of breast cancer.

Address correspondence to Lizbeth López-Carrillo, National Institute of Public Health, Av. Universidad No. 655, Col Sta. María Ahuacatitlán, Cuernavaca, Morelos C.P. 62508 Mexico.

We acknowledge Devra Davis and Malaquías López-Cervantes for their comments on the manuscript, Reina Collado-Ruiz, Celina M. Rueda-Neria, and Betania Allen for typing and editing, and the General Directorate of Preventive Medicine for providing information about the Mexico malaria campaign. This work was supported by the MacArthur Foundation, the Mexican Secretary of Health, and the COPERA Program sponsored by the Government of Mexico City.

Received 22 November 1995; accepted 15 March 1996.

Production and Consumption of DDT in Mexico and the United States

After the Second World War (1945), industrial production of DDT began, largely for the treatment of lice in soldiers. The production and consumption patterns of DDT have varied substantially between developed and developing countries. Malaria control campaigns have been undertaken in both developed and developing regions. Agricultural uses have occurred solely in developing countries. The production and uses of DDT in Mexico and the United States are a good example of these differences (*29*).

In Mexico, production of DDT by two firms began in 1959 (Table 1). In the 1960s in the United States, Rachel Carson's book *Silent Spring* (*30*) generated widespread concern about the indiscriminate use of pesticides. Eventually this led to the regulation of DDT in 1969 and the ban on domestic use in the United States in 1972. However, in Mexico during the same decade, DDT and other organochlorine pesticides were widely used, and during the 1971–1984 period, 60,609 tons of these products were sprayed; DDT accounted for approximately 10% of these products (*31*). During this same period, (1971–1984), the capacity for DDT production in Mexico was 8000 tons annually, representing between 43% and 45% of the total national capacity for organochlorine pesticide production (*29*).

The evolution of DDT production and consumption in Mexico between 1971 and 1991 is shown in Figure 1. During the second part of the 1970s and the beginning of the 1980s, both production and consumption varied between 3400 and 4100 tons annually. Between 1982 and 1986, production and consumption decreased notably, almost to zero, although beginning in 1986 production began to grow again and consumption followed in 1987 (*31*).

The pronounced decrease in production and consumption of DDT observed during the first half of the 1980s in Mexico was principally due to two causes. First, a severe economic crisis affected Mexico in 1982, which resulted in a drastic drop in production and commercial activities in the country. Second, an international trend reduced the use of organochlorine pesticides, which were widely recognized to persist in the environment (*32*). This trend resulted in restriction of DDT use in official Mexican sanitary campaigns, and therefore a notable decrease in demand and production of DDT.

As shown in Figure 2, approximately 226,000 tons of DDT were used in the antimalaria campaign during 1971–1993, averaging 500 g per household sprayed. Since 1988, malaria incidence has reportedly decreased by 90%, and the proportion of rural towns with blood samples positive for malaria has decreased by 75%. However, current use of DDT in Mexico is still greater than in other Latin American countries, as can be seen in Figure 3 (*17–33*).

To illustrate the widespread production and marketing of DDT in Mexico, it should be noted that in 1987 there were two large firms that produced DDT as an active ingredient. This product was used in turn by 23 firms to develop 6 different formulations, resulting in 35 different registered brands of pesticides sold in the national market. Currently DDT is produced in only a few countries; in Mexico DDT is produced in a single industrial plant, located in the city of Salamanca. Less than 5000 tons of DDT have been exported from Mexico since 1971 (*28,34*).

Recently the Mexico Secretary of Health made a commitment to eliminate

Table 1. Evolution of DDT use in the United States and Mexico

Year	United States	Year	Mexico
1945	Use begins	1945	Use begins
1962	Publication of *Silent Spring* (*30*)	1959	Production begins
1969	Regulation begins	1960–70	Use spreads
1972	Domestic use banned	1971–84	60,609 tons of organochlorine pesticides, including 6,061 tons of DDT per year

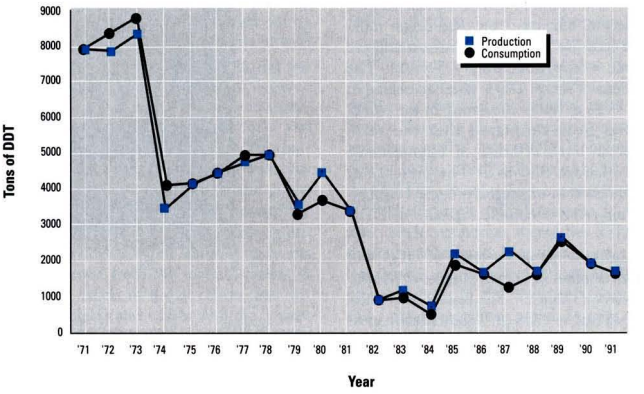


Figure 1. Apparent DDT production and consumption in Mexico, 1975–1991 (*31,34*).

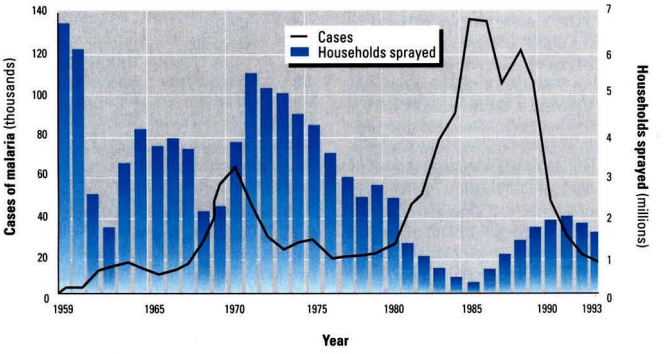


Figure 2. The Mexican malaria campaign, 1959–1993. The number of cases of malaria and households sprayed are shown (*17*).

the use of DDT and to look for alternatives for malaria control with technical support from United States and Canada (*35*).

DDT Levels in Serum, Adipose Tissue, and Breast Milk

Due to the lipophilic nature of DDT and its principal metabolite, dichlorodiphenyl dichloroethene (DDE), these compounds have been found in diverse human samples of serum, adipose tissue, and breast milk. The half-life of DDT in human adipose tissue is approximately 7.5 years. The amount of serum DDT varies according to the levels of lipids circulating in the blood. It has been estimated that the ratio between levels of DDT in adipose tissue and blood is 300 to 1. The presence of DDE levels in organisms is a good biological indicator of chronic exposure to DDT (*36*).

Information about levels of DDT and its metabolites in human samples in Mexico is scarce (Table 2). The results of a study carried out in 1975 (*37*) showed that levels of DDE in abdominal adipose tissue samples, expressed in parts per million in a lipid base (μg/g of extractable lipids), ranged from 2.65 ± 2.35 in 9 adipose tissue samples from necropsies in the city of Puebla (located in the central region of Mexico) to 18.36 ± 33.27 in 19 similar samples obtained in the city of Torreon (located in the northern part of Mexico). Corresponding levels detected in 9 samples from biopsies from Mexico City had a mean value of 6.05 ± 3.49 (*37*). The results of another study carried out in a city on the northern border of Mexico (Ciudad Juarez) showed that accumulated levels of DDT and its derivatives in 62 human adipose tissue samples obtained in 1977 were 20.59 ± 13.18 ppm, and in 1992, Waliszewski et al. (*38,39*) found average levels of DDE to be 18.91 ± 23.29 ppm in necropsy tissue from individuals who had lived in the state of Veracruz (Gulf of Mexico). The initial results obtained by our research team in 1995 showed that the geometric mean of DDE in adipose breast tissue samples from 160 women living in Mexico City is 6.66 ± 1.66 ppb.

Although the representativeness of the biological samples analyzed in some of these studies can be questioned, these studies suggest that there is a DDT accumulation gradient that is greater in tropical areas and/or regions with greater agricultural activity (Veracruz, Torreon, Ciudad Juarez). These data also show that inhabitants of urban areas are exposed to DDT (Torreon, Ciudad Juarez, Puebla, Veracruz, and Mexico City).

Since the end of the 1970s, Mexican studies have episodically documented the presence of DDE and total DDT in breast milk, with levels varying between 0.20 and 0.26 mg/kg (ppm) of total milk. Thus, levels of DDE in breast milk in Mexico are two to three times greater than corresponding levels in samples analyzed in the United States during that same period (*40*).

Our 1995 study showed that women living in Mexico City had DDE levels in of breast milk of 0.594 mg/kg in a lipid base. In contrast, in women living in tropical Mexico these levels reached an average level of 5.02 mg/kg, which is extremely high (Waliszewski et al., submitted). Thus, there is a 10-fold difference in DDE levels within Mexico.

As mentioned previously, use of DDT in Mexico has been restricted to sanitary campaigns against malaria. These campaigns have been carried out in all states, except those which are not considered endemic (Tlaxcala, Mexico City, Baja California Norte). Despite this, high accumulation levels of DDE have been found in the biological samples from Mexico City residents. It is quite likely that exposures to DDT have occurred in these urban areas not only from malarial campaigns but also from other sources, such as lipophilic foods.

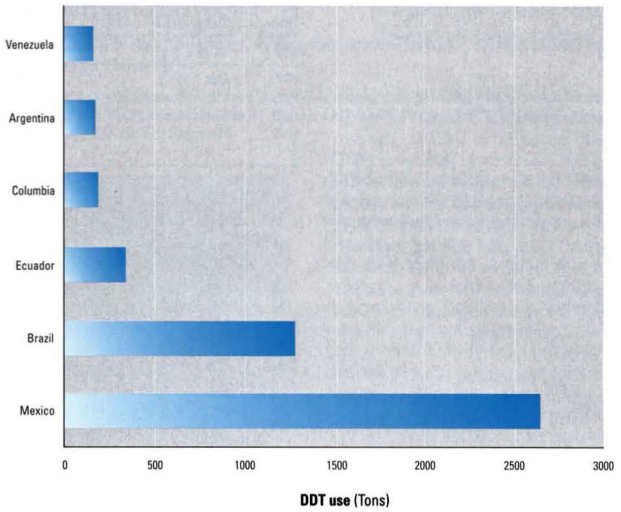


Figure 3. DDT use in malaria prevention programs in selected Latin American countries, 1993–1994; including 100% and 75% formulations. The 1994 values are estimated (*33*).

Table 2. DDE and total DDT levels in Mexican human samples

Reference	City	Sample type	*n*	Anatomic site	DDE, mean ± SD (ppm)	DDT, mean ± SD (ppm)
(37)	Torreon	Necropsy	19	Abdomen	18.36 ± 33.27	21.47 ± 37.10
	Mexico City	Biopsy	9	Abdomen	6.05 ± 3.49	8.31 ± 4.95
	Puebla	Necropsy	9	Abdomen	2.65 ± 2.35	3.4 ± 3.25
(38)	Ciudad Juarez	Biopsy	62	NR	NR	20.59 ± 13.18
(39)	Veracruz	Necropsy	90	Abdomen	18.91 ± 23.29	24.14 ± 27.88
This study	Mexico City	Biopsy	160	Breast	6.66 ± 1.66 (ppb, geometric mean)	NR

NR, not reported.

DDT Levels in Foods

According to the information provided by the Public Health Laboratory of the Ministry of Health, out of a total of 439 food samples analyzed between February 1993 and March 1995, diverse organochlorine and organophosphate pesticide residues were found in 146 (unpublished data).

The foods that contained DDT and its derivatives were principally meats and dairy products. For example, in 43.5% of the milk samples analyzed (86/202), *p,p'*-DDT, DDE, and *p,p'*-TDE were found. DDT levels varied widely, from 0.01 ppm to 0.082 ppm. Likewise, in 13 of the 30 meat samples (30%), DDE and *p,p'*-DDT were found at levels from 0.001 ppm to 0.06 ppm. Sixty-eight percent of the milk sam-

ples and all the meat products came from states located in the central–southern part of the country.

Butter samples (345) from the state of Veracruz (Gulf of Mexico) were analyzed in 1994. The results showed that DDE levels did not exceed those recommended by the World Health Organization (1.25 ppm) (Waliszewski et al., submitted). The same authors found that in 192 cows' milk samples, DDE levels did not exceed the corresponding Food and Agriculture Organization recommended level (Waliszewski et al., submitted); levels were also acceptable in 53 samples of bovine liver fat samples from the same area (*41*).

Information about residues of DDT and its derivatives in Mexican foods should be interpreted with caution. It is possible that the results described above are not representative of the actual DDT contamination values, since none of the samples analyzed was obtained through probabilistic sampling methods.

Methodological Limitations of Epidemiological Studies of DDT Exposure and Breast Cancer

A number of articles have discussed the methodological limitations of the eight epidemiological studies that have provided controversial results on DDT exposure and breast cancer (*42*). Given the small sample size of most of these studies, they lacked the minimum power necessary to detect a difference, if one exists, between DDT levels in breast cancer patients and the corresponding levels in women without the disease. Another criticism has been the lack of control of confounding variables, principally parity, breastfeeding, and obesity, which are factors associated both with breast cancer incidence and accumulation or elimination of DDT from the body.

In addition to these limitations, other factors could account for some of the discrepancies in the results. For example, different tumor types may have distinct susceptibilities to xenoestrogens, so that estrogen-positive and -negative tumors may have different etiologies. In addition, levels of DDT or metabolites can be reported in either a lipid base or a wet base, thus affecting comparability across the studies.

In the only study that considered the presence of estrogenic receptors in patients with breast cancer (*5*), a highly significant difference was found between the levels of DDE in adipose tissue and serum in women with breast cancer (cases) and controls with benign breast disease (× DDE adipose tissue: 2732 ± 2749.9 µg/kg versus 765 ± 52.9 µg/kg, serum: 8.5 µg/l versus 3.5 µg/l). The groups of women compared were similar in terms of age, parity, and weight loss during the year before diagnosis. However, there was a greater prevalence of non-breastfeeding among cases (88.9% versus 76.5%). In spite of a small sample size (9 cases and 17 controls), the authors estimated an 8.9 times greater breast cancer risk in those women in whom DDE levels were above 1292 µg/kg in adipose tissue (*5*).

In contrast, the epidemiological study with the largest sample size, carried out by Krieger et al. (*24*), compared women with breast cancer and women without the disease among a cohort established between 1964 and 1971. The 150 cases and controls consisted of 50 whites, 50 blacks, and 50 Asian-Americans. Although approximately 50% of the patients with breast cancer showed higher DDE levels, the difference between these levels and those of the control group was not statistically significant. However, when Asian-Americans were removed from the analysis, a two- to threefold excess of breast cancer was evident for blacks and whites with the highest levels of DDE in the sera. In addition, there was no information about breastfeeding or the proportion of estrogen-dependent breast tumors. Levels were not adjusted by total lipids, and no information of DDE levels in adipose tissue was provided, which is the best way to measure chronic DDT accumulation. This could have influenced the observed results (*43*).

A similarly designed study, controlled for breastfeeding, found that breast cancer risk was 3.68 times greater in women with DDE serum levels of 19.1 ng/ml as compared to women with DDE serum levels of 2.0 ng/ml (*4*). Another study reported that *p,p′*-DDE levels in adipose tissue of women with breast cancer are greater than corresponding levels in those with benign breast disease (*3*).

In a study that compared only nine samples of adipose breast tissue from breast cancer patients and five adipose tissue samples from women who died in accidents, greater concentrations of *o,p′*-DDT were found in the women with breast cancer (*2*). Finally, two more studies with the same type of limitations mentioned earlier did not report higher levels of DDT or its metabolites in women with breast cancer in comparison to women without the disease (*26,27*).

It is difficult to conclude whether exposure to DDT contributes to an increase in breast cancer. All of the recent studies have yielded evidence of a dose–response relationship, although these are subject to interpretation. There is an evident need for additional investigations that surmount the methodological limitations described here.

Conclusions

Many questions about DDT exposure and its potential impact on health are being researched at a number of levels. In terms of biomedical research, knowledge should be generated about the possible carcinogenic mechanisms of DDE in humans. Also, data are lacking about the levels of DDT accumulation in adipose tissue and serum, as well as rates of elimination of this compound in breast milk, in representative populations in developed and developing countries. High-risk populations have been identified, principally in urban and agricultural areas. In Mexico there is a need to develop systematic and representative data on DDT contamination of foods, which will probably explain the high levels found in human samples.

Identification and assessment of less toxic and less persistent alternatives for controlling malaria and educational, population-based interventions to reduce DDT exposure both in the work environment and among the general population are also needed. These interventions could be directed toward promoting use of protective gear by workers and health education for populations not occupationally exposed.

The continuing epidemiological study being carried out by the National Institute of Public Health of Mexico, which seeks to evaluate the association between DDE accumulation levels in serum and adipose tissue and breast cancer in Mexican women, is promising in terms of its methodological characteristics. These characteristics include sufficient statistical power (150 cases and 300 controls), a wide range of DDT exposure, control of confounding reproductive and dietary variables, assessment of DDT and DDE levels in lipid base, and information about estrogenic receptors in a subgroup of the cancer patients.

Efforts should continue to find alternatives to DDT while additional study results about its role in breast cancer are generated. Evidence on the long-term ecological consequences of DDT for wildlife are indisputable. The absence of clear-cut proof that DDT causes breast cancer should not be used as an excuse for further delays in phasing out this persistent, toxic organic pollutant. As many of the known causes of breast cancer cannot readily be altered, those causes that can be controlled become all the more important for public health.

References

1. Bustos S, Denegri JC, Díaz F, Tchernitchin AN. *p,p′*-DDT is an estrogenic compound. Bull Environ Contam Toxicol 41:496–501 (1988).
2. Wassermann M, Nogeira DP, Tomatis L. Organochlorine compounds in neoplastic and

adjacent apparently normal breast tissue. Bull Environ Contam Toxicol 15:478–484 (1976).
3. Falck F, Ricci A, Wolff M, Godbold J, Deckers P. Pesticides and polychlorinated biphenyls residues in human breast lipids and their relation to breast cancer. Arch Environ Health 47:143–146 (1992).
4. Wolff MS, Toniolo PG, Lee EW, Rivera MK, Dubin N. Blood levels of organochlorine residues and risks of breast cancer. J Natl Cancer Inst 8:648–652 (1993).
5. Dewailly E, Dodin S, Verreault R, Ayotte P, Sauve L, Morin J, Brisson J. High organochlorine burden in women with estrogen-receptor positive breast cancer. J Natl Cancer Inst 86:232–2234 (1994).
6. Garabrant DH, Held J, Langholz B, Peters JM, Mack TM. DDT and related compounds and risk of pancreatic cancer. J Natl Cancer Inst 84:764–771 (1992).
7. Faustini A, Forastiere F, Di-Beta L, Magliola EM, Perucci CA. Cohort study of mortality among farmers and agricultural workers. Med Lav 84:31–41 (1993).
8. Flodin U, Fredriksson M, Persson B, Axelson O. Chronic lymphatic leukaemia and engine exhausts, fresh wood, and DDT: a case referencie study. Br J Ind Med 45:33–38 (1988).
9. Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmesiter LF, Van Lier SF, Dick F. Pesticides exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 50:6585–65891 (1990).
10. Bercovici B, Wassermann M, Cucos S, Ron M, Wasserman D, Pines A. Serum levels of polychlorinated biphenyls and some organochlorine insecticides in women with recent and former missed abortions. Environ Res 30:169–174 (1983).
11. Saxena MC, Siddiqui MK, Seth TD, Krishna-Murti CR, Bhargava AK, Kutty D. Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature of full-term delivery. J Anal Toxicol 5:6–9 (1981).
12. Wassermann M, Ron M, Bercovicic B, Wassermann D. Premature delivery and organochlorine compounds: Polychlorinated biphenyls and some organochlorine insecticides. Environ Res 28:106–112 (1982).
13. Nurminen T, Rantala K, Hokmerg PC. Agricultural work during pregnancy and selected structural malformations in Finland. Epidemiology 6:23–30 (1995).
14. Gladen BC, Rogan WJ. DDE and shortened duration of lactation in a northern Mexican town. Am J Public Health 85:504-508 (1995).
15. Loganathan BG, Kannan K. Global Organochlorine contamination trends: an overview. Ambio 27:187–191 (1994).
16. Forget G. Pesticides and the third world. J Toxicol Environ Health 32:11–31 (1991).
17. OPS/OMS. XXIV Conferencia Sanitaria Panamericana XLVI. Reunión del Comité Regional. Situación de los programas de Malaria en las Américas Informe XLVII. Washington, DC:Oficina Panaméricana para la Salud/ Organización Mundial para la Salud, 1994.
18. OMS. Resistencia a los Incecticidas. In: Métodos químicos de lucha contra artrópodosvectores y plagas de importancia en Salud Pública. Ginebra:Orgización Mundial para la Salud 1984;13–15.
19. Davis DL, Bradlow HL. Can environmental estrogens cause breast cancer? Sci Am 273: 166–172 (1995)
20. Guillette LJ Jr, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 102:680–688 (1994).
21. Secretaria de Salud. Perfiles estadísticos no. 7: Tumores. Series Monográficas. México City, 1993.
22. Secretaria de Salud. Morbilidad por neoplasias malignas 1982–1989. México City, 1993.
23. Kelsey J, Gammon M, John E. Reproductive factors and breast cancer. Epidemiol Rev 15:36–47 (1993).
24. Brinton L, Schaire C. Estrogen replacement therapy and breast cancer risks. Epidemiol Rev 15:66–79 (1993).
25. Henrich J. The postmenopausal estrogen breast cancer controversy. J Am Med Assoc 278: 1900–1902 (1992).
26. Austin H, Keil JE, Col P. A prospective follow-up study of cancer mortality in relation to serum DDT. Am J Public Health 79:43–46 (1989).
27. Mussalo-Rauhamaa HM, Hasanen E, Pyysalo H, Antervo K, Kauppila R, Pantzar P. Occurrence of beta-hexachlorocyclehexane in breast cancer patients. Cancer 66:2124–2128 (1990).
28. Krieger N, Wolff M, Hiatt R, Rivera M, Vogelman J, Orentreich N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J Natl Cancer Inst 86:589–599 (1994).
29. Comisión Petroquímica Mexicana-SEMIP. La Industria de Los Plaguicidas en México. México City, 1987.
30. Carson R. Silent Spring. Boston:Houghton-Mifflin, 1962.
31. ANIQ. A.C. Anuario Estadístico de la Industria Química Mexicana 1994. México City, 1994.
32. Ortega J, Espinosa F, López L. El control de los riesgos para la salud generados por los plaguicidas organofosforados en México: retos ante el tratado de libre comercio. Salud Publica Mex 36: 624–632 (1994).
33. PAHO. Status of malaria programs in the Americas XLII report. PAHO document CSP24/INF/2. Washington, DC:Pan American Health Organization, 1994.
34. ANIQ. A.C. Anuario de la Industria Química Mexicana 1978. DF, México:Asociación Nacional de la Industria Química Mexicana, 1979.
35. Enciso A. DDT, mercurio y clordano se añaden a los PCB. Acuerdan los firmantes del TLC eliminar el uso de tres tóxicos. La Jornada, 9 Febrero 1996.
36. Woodruff T, Wolff M, Lee DD, Hayward D. Organochlorine exposure estimation in the study of cancer etiology. Environ Res 65:132–144 (1994).
37. Albert L, Méndez F. Organochlorine pesticide residues in human adipose tissue in Mexico: results of a preliminary study in three Mexican cities. Arch Environ Health 35:262–269 (1980).
38. Redetzke K, Gonzalez A, Applegate H. Organochlorine pesticides in adipose tissue of persons from Ciudad Juarez, Mexico. J Environ Health 46:25–27 (1983).
39. Waliszewski SM, Pardio-Sedas VT, Infanzon RM, Rivera J. Detemination of organochlorine pesticides residues in human adipose tissue: 1992 study in Mexico. Bull Environ Contam Toxicol 55:43–49 (1995).
40. Albert L. Residuos de plaguicidas organoclorados en leche materna y riesgo para la salud. Bol Off Sanit Panam 91:15–29 (1981).
41. Waliszewski SM, Infanzon RM, Rivera J. Levels of organochlorine pesticides in bovine kidney fat. Environ Toxicol Chem 14:342–345 (1995).
42. Houghton DL, Ritter L. Organochlorine residues and risk of breast cancer. J Am Coll Toxicol 14:71–89 (1995).
43. Savitz DA. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J Natl Cancer Inst 86:1255–1256 (1994).

an institute of the National Institutes of Health

NIEHS SUPERFUND INFORMATION SYSTEMS

- **NIEHS Superfund Basic Research World Wide Web Page**

  URL: http://www.niehs.nih.gov/sbrp/home.htm

- **NIEHS Superfund Basic Research Electronic Bulletin Board (BBS)**

  1-800-850-7235 (8 data bits, 1 stop bit, no parity, ANSI)

For additional information:

Bradley C. Blackard
tel. (919) 544-0544
e-mail: blackard@niehs.nih.gov

BASIC RESEARCH PROGRAM

The National Institute of Environmental Health Sciences

Environmental Health and Hazardous Waste Issues Related to the U.S.–Mexico Border

Dean E. Carter,[1] Carlos Peña,[1] Robert Varady,[2] and William A. Suk[3]

[1]College of Pharmacy and [2]Udall Center for Studies in Public Policy, University of Arizona, Tucson, AZ 85721 USA; [3]National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 USA

Environmental health and environmental quality issues along the U.S.–Mexico border have been of concern for several years. The enactment of the North American Free Trade Agreement and the presence of the *maquiladoras* (foreign-owned industries using imported raw materials) have intensified those concerns recently. Efforts to assess these issues are complicated by the fact that many of the issues affecting the border region are within federal jurisdiction, but the problems are regional and local in nature. Thus, state and local governments become involved with public concerns about real and potential problems. One major problem is that environmental health data from this region are lacking, particularly from Mexico. Some new agencies such as the Border Environment Cooperation Commission, the United States–Mexico Border Health Commission, and the North American Commission on Environmental Cooperation have joined several existing agencies at the federal and state level to address environmental quality and health. Several studies have been initiated to determine air and water quality, but little is being done in the areas of hazardous waste and health assessment. Several problems are anticipated in the generation of such data, such as its format and accessibility. Data gaps and research needs are discussed. *Key words:* environmental health, environmental quality, hazardous waste, Mexico, NAFTA, U.S.–Mexico border. *Environ Health Perspect* 104:590–594 (1996)

The North American Free Trade Agreement (NAFTA) has resulted in increased trade between the United States and Mexico. With more trade, there is a growing concern about environmental health and hazardous waste issues in the two countries, particularly near the border. Americans are concerned that pollution may cross from Mexico to the U.S. and that companies expanding into Mexico may get an economic advantage from less stringent environmental enforcement; Mexicans are concerned that industrial expansion from American companies will increase hazardous waste problems. A workshop was held to address some of these problems (*1*); the workshop followed a previous U.S.–Mexico conference (*2*). This commentary attempts to put the results of that workshop in the larger context of the structures that are in place to address border environmental problems.

The workshop was co-sponsored by the National Institute of Environmental Health Sciences (NIEHS), the U.S. Environmental Protection Agency Office of Research and Development (EPA), and the Centers for Disease Control and Prevention (CDC) under the auspices of the Interagency Coordinating Committee for U.S.–Mexico Border Environmental Health, in cooperation with the Program for the Environment at the National University of Mexico (Universidad Nacional Autónoma de México, Programa Universitario de Medio Ambiente). Its goals were to assemble a cross-section of people from federal agencies, state agencies, local action groups, and universities from the United States and Mexico to present individual perspectives on the problems of hazardous waste on the border, identify current databases of environmental health and quality, and identify data gaps.

Hazardous waste at an international border presents some unique problems for citizens, regulators, and industry because of overlapping jurisdictions. International borders are under federal jurisdiction, so laws regulating the movement of hazardous waste across the border must be negotiated by treaty between the two countries and must be consistent with the laws of each country. However, hazardous waste problems usually affect local communities composed of relatively small populations and limited political power. Most often, local and state authorities are the first entities to hear about citizen concerns and are asked to intervene (*3*). Concerns about hazardous waste may be stimulated by observations and/or studies that may or may not be scientifically rigorous but, nonetheless, result in great concern about the potential problem or general perception of it. In most cases the issues get passed up the government ladder and must compete with other hazardous waste problems in the respective states and federal agencies. A major difficulty exists when trying to evaluate the concern or when formulating solutions because an entire new set of agencies is involved at the local, state, and federal level. For example, if there was a serious chemical spill in the Rio Grande, it might involve federal agencies from Mexico and the United States, six different state governments (four in Mexico—Chihuahua, Coahuila, Nuevo Leon, and Tamaulipas—and two in the United States—Texas and New Mexico), and numerous border communities that are contiguous and separated only by a fence (e.g., El Paso, Texas, and Ciudad Juárez, Chihuahua).

The jurisdictional issue is further complicated by other differences between the two countries. National differences include language, economic strength, environmental awareness by the population, and public health infrastructure. As a developing nation, Mexico has had to emphasize providing basic services to its growing and largely youthful population. Problems such as nutrition, clean water, sewers, and communicable disease control have been more pressing than other environmental problems.

This particular region has unique characteristics that add to potential hazardous waste concerns. The border is an arid region where water is a precious resource and any pollution of that resource would create serious problems (*4*). The border region also features a distinct industrial arrangement wherein *maquiladoras* (foreign-owned industries using imported raw materials) exist south of the border; the sole function of these facilities is to import raw materials mostly from the United States and return the finished products for sale in foreign markets. By law, all hazardous materials either imported or generated as by-products by these companies are to be returned to the United States for disposal. Regulations regarding the import and export of hazardous waste create an incentive for illegal disposal.

NAFTA

Although problems of environmental quality in general and toxicity in particular are not new in the U.S.–Mexico border region, preventive and remediative measures have been inadequate and uneven. However, the

Address correspondence to D.E. Carter, Department of Pharmacology/Toxicology, College of Pharmacy, University of Arizona, 1703 E. Mabel, PO Box 210207, Tucson, AZ 85721-0207 USA.
Received 21 June 1995; accepted 22 February 1996.

negotiations leading to NAFTA and its passage in 1993 have heightened public awareness of the border and of the potential effects of large-scale economic development. One result of this attention and dialogue has been the adoption of a new cooperative, binational approach to alleviating environmental and public health problems.

This approach, formalized in the 1993 NAFTA environmental side accords, seeks to improve environmental infrastructure on the one hand and to assure environmental sustainability and community support on the other. The strategy relies on new investment to improve the region's capacity to manage resources and improve the general quality of life, but it also provides for public input to the process and establishes environmental criteria for new projects.

This post-NAFTA process is still in the early developmental stages: its first tentative steps were taken during the summer of 1994. The binational framework within which this process will operate is innovative but experimental. The longest-standing modes of cooperation between the United States and Mexico have been water-related, principally governing the shared surface waters of the Rio Grande/Rio Bravo. In this regard, the century-old International Boundary and Water Commission (IBWC) is the region's oldest and most durable binational institution. Its purview has included the entire 3000-km length of the border, and its primary concerns have been issues of water quality and sewage treatment.

Partly as a result of the mutual distrust between the two neighboring countries and partly as a reflection of societal indifference to environmental concerns, issues other than those governing surface water remained virtually unaddressed until the early 1980s. In 1983 the two nations' presidents signed the La Paz Agreement, or Reagan-de la Madrid Accord, which for the first time addressed such matters as transboundary air and water pollution and hazardous materials. These issues, considered by joint national ad-hoc working groups, were discussed, and at least two major concerns (San Diego-Tijuana sewage and Arizona-Sonora "Gray Triangle" air pollution) were addressed during the late 1980s.

The post-NAFTA configuration relies on several new concepts and a number of new transnational institutions to implement these ideas. First, recognizing that sizable infusions of new capital are needed by communities on both sides of the border, the side accord to the free-trade agreement establishes a new regional lending institution, the North American Development Bank (NADBank). This bank, fueled by private investment capital, is to "finance public and private investment in environmental infrastructure projects" (*5*). NADBank's decision-making, however, is tied to another new institution, the Border Environment Cooperation Commission (BECC), also created by the NAFTA side accord. According to its charter, BECC is to certify projects for NADBank funding. Additionally, the BECC is intended to provide a new voice to previously underrepresented and disadvantaged border communities by assisting them in developing and implementing environmental infrastructure projects (*5*). To accomplish this, BECC's board includes two members (one from Mexico and one from the United States) representing the community at large.

Complementing NADBank and BECC at the trinational level, a third institution has been established: the trilateral (United States, Canada, Mexico) North American Commission on Environmental Cooperation (CEC). It too solicits public input and seeks to ensure that grassroots community concerns form a meaningful part of economic development resulting from NAFTA.

These organizations are nascent, and their agenda is just beginning to be implemented, so it is too early to gauge their effectiveness. Nevertheless, it has become clear to residents and public agency officials of the border region that the area's environmental and health concerns need to gain greater attention and, not withstanding U.S. congressional politics, a larger share of resources. Insofar as free trade implies increased industrialization and therefore larger volumes of hazardous chemicals, and given that the new focus will lead to better data gathering and enhanced monitoring, it is safe to predict that problems of toxic pollutants are likely to become increasingly prominent.

Organizations Involved with Border Environmental Problems

The new approach to addressing environmental concerns in the border region has been adopted by the two countries precisely because they agree that existing mechanisms, organization, and financing arrangements have proven insufficient. Still, it would be incorrect to assume that NADBank, BECC, and CEC will operate in a social and institutional vacuum. They will, of course, function in a complex setting that includes numerous ministries, agencies, state and local governments, and nongovernmental organizations. In fact, by design, the NADBank and the commissions comprise these very elements within their memberships.

The existing organizations that retain jurisdiction over various aspects of environmental and health problems can be considered to function at three levels: international, national, and state.

International groups. In addition to the three new institutions discussed above, the IBWC, the Interagency Coordinating Committee for U.S.–Mexico Border Environmental Health, the Pan American Health Organization (PAHO), and the La Paz Agreement Technical Working Groups are the most important groups that function transnationally in the U.S.–Mexico border region. Recently, the United States passed a bill to establish the U.S.–Mexico Border Health Commission to address health concerns along the border.

PAHO maintains a regional office in El Paso, Texas, which has dealt with health issues of concern at the border. PAHO's concerns have largely been communicable diseases and not environmental health issues, although environmental issues were addressed in their Project Consenso study. Thus, PAHO has added environmental health concerns to its agenda and should be a source of data in the future.

Environmental health issues stimulated the formation in 1992 of an Interagency Coordinating Committee for U.S.–Mexico Border Environmental Health. The lead agencies for the United States are CDC and EPA; involved agencies include NIEHS, the National Institute for Occupational Safety and Health (NIOSH), the Agency for Toxic Substances and Disease Registry (ATSDR), and the Food and Drug Administration (FDA). Federal agencies from Mexico include the Ministry of Health and the Ministry of National Resources, Environment, and Fisheries. The four border states on the U.S. side (Arizona, California, New Mexico, and Texas) are also represented along with the PAHO Border Office. This committee is developing a Border Action Program to address the most immediate environmental problems, and its activities involve data inventories, monitoring, technology transfer, training, and infrastructure. The disposition and public access mechanism to data accumulated is still being discussed.

A new organization called the United States–Mexico Border Health Commission was authorized in October 1994 by the U.S. Congress and now awaits a funding appropriation and an agreement with Mexico before it will be established. Its duties include: 1) conducting a comprehensive needs assessment in the border region to identify, evaluate, prevent, and resolve health problems; 2) implementing the actions recommended by the needs

assessment through assisting in the coordination and implementation of the efforts of public and private entities to prevent and resolve health problems and to educate such populations concerning health problems; and 3) formulating recommendations to the governments of the United States and Mexico concerning a fair method by which the government of one country could reimburse a public or private entity in the other country for the cost of health care service provided.

The law does not clarify how this commission will interact with the other international agencies described above or how any of the recommendations of the committee will be funded. If the two governments can work together in the development of this commission, it could serve as a beneficial group that could combine the efforts of several other groups.

National agencies. Environmental issues at the border reflect the spectrum of concerns including human health, ecology and biological diversity, and environmental quality and industrial activity. In the United States, several parent agencies are directly involved in research, regulation, and community action of environmental health concerns: CDC, NIOSH, ATSDR, and NIEHS. The U.S. EPA has responsibilities in environmental health, environmental quality, and ecology. Other federal agencies that have specialized interests in this area, particularly with regard to potential data generation, are the Department of Interior and the U.S. Geological Survey, Bureau of Land Management (BLM), National Park Service, Bureau of Reclamation, U.S. Forest Service, and U.S. Department of Agriculture (USDA). In Mexico, the main ministry was SEDESOL up until December 1994. The new agency is Secretaría de Recursos Naturales, Medio Ambiente y Pesca (Ministry of Natural Resources, Environment, and Fisheries) and others involved are the Ministry of Health and the Ministry of Labor. The Ministry of Natural Resources, Environment, and Fisheries has representatives in each state of Mexico, but authority appears to be centralized in Mexico City.

State agencies. Each of the 10 states on the two sides of the border has separate offices of Health and Environmental Quality (generally called Ecology in Mexico). In the states on the U.S. side, there are offices specifically for border-related problems. In addition, these states generally have good communications with their cross-border counterparts. For example, Arizona and Sonora have an Arizona–Sonora Commission as a part of the governor's office that includes environmental concerns in its responsibilities, although its primary concern is economic development.

In addition, the Border Governors Association comprises all the border states in both countries. The governors meet regularly, and the association has appointees to a committee specifically responsible for environmental concerns.

Some border counties and cities have offices responsible for public health and environmental quality. These offices are often the first to become aware of environmental concerns. Generally, the cross-border cities have a long history of cooperative action in the identification and solution of common problems.

Finally, most of the region's universities in the U.S. and Mexico have outreach, education, and research programs concerning the environment. For example, The Udall Center for Studies in Public Policy at The University of Arizona has been particularly active in studying environmental problems along the U.S.–Mexico border.

Environmental Health and Environmental Quality Data

Reliable, thorough, and accurate information bases are essential to health researchers. Yet the border region does not have a central repository or even an inventory for data concerning environmental health or environmental quality. Of greater concern is the fact that virtually none of the existing studies has been published in peer-reviewed publications. The Interagency Coordinating Committee for U.S.–Mexico Border Environmental Health is compiling information on data sources for its evaluation of the border environmental problems and plans to make this information available in a user-friendly mode; but nevertheless, nothing currently exists.

The status of environmental health data is of particular concern. There have been reports of abnormally high incidences of neural tube defects including anencephaly in the lower Rio Grande Valley, of cancer (particularly multiple myeloma) and lupus in a neighborhood in Nogales, Arizona, and adverse pregnancy outcomes among workers in *maquiladoras* (*4*). However, these reports are not the result of rigorous epidemiological studies and have not been published except as reports or by the news media. Studies have been funded to examine the populations in the lower Rio Grande and in Nogales, Arizona, but the complete results from those studies will not be available for some time. These investigations may find it particularly difficult to link illness with hazardous chemicals because of a lack of baseline data and the presence of other factors such as lack of adequate nutrition, initial access to health care, and existence of infectious diseases. A major problem exists with regard to disease registries and health data in Mexico because their health statistics do not distinguish the border states from the rest of Mexico (*6*).

The status of environmental quality data is better; there is monitoring of air quality on both sides of the border and monitoring of water quality in the United States. Many of these efforts have been established recently or are in the process of being established, and results should be available in the timeframe of months to a few years. Air quality data are being collected on air toxics as well as criteria pollutants in El Paso/Ciudad Juárez and Brownsville, and they are available from the National Aerometric Information Retrieval System by contacting the Texas Natural Resources Conservation Commission (TNRCC) or EPA Region 6. A pilot environmental monitoring study of air quality in the Rio Grande Valley has recently been completed and is available. The TNRCC, the U.S. Geological Survey, the IBWC, and some local health departments routinely survey water quality in the Rio Grande. For example, TNRCC monitors at 39 main-stem sites and 14 major tributaries for toxic chemicals in water, sediment, and fish tissues. Data are available from TNRCC or EPA Region 6 databases. U.S. Geological Survey monitors at 6 main-stem sites and 5 tributaries and the data are available from STORET or USGS WATSTORE. The Rio Grande Toxic Substances Study conducted by agencies in the U.S. and Mexico to screen the Rio Grande for the prevalence, magnitude, and impacts of toxic chemical contamination was completed recently, and the data are available from TNRCC and the national EPA database. This represents a considerable amount of data from Texas and New Mexico alone, and the data reside in a variety of locations. The Consortium for International Earth Science Information Network (CIESN) is developing an inventory of the environmental databases that exist along the border, but nothing exists at this time to coordinate these data.

Findings to this point show nonattainment for PM_{10} (particulate matter <10 μm), carbon monoxide, and ozone in the El Paso/Ciudad Juárez area, but not much water contamination has been found. Based on the region's economy, one would expect environmental concerns to be primarily from pesticides and heavy metals.

The impact of emissions and disposal practices for hazardous materials from the

maquiladoras has not been addressed in a systematic way by the Mexican government. Neither government appears to have data on hazardous waste disposal sites or on soil contamination by hazardous chemicals. In a border region that spans almost 3000 kilometers, there is ample opportunity for illegal dumping activity with small chance of detection.

Environmental Problems

Significant barriers must be overcome to address the environmental health and quality problems. The major concerns expressed at the workshop were with the amount, format, and availability of information, particularly health data. There is a need for baseline information that is identified by a geographical coding system so that smaller areas can be studied according to their exposure levels. Mexico needs to include the border region in its health statistics database and to gather that health data in a standardized format to build on existing vital statistics (birth and death). The location and accessibility of health data is of concern; there is a pressing need to disseminate information about where to get information, and this must be done on a binational basis.

Specific Data Gaps

There are specific data gaps in the environmental quality information that are not being addressed. Current environmental quality monitoring focuses on air quality and surface water quality near the largest urban centers, but hazardous waste in other parts of the environment is not being addressed. Specifically, environmental levels of pesticides and potential human exposure routes are not being targeted despite the likelihood that pesticides are a significant problem. Also, the area has a large number of *colonias* (unplanned communities without zoning regulations that do not have municipal water and sewer delivery). Their source of water is from private wells and their water levels of hazardous wastes should be examined. Most of the monitoring activity is in Texas and New Mexico, and monitoring of surface water in the western United States and Mexico border states should be as extensive as that done for the Rio Grande environmental assessment studies.

Data gaps in environmental health information are a greater problem because baseline health information that is necessary to identify chemically induced disease does not exist (*6*). Basic health data should be expanded to include additional data that would better control for confounding factors and improve the geographic coding of data so that smaller areas can be studied according to their exposure levels.

Community Participation

The NADBank, the vehicle for funding environmental infrastructure projects, has been designed to include two checks on its ability to finance: first, it will need to assure that its sponsored efforts protect the border region's fragile, semiarid environment. Second, the NADBank will have to demonstrate that its decision-making is responsive to community input. While both of these features are innovative ways to approach international lending, the second characteristic, promoting democratic policy making, is especially unique to the North American continent. This heretofore untried mode of resource allocation is certain to challenge traditional thinking, especially traditional fiscal thinking. As NADBank begins to try to finance BECC-certified projects, it will be interesting to see how community preferences and concerns will be assessed and implemented.

Border communities, long ignored by distant policy makers and administrators, have become accustomed to what they perceive as environmental neglect and associated health problems. The largely Latino populations in these areas increasingly have begun to view their situation as a manifestation of environmental racism, a term that describes the effects of benign or deliberate neglect of communities and the consequences for public health (*7*). This perception commonly adds to fears that unexplained disease clusters result from degradation of the environment that residents have linked to industrialization. Among the most important promises of the post-NAFTA order is that government agencies in the United States and Mexico will become more sensitized to such community apprehensions and accordingly more willing to include the citizenry in project design and implementation.

Research Needs

By identifying the data gaps in the study of hazardous wastes along the border, certain research needs became apparent. First, reliable biological markers from chemically induced effects must be selected and specified because of the lack of reliable baseline epidemiological data, particularly in Mexico. To identify environmental health problems in the border region, the baseline incidence of disease must be determined. Health registries have been recently established in U.S. border states, but there are no plans to establish such registries in Mexican border states. The development of biological monitors for chemical exposure and for chemically induced disease would be particularly useful in identifying hazardous waste problems in the border region. Furthermore, monitors of exposure to pesticides and to metal ions would also be beneficial at this time, although the use of all industrial solvents is expected to increase as the area develops.

Second, the influence of nutrition and underlying disease on chemical toxicity should be investigated. In the United States, the border region is populated by a substantially lower socioeconomic class than in the rest of the country, a characteristic that is often linked to higher incidence of nutritional deficiencies and more frequent exposure to infectious diseases. This population is at particular risk from exposure to hazardous waste. This combination of risk factors has not been adequately addressed in research studies.

Third, the incidence of lupus needs further investigation and, in particular, the role of chemicals in the development of that disease state. An excessive incidence of lupus has been identified in a border environmental health study, but its etiology remains unclear. Research to develop models of this disease as well as epidemiology studies would be valuable.

Finally, research in environmental data analysis is needed to obtain maximal benefit from the data being generated at the border. Several state and federal agencies have established programs to analyze chemicals in air, water, and soil. Such data have begun to come online, but there has been no comprehensive plan to analyze that data for trends, source of hazardous waste, profiles of chemical waste, etc. This dilemma may partially reflect the multiple sources of these data but also may be due to a lack of techniques to analyze these data effectively.

In summary, it is apparent that more well-targeted research is needed that evaluates the health impact of various wastes and waste treatment processes, improving the database and extrapolation methodologies upon which risk assessments are founded. The ultimate goal of such health investigations is to generate accurate and effective information that helps determine what type of intervention or prevention actions are necessary, if any.

Conclusions

The major problem to be addressed in a binational setting is determining the extent and origin of hazardous chemicals that cross or threaten to cross the U.S.–Mexico border. There is a general concern that the border region is seriously contaminated with hazardous waste chemicals and that there will be a substantial cost to clean it

up. An estimated cost of $1 billion dollars was mentioned in an article by Vandermeer (*8*). Further, there is concern that there is a general lack of data from the region. Several agencies currently are addressing the lack of environmental quality data, but the collection of environmental health data will be substantially slower. It is likely that there will be a large amount of data available soon that may defy analysis because it will be spread over so many sources. The problem may be evaluating all the data sources for completeness and reliability and assessing the true data gaps because of the sheer volume of information.

This conference has made recommendations, but it is unclear to whom they should be presented because of the uniqueness of the national border area. It is clear that there is heightened awareness of the problems at the border and that resources will be made available to address them. The procedures used and the people who will be involved in the decisions will be most important in defining how this binational relationship will actually work and in what will actually be accomplished. In a sense, this effort will serve as a laboratory for binational relationships that involve science, health, and policy.

References

1. Hurt S, Carter D, eds. Summary report of the Workshop on Health and Hazardous Waste Issues Related to the U.S.–Mexico Border. Tucson, AZ:University of Arizona, 1994.
2. Dieter MP. Fate, transport, and interactions of metals. Environ Health Perspect 101:344–345 (1993).
3. Ingram H, Laney N, Gillilan D. Divided waters: bridging the U.S.–Mexico border. Tucson, AZ:University of Arizona Press, 1995.
4. Varady RG, Mack MD. Transboundary water resources and public health in the U.S.–Mexico border region. J Environ Health 57:7–14 (1995).
5. U.S. Department of the Treasury. Agreement between the government of the United States of America and the government of the United Mexican States concerning the establishment of a border Environment Cooperation Commission and a Border Environment Finance Facility. Washington, DC:U.S. Government Printing Office, 1993.
6. Mack M. Udall Center survey of data sources and gaps regarding environmental health problems affecting residents along the U.S.–Mexico Border. Udall Center for Studies in Public Policy, University of Arizona, Tucson, Arizona, 1994.
7. Bullard, Robert D. Overcoming racism in environmental decisionmaking. Environment 36:10–20, 39–44 (1994).
8. Vandermeer DC. NAFTA prompts health concerns across the borders. Environ Health Perspect 101:230–231 (1993).

Monograph based on conference to be published in EHP

Sixth International Meeting on the

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

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

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

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

Objectives

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

Topics to be covered include:

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

For more information:
University of Rochester School of Medicine and Dentistry
Office of Continuing Professional Education
601 Elmwood Avenue, Box 677
Rochester, New York 14642 USA
FAX (716) 275-3721

Unless we realize that the technologies we are dealing with are of international consequence, that the technologies are very serious, we are headed down a very dangerous and possibly disastrous path.

Robert P. Gale, on nuclear power accidents such as Chernobyl

USA Today, 27 April 1988

Forum

Questions about Grilling

Accompanying each savory morsel of charbroiled meat are cancer-causing heterocyclic amines (HCAs). New studies indicate that HCAs may be involved in diseases such as cardiomyopathy, mammary cancers, and colon cancer. Even the fumes of cooked meats contain HCAs, which may pose respiratory risks for those who don't eat meat.

HCAs, formed during cooking when naturally occurring amino acids in meat react with muscle creatine, are among the most potent mutagens and carcinogens known. In a recent study completed at York University in London and the Lawrence Livermore National Laboratory (LLNL) in Livermore, California, when patients scheduled for colon surgery were given trace doses of radiolabelled HCAs (equivalent to the amount in four well-cooked, quarter-pound hamburgers) beforehand, minute amounts were recovered from DNA in the tissue removed during surgery. "Even at these small levels, the carcinogen binds to DNA, showing how powerful it is," said Mark Knize, a biomedical scientist at the LLNL.

Early investigations of the health risks of HCAs have focused on ingestion as the primary route of human exposure and the colon as the primary target. Recent animal studies suggest that HCAs target other organs as well. In rats, HCAs trigger cardiomyopathy, the inflammation and deterioration of heart tissue that occurs with age. The primary cause of human cardiomyopathy remains unknown, but, "dietary factors like HCAs may play a role," says Elizabeth Snyderwine, chief of the Chemical Carcinogenesis Section at the National Cancer Institute.

Snyderwine and colleagues Richard Adamson and Unnur Thorgeirsson noticed that monkeys with HCA-induced liver cancer also developed an unexpectedly high incidence of degenerative heart damage. When they gave HCAs to adult rats (100 milligram per kilogram per day by gavage for two weeks) and cultured rat myocytes (200 micromolars for two hours), both experiments caused abnormal mitochondria, loss of myofilaments, and cell death. Studies in human cell lines and epidemiological evidence are needed to determine whether HCAs contribute to human cardiomyopathy.

Snyderwine's team also found that HCAs target the mammary glands of rats and are passed to their offspring through nursing. A study published in the 20 July 1994 issue of the *Journal of the National Cancer Institute* showed that when lactating rats were fed a single oral dose of 10 mg/kg of HCAs, metabolites showed up in the urine of nursing, five-day-old rat pups. "This might be a model for the human situation," says Snyderwine. Humans are continuously exposed, starting early in life, to low doses of carcinogens like HCAs, which are promoted, not just later but early on as well, by factors like dietary fat to produce cancer. However, it's too early to say that eating meat and breast feeding don't mix. Antioxidants and other nutrients may counteract HCAs, and no investigation has been done in humans.

Kimberly Thigpen

Food for thought. Grilling meats releases heterocyclic amines which are potent animal carcinogens and may play a role in cancers in humans.

Because epidemiological studies (cited in the July 1994 issue of the *Journal of Agricultural and Food Chemistry*) find an increased risk of respiratory tract cancers among cooks, researchers at the LLNL analyzed the amount of HCAs produced by frying beef hamburgers, bacon, and soy-based tempeh burgers. The results, published in the October 1995 issue of *Food Chemistry and Toxicology*, showed the total HCAs in the smoke condensate were 3 nanograms per gram (ng/g) from bacon and 0.37 ng/g from hamburgers, compared with 163 ng/g in cooked bacon and 110 ng/g in cooked beef. Levels of HCAs in tempeh, which lacks creatine, were nondetectable.

Airborne HCAs present the greatest risk to professional cooks who stand over a stove all day, says Knize. A fume hood could decrease the risk. For home cooks, eating meat, rather than breathing in the cooking fumes, poses the greatest hazard.

The consumption of HCAs in the United States averages 26 ng/kg/day. "The amounts are small, compared to other pollutants," says Knize, "but [HCAs] are powerful mutagens and carcinogens." And the recent culinary trend to switch from beef to chicken may not be quite so healthy when it comes to HCAs. In his study in the 15 October 1995 issue of *Cancer Research*, Knize found that pan-fried, oven-broiled, and grilled chicken contain two- to seven-fold more HCAs than fried beef.

Meat connoisseurs can lessen their intake by cutting away the HCA-rich char that forms during cooking. Reducing cooking temperatures helps, too. The LLNL researchers found that beef cooked at 198° C and 277° C contained 10.5 ng/g and 110 ng/g of HCAs, respectively.

A new grill, invented by microbiologist Richard Basel at Lebensmittel Consulting in Fostoria, Ohio, allows people to enjoy grilled meats without the carcinogens. Named the Safe Grill, it blocks both HCAs and polyaromatic hydrocarbons, which form when fat drips into the fire. A special filter placed between the fire and the meat blocks carcinogens from rising and coating the meat. The filter contains special fractionation packing that separates the desirable lower-boiling-point flavor compounds from the undesirable higher-boiling-point carcinogenic compounds, allowing only the desirable flavor compounds to pass through.

In addition, the Safe Grill cooks meat at a

lower temperature, which prevents flames from directly charring the meat. Taste panels judged meats cooked on the Safe Grill to be more flavorful and tender than those cooked on conventional grills. The invention of the Safe Grill was funded by National Cancer Institute to reduce the threat of cancer from foods.

Greening the Olympic Games

The International Olympic Committee (IOC) is now a champion of the environment. In 1991 the IOC, with the United Nations Environment Programme (UNEP), amended the Olympic charter to include a policy that requires each candidate for hosting future Olympic games to include an environmental plan as part of its bid.

"It is natural that the International Olympic Committee, as leader of a worldwide humanistic movement, should be concerned with the integration of the activities of the Olympic movement with the well-being of the world in which we live," said Richard W. Pound, an IOC executive board member, in the IOC's *Olympic Message* in March 1993. "The IOC must seek a balance between the needs of our generation and those of the next and succeeding generations. It is, after all, the youth of the world who will inherit the earth which we leave them."

Lillehammer, home of the 1994 Winter Olympic Games, was awarded the games before the environmental plan requirement was passed, but was the first city to voluntarily address environmental issues, and proclaimed the 1994 events the first "Green Games." Environmental activist groups were highly involved in the planning and implementation of the games. The city emphasized environmental protection in land use and venue construction, as well as during the games through programs such as recycling and composting.

This year's Olympic Games host, Atlanta, Georgia, was awarded the games in 1990, also before the IOC environmental requirement went into effect. The Atlanta Committee for the Olympic Games (ACOG) is not proclaiming the Atlanta events as the second green games, but is concentrating instead on being known as the "Centennial Games." The committee has, however, taken measures to be environmentally friendly.

On 13 July 1995, ACOG presented an environmental policy statement detailing ACOG's efforts on behalf of the environment to the IOC at the World Conference on Sport and the Environment held in Lausanne, Switzerland. Early in the planning for the games, ACOG developed the Olympic Environmental Support Group (OESG), a citizen advisory group of 23 people, to educate and advise ACOG on environmental issues and recommend environmentally responsible decisions on Olympic issues. The group assisted in setting environmental guidelines for venue sites, making recommendations for solid waste management and recycling, and developing ACOG's environmental policy statement.

Stadium sunrise. Parts of a new stadium built for the 1996 Olympic games, including the track surface, will be reused after the games.

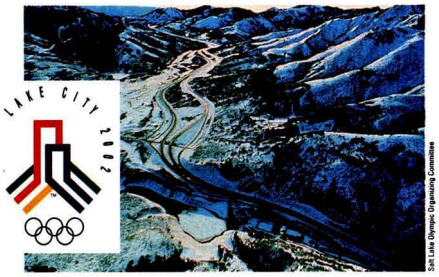
Environmentally friendly measures taken include constructing the new Olympic stadium on the site of the parking lot for the Fulton County stadium, which will be used during the games and then torn down. The asphalt and concrete from the old parking lot and stadium will be used as filler in constructing a new parking lot. In addition, the new stadium's capacity will be reduced after the games from 85,000 seats to 45,000. The materials removed will be reused or recycled, including the Olympic track surface, which will be placed at a local university.

ACOG has also tried to avoid unnecessary waste in construction. According to the committee's statement, many of the venues will use rented equipment and furniture to avoid consuming excess resources and generating construction debris. For example, at a rowing/canoeing venue on Lake Lanier, spectator seating will be rented and placed on a temporary platform on the lake to avoid clearing trees on a nearby hill for spectators, as was originally planned. ACOG has also reduced the number of outdoor trap and skeet shooting ranges at the Wolf Creek shooting venue in order to minimize the impact of lead shot deposit on nearby wetlands. ACOG is advocating the use of alternative shot in future games because of the environmental damage caused by lead shot. In the area of transportation, ACOG will encourage the use of Atlanta's mass transit system rather than automobiles. Two hundred of the 2,000 buses designated for the games are powered by natural gas.

In late 1995, ACOG hired an environmental consulting firm, CH2M HILL, as an advisor to assist with activities such as waste management services. According to CH2M HILL, one of the major accomplishments of the Atlanta games will be waste management. "The Atlanta games should establish some new benchmarks in solid waste management at a sporting event of this magnitude," said Bill Wallace, CH2M HILL's Olympic program manager. According to Bill Steiner, an EPA consultant to ACOG, the committee estimates that athletes and fans will generate about 9,000 extra tons of garbage over the 30-day period of the games, not including waste from venue construction. ACOG will attempt to divert about 85% of the waste away from landfills through recycling and composting, Steiner said. For example, horse manure from the equestrian sites will be composted for fertilizer.

Local environmental groups seem satisfied with the way ACOG has handled environmental protection in planning for the Olympics. Carolyn Hatcher, president and CEO of the Georgia Conservancy, a nonprofit environmental advocacy group, served as co-chair of the OESG. She said, "ACOG officials were eager to be responsive on environmental concerns, but they were working within . . . budgetary and timetable [constraints]. They did a good job, considering the constraints they were operating under. They weren't able to do everything they would've liked to have done, but they did many very positive things."

Marcia Bansley, executive director of Trees Atlanta, a nonprofit organization that plants and conserves trees, agrees. "ACOG is doing the best they can with limited resources," she said, pointing out that these Olympics are being funded entirely through private sources, without tax dollars. Therefore, many private groups are helping to foot the bill on behalf of the environment during the games. Trees Atlanta has raised $4.5 million to plant trees around the city. "This will be an environmental improvement," Bansley said. "We have a terrible pollution problem when it's extremely hot, and downtown is the hottest part of the city. Trees are the best way we can cool the area off. If we can cool this area down, we can help our air quality. Trees also help stop



Salt Lake Olympic Organizing Committee

Sportsmanlike conduct. Organizers are working to protect the environment at the site of the Olympic Winter Games of 2002.

runoff from nonpoint source pollution."

The Atlanta Bicycle Campaign (ABC) is also taking measures to help control air pollution during the games by encouraging the use of bicycles for transportation. ACOG and the city of Atlanta are helping the group with publicity and parking, and ABC will staff the bicycle parking lots. "Our goal is to provide access to the Olympics by bicycle," said Dennis Hoffarth, director of ABC's Olympic Bicycle Access Project. "Atlanta already has a severe air pollution problem, and this campaign will help with pollution control.

Salt Lake City is also facing the challenge of protecting the environment when it hosts the Winter Olympics in 2002. "The environment has always been a contentious issue [here] because the small mountains around the city contain delicate watersheds," said John Hoagland, a winter sports and resorts specialist for the U.S. Forest Service, and a member of the Environmental Advisory Committee of the Salt Lake Olympic Organizing Committee.

During preliminary planning, the organizing committee selected two major canyons outside the city to serve as ski venues, but environmental groups protested and the committee withdrew the proposal. "Withdrawing the venues from those canyons calmed down the environmental community," Hoagland said. "Now the community is more supportive of the Olympics."

The organizing committee is cooperating with environmental groups through representatives on the advisory committee. A preliminary environmental platform is now in place in which the organizing committee says it "intends to carry on and improve on the environmental progress initiated in Lillehammer." Plans include requiring contractors to guarantee that the environment will be restored after the games, ensuring that cultural events such as the opening ceremony have an environmental theme or message, educating students and the community on the importance of a healthy environment to human health, mandating that spectators use mass transit, and contracting with green vendors and green hotels. "The city has always been sensitive to the environment," Hoagland said, "and Lillehammer cranked up the heat a little bit."

Airing the Word on Pollution

The American Medical Association (AMA) passed a policy resolution in December 1995 urging its members to help spread the word to health care colleagues, patients, and the public about the negative health effects of indoor and outdoor air pollution. The resolution was proposed by the National Association of Physicians for the Environment (NAPE), which sponsored a conference on 18 November 1994 to examine the impact of air pollution on body organs and systems.

"It is important that people understand that air pollution can affect not only the lungs, but virtually every organ and system in the body," said John Kimball Scott, an otolaryngologist and president-elect of NAPE, who served as floor manager of the AMA resolution, in a press release announcing its passage.

According to the conference summary, published 20 September 1995, air pollutants can enter the body through various ways—not just by inhalation. They can be absorbed through the skin or ingested by eating food or drinking water that has been contaminated, possibly through bioaccumulation in the food chain. The pollutants in food and water that humans and animals are most likely to be exposed to include pesticides, PCBs, dioxin, and heavy metals such as cadmium, lead, and mercury, says the report. Such pollutants can cause a variety of adverse health effects including respiratory ailments, damage to the blood system leading to anemia or leukemia, heart disease, including hypertension and cardiac arrhythmias, and damage to the urogenital system resulting in kidney disease, bladder cancer, and reproductive problems. In addition, the skeletal system stores heavy metals such as lead that may accumulate over time. During times of bone loss such as pregnancy, lactation, or osteoporosis, the stored toxins may be released back into the body causing health problems, especially in women, newborn children, and senior citizens.

Air pollutants can also cause immune suppression or overstimulate the immune response, which can lead to allergies and immune-mediated diseases. Air pollutants have also been linked to psychological disorders and toxic damage to the nervous system and the brain, especially in developing fetuses or young children. In addition, air pollutants are thought to have detrimental effects on the reproductive and endocrine systems, but according to the conference summary, these effects require more research to be fully understood. The report points out that certain populations, including children, the elderly, and minorities, are at a higher risk of being affected by air pollutants.

Not only should people be concerned about the direct impact of air pollution on human health, says the report, but they should also be concerned about the adverse effects of air pollution on plants, animals, and ecosystem functions, which affect agriculture, fishing, wildlife, tourism, and recreation. "Human health is inseparable from the health of the natural world," says the report.

The report emphasizes the need for more research and public education on the consequences of air pollution. "There is no question that air pollution can be a serious public health hazard and that prevention of air pollution will lead to disease prevention," says the report.

A Knock-out for NSAIDs

Every year millions of Americans take aspirin and other drugs such as ibuprofen and naproxen for relief of headaches and other minor aches and pains, for chronic pain relief of arthritis, and as a preventive against colon cancer and heart attacks. Referred to collectively as nonsteroidal antiinflammatory drugs (NSAIDs), they are the most widely used drugs in human medicine.

Most people who take NSAIDs do not experience severe side effects, although in some people, especially those taking the drugs chronically, NSAIDs can cause stomach ulcers and irritate the stomach's lining. NSAIDs that retain their positive benefits and do not cause adverse side effects could significantly benefit the individuals taking them.

Collaborative research by investigators at the NIEHS and the University of North Carolina at Chapel Hill (UNC-CH), has produced two strains of transgenic mice that may lead to better NSAID development. The mice should help scientists obtain a clearer idea of how NSAIDs work. "The importance of this research goes beyond aspirin/NSAIDs," said Robert Langenbach, a microbiologist at the NIEHS who, with other investigators, developed one of the mouse strains. "It may lead to better treatments and prevention of

diseases like arthritis and, as importantly, colon cancer, because these mice give us a better tool for understanding how these diseases may actually develop," he said.

Langenbach and Scott Morham, an American Cancer Society post-doctoral fellow at UNC-CH, used powerful genetic engineering techniques to eliminate, or "knock out," the genes *Ptgs1* and *Ptgs2* that produce the enzymes cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2), respectively, in mice. These enzymes are the first enzymes in the prostaglandin biosynthesis pathway.

Prostaglandins, hormone-like compounds, are believed to play a role in cell proliferation, inflammation, and many other biological processes. Scientists had believed that inhibition of COX-1 synthesis by NSAIDs was the cause of the adverse side effects such as occasional stomach upset and more seriously, at higher, continual doses, ulcers and kidney damage. Evidence for this belief was that ingested prostaglandins protected against these effects. Scientists also thought that NSAIDs blocking COX-2 produced the beneficial effects. "NSAIDs inhibit these enzymes. When you knock out the gene, it eliminates the enzyme and that mimics the drugs," Morham said.

However, Langenbach discovered that the mouse lacking COX-1 displayed no ulceration. "Probably the most surprising finding was that the COX-1 knock-out mouse is really quite a healthy animal," he said. "This animal is surviving very well with the basal level of prostaglandins reduced by greater than 99.5%." Given the role prostaglandins are supposed to play, most scientists would have thought that these animals would not have survived. Also surprising was that when the investigators administered one particular NSAID, the mice were more resistant to developing ulcers. "It might suggest that it is the interaction of the enzyme itself with the drug that is causing this ulcerative problem," Morham said.

Morham's mouse lacking COX-2 also displayed an unexpected result. This mouse was able to mount an inflammatory response, but was born with kidney problems. Previously, researchers believed that COX-2 was primarily involved in the inflammatory process, "but our data show that both genes contribute somehow to the process," Langenbach said. "Quite a bit of what we see is contradicting current dogma," Morham added.

"The results," Langenbach said, "suggest that what we know about the role of prostaglandins may have to be modified in terms of how important they are, or that there are other pathways and molecules in the body that can compensate for their lack. The therapeutic effect of aspirin-like drugs may be on other gene products rather than these, or in addition to these; we really cannot say."

"Signaling by prostaglandins is very important in almost all biological processes. This research should have an impact on our understanding of how prostaglandin-signaling molecules may be involved in fundamental processes such as the development of colon cancer," said David DeWitt, a biochemist at Michigan State University.

To produce the mice, the investigators used a molecular biology/biotechnology technique developed by Oliver Smithies, a pathologist at UNC-CH in whose laboratory this work was started. The investigators inactivated the gene of interest, or "knocked it out," in cultured cells. The modified genetic material is inserted into mouse embryonic stem cells. This fragment scans the existing genetic material, locates a matching strand, and binds there. Cells with the altered gene are inserted into a developing mouse embryo and the resulting offspring carry the defective gene. A male and a female mouse with the defect are mated to produce the "knock-out mice." "These mice are highly valuable for studying the function of a gene in a whole animal model," Langenbach said.

Langenbach is continuing to use the mice to study how NSAIDs cause stomach ulcers, and is also studying the role of *Ptgs* genes in the development of various cancers, including colon cancer, and studying

EHPnet

Doctors and the Environment

"Pollution prevention is disease prevention" is the theme of the National Association of Physicians for the Environment (NAPE), founded in 1992 to help physicians and medical specialty organizations examine the impacts of environmental pollutants on health; educate physicians, patients, and the public about these impacts; and work for the reduction or elimination of environmental pollutants. NAPE also works to involve physicians in global environmental issues such as biological diversity.

NAPE has developed a site on the World Wide Web called "NAPEnet" to provide information about its activities and to make scientific information about health and the environment widely available. Located at http://intr.net/napenet, the site offers four major links: About NAPE, What's New on NAPEnet, Documents on NAPEnet, and News Releases.

National Associati of Physicians for the Environment

About NAPE provides the history of NAPE and offers links to a NAPE fact sheet, newsletters, an annual report of activities for 1994, and membership information.

What's New on NAPEnet offers links to recent additions to the site. Among these is a link to *1996 Summer Olympics and Your Health: Sun Protection While at the Olympics*, which offers warnings about the risks of skin damage, eye damage, and potential immune system effects from sun exposure, as well as advice on how to prevent such damage.

Documents on NAPEnet offers links to items such as conference reports and UV index documents. The full text of the conference report, *National Conference: Air Pollution Impacts on Body Organs and Systems Summary of Proceedings* (see Forum article, "Airing the Word on Pollution"), is available under Conference Reports.

News Releases offers press releases about the work of NAPE and its member organizations. For example, there is a link to a press release from 8 December 1995 on the American Medical Association's resolution on the importance of the protection of biodiversity on human health. The release highlights the AMA resolution to encourage its members to take part in a national effort to inform their colleagues, patients, and the public of the importance of protecting biological diversity, particularly because of the value of pharmaceuticals and biologicals that are derived from nature.

NAPE is working to spread the word on environmental health issues through NAPEnet and other outlets because the association maintains that every environmental and pollution problem is, or will become, a medical or public health problem.

EHP online: http://ehpnet1.niehs.nih.gov

how these genes may interact with other genes to predispose people to cancer.

Morham is looking further at the inflammatory responses of the COX-2 knock-out mice, and is also studying ulceration in these mice. Both researchers hope this work will produce better NSAIDs in the future, as well as benefit patients who take these drugs.

Who Pays to Clean Up Livestock Waste?

Widespread coverage by both the popular and scientific press in the last year pointed out the seriousness of environmental problems associated with livestock waste, particularly waste lagoons. Feces and urine from confinement buildings are typically washed into earthen lagoons, from which they can leak into groundwater at a rate of 500 gallons per acre each day, according to the Washington, D.C.-based Sustainable Agriculture Coalition, a public interest environmental group. Lagoons can also spill directly into surface waters. In the wake of last year's spills that dumped millions of gallons of animal waste into North Carolina and Iowa waterways, Congress recently adopted a bill in the 1996 Farm Act intended to address the livestock waste problem.

Known as the Environmental Quality Incentive Program (EQIP), the bill provides technical assistance to livestock operators such as incentive payments to keep farmers from spraying liquid waste from lagoons along stream banks, and cost-share assistance for building livestock waste facilities. Farmers would be eligible to receive as much as $10,000 a year with a cap of $50,000.

In a March letter to Alice Rivlin, director of the Office of Management and Budget, EPA Administrator Carol Browner lauded EQIP and recommended that President Clinton sign the 1996 Farm Bill. EQIP also enjoys overwhelming support in Congress and is supported by environmental groups, with one caveat. Environmentalists favored the Senate version of EQIP, which had set a limit on the size of farms that are eligible to receive cost-share funds; livestock operations would have to be smaller than those defined as point sources of water pollution in the Clean Water Act (i.e., 1,000 beef cattle, 2,500 hogs, or 100,000 poultry). In contrast, while the version of EQIP that passed prohibits "large confined livestock operations" from receiving these cost-share funds, it stops short of defining "large" and leaves that decision to the discretion of the Secretary of Agriculture.

Some livestock operations can have more than 100,000 beef cattle, 10,000 hogs, and 400,000 chickens. The question being asked is whether operations this large should be eligible for federal cost-sharing funds to build animal waste management facilities. The answer depends on who you talk to. "We support LEAP [the Livestock Environmental Assistance Program, which was the House version of EQIP and set no size limits]," says National Pork Producers Council spokeswoman Deborah Atwood. "This is an environmental bill, not a structure bill. The numbers are irrelevant." LEAP [would] give USDA Secretary Dan Glickman the freedom to protect the most impaired watersheds from the effects of livestock waste, she says. (EQIP also leaves the size of operations eligible for funds to the discretion of the USDA secretary).

Some environmentalist groups disagree. "We think it is a structure issue," says Lonnie Kemp, policy director of the Canton-based Minnesota Project, a nonprofit organization devoted to rural and environmental issues. "Big factory farms get loans and investors and should be able to pay for waste management facilities." However, Kemp does support EQIP for operations smaller than the Clean Water Act limits, saying that financial incentives are an excellent way of encouraging farmers to minimize their impact on the environment. There are also some dissenters in Congress who, like Kemp, think EQIP should set eligibility size limits. "We should target the money to family farmers," says Mark Rokala, spokesman for Representative David Minge (D-Minnesota). "It can cost $30,000 to $50,000 to get feedlots to prevent [environmental] impact, which is significant cost for a guy with 1,000 head of cattle."

A more fundamental question about EQIP is whether waste lagoons are safe for the environment. Again, the answer depends on who you talk to. Waste lagoons are adequate when managed properly but many operators overfill them, making them more likely to spill over, says Deanne Morse, livestock waste management specialist at the University of California at Davis. Others say that waste lagoons are not safe even when managed properly, and that the real issue in livestock waste is large versus small operations. "There is as yet no workable technology for safely dealing with concentrated livestock waste from large operations," says Ferd Hoefner, the Sustainable Agriculture coalition's Washington representative. The coalition favors small family farms because they don't generate huge concentrations of animal waste and therefore can avoid the problem altogether, he says.

In response to concerns about the trend towards ever-increasing concentration in the livestock industry, the USDA appointed an advisory committee in February. The 21-member committee is expected to report on a variety of issues, including the effects of large livestock operations on the environment, by early June.

Rather than help farmers build waste lagoons, the federal government should develop and encourage alternative methods of managing livestock waste, says Paul Sobocinski, a farmer in Wabasso, Minnesota, who is also a staff member of the Land Stewardship Project, based in Marine, Minnesota. Existing alternative methods, which are more feasible for small livestock farms and are widely used in Europe, include dry bedding, which entails keeping the animals on straw and then composting the waste-laden straw.

"I don't need EQIP," says Dwight Ault of Austin, Minnesota, who uses the manure from his 700 hogs to fertilize his crops. "It will benefit the people who are the real polluters and is a short-term fix at best. In the long run it will do more damage than good because it will continue the push for largeness. Bigger is not necessarily better."

Pamlico-Tar River Foundation

Cleanup costs. New legislation provides funds for cleanup of livestock waste such as the spills that caused fish kills in Iowa and North Carolina rivers last year.

Lead and Delinquency

Part of society's recent increase in violence

may be due to lead poisoning. According to a study by Herbert Needleman of the University of Pittsburgh School of Medicine and colleagues published in February in the *Journal of the American Medical Association*, boys with higher bone-lead levels are more likely to be aggressive and delinquent.

"This is probably the most critical study that has been done on lead in the last five years," says Janet Phoenix, manager of public health programs at the National Lead Information Center of the National Safety Council, an international public interest organization. "The social implications are enormous."

Lead has been linked with behavioral problems since the early 1940s, when pediatrician R.K. Byers noted that some children he had treated for acute lead poisoning subsequently developed violent, aggressive behavioral difficulties such as attacking teachers with knives or scissors. Needleman's study, supported in part by the NIEHS, is the first to link asymptomatic levels of lead with aggressive behavior and delinquency.

Needleman and his colleagues studied 301 boys from primary schools in Pittsburgh. The researchers measured bone-lead levels by K X-ray fluorescence when the boys were about 12 years old. Based on the relative lead content of their tibias, the boys were divided into high- and low-lead groups. Bone-lead levels reflect lifetime exposure to lead because, like calcium, lead is stored in bones. The boys in the high-lead group had normal levels of lead in their blood by the time of the study, showing that their lead exposure had occurred in the past.

The researchers evaluated the boys' behavior at 7 and 11 years of age based on reports from three sources: the boys themselves, their parents, and their teachers. These data were from widely respected tests of antisocial behavior that had been administered by the Pittsburgh Youth Study, a longitudinal study of the developmental course of delinquency. At 11 years, the boys were given a self-reported delinquency interview, which comprises 35 questions such as how many times in the past six months a subject has "been drunk in a public place" or "attacked someone with a weapon." The parents and teachers filled out the child behavior checklist, which contains 113 symptoms of childhood behavioral disorders such as cruelty or bullying, shoplifting, setting fires, and apparent lack of guilt after misbehaving.

When the high-lead boys were 7 years old, neither they nor their parents reported significant behavioral problems, and their teachers reported only borderline tendencies toward symptoms such as social problems, delinquency, and aggressive behavior. By the time these boys were 11, however, they reported significant increases in antisocial acts, and their parents and teachers reported significant increases in symptoms such as delinquent and aggressive behavior. The researchers corrected for confounding factors such as the mothers' intelligence, the presence of the father, and socioeconomic status.

Many U.S. children have toxic bone-lead levels and—provided that their results are found to extend to the population at large—Needleman and his colleagues conclude that lead makes a substantial contribution to delinquent behavior. Other researchers hail the Needleman study as the first to rigorously demonstrate a link between lead and antisocial behavior. The study was well-designed and its implications are likely to be valid, according to Terrie Moffitt of the Department of Psychology at the University of Wisconsin at Madison. Self-reporting is trustworthy when the period reported on is less than a year, the interviews are private and face-to-face, and confidentiality is guaranteed, she says, and the Needleman study met these conditions. Furthermore, Needleman's conclusions are strengthened by the fact that reports from three sources—the boys, their parents, and their teachers—all linked lead with antisocial behavior.

Lead is a neurotoxin and human studies indicate that its neurological effects are likely to be irreversible. However, delinquency is also associated with factors such as weak parent-child attachment, lax parental supervision, and school failure. Addressing these issues can mitigate the effects of lead. "These kids need help. They need support from teachers and parents," says Phoenix. "No one knew they were lead-poisoned." The good news is that environmental lead exposure can be avoided. "Lead-related delinquency is the easiest to prevent," says Needleman. "We should be able to wipe this disease out by removing old lead-based paint."

Closing Chernobyl

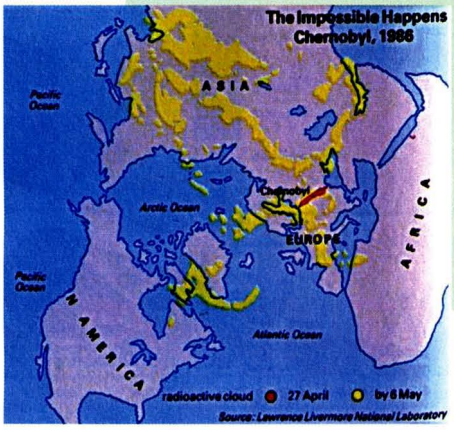
Almost 10 years after the explosion and full-scale meltdown of a graphite core at the Chernobyl nuclear power plant in Ukraine, officials finally agreed to close the plant.

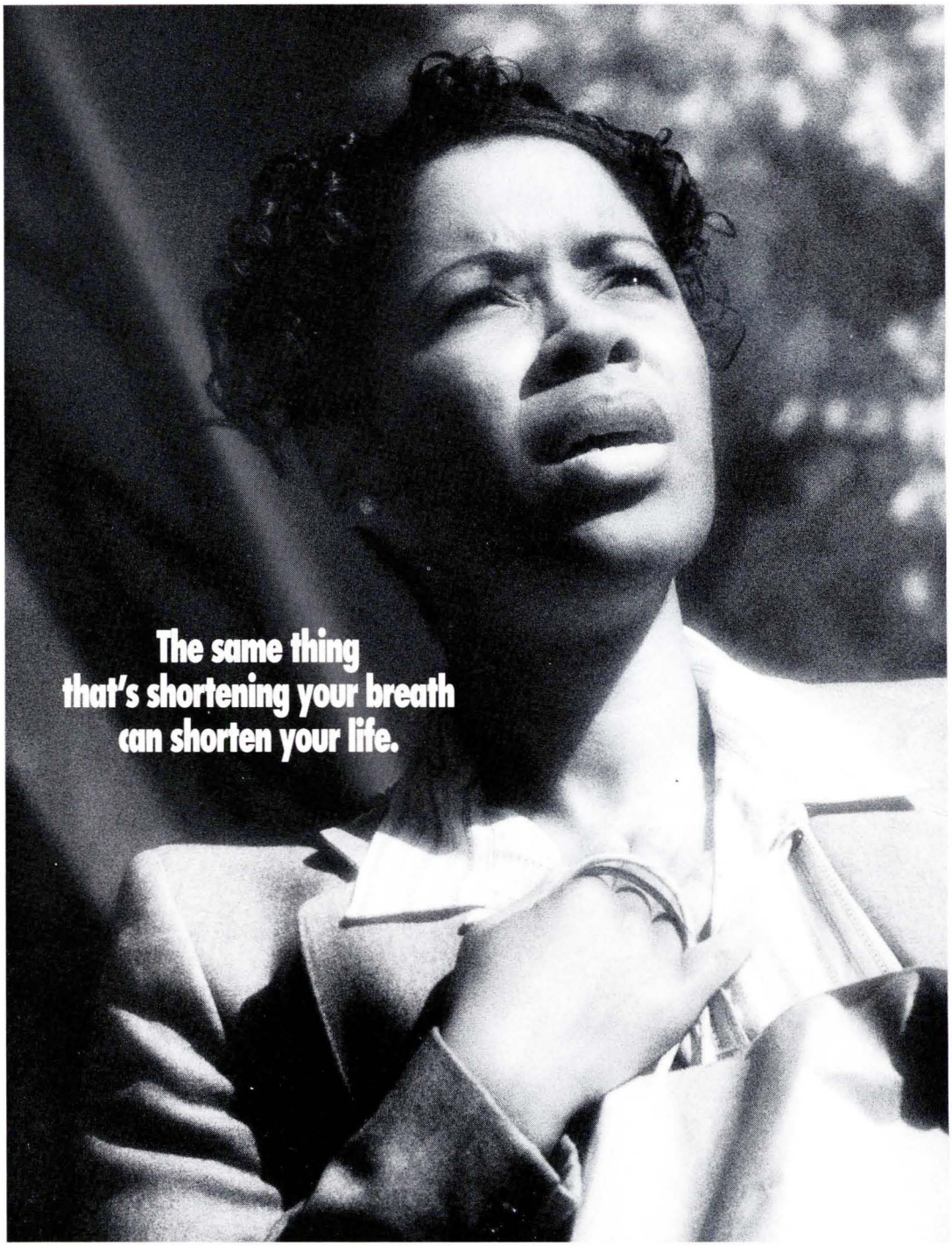
The governments of Ukraine, the Group of Seven (G-7) industrialized nations, and the Commission of the European Communities signed a memorandum of understanding on 20 December 1995 that outlines a comprehensive program for the closure of the Chernobyl nuclear power plant by the year 2000. The program's provisions include a focus on nuclear safety; the development of a financially sound electric power market with market-based pricing to encourage energy efficiency and conservation; and a social impact plan to address the effects of the closure of the plant on its employees and their families. Representatives of Ukraine, the G-7, and international financial institutions plan to meet annually to monitor the implementation of the program.

The memorandum allocates $2.3 billion in aid, including $349 million for nuclear safety and decommissioning activities and $1.9 billion for new energy investments. The funding will come from grants by G-7 countries and loans from international financial institutions, although the financing has not yet been worked out.

Financial details and the fact that the agreement is not legally binding have caused environmental groups to remain skeptical about the agreement. "If the West does not provide what Ukraine feels is sufficient capital, it's quite possible that Chernobyl might not be shut down," said Miriam Bowling, a research associate for the Natural Resource Defense Council's nuclear program. In addition, Bowling said, Ukraine faces political pressure from the Russian government, which prefers that Chernobyl stays open.

Environmental groups are also disappointed that the agreement includes the exchange of the closure of Chernobyl for the completion of two more nuclear reactors in Ukraine. However, as Bowling said, "It is a very important agreement, and it's great to see the words 'Chernobyl' and 'closure' on the same piece of paper."





If you cough a lot, wheeze, are often short of breath or frequently feel tightness in your chest, see a doctor. You may have asthma.
But with proper treatment, you can control your asthma. And your life.

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

National Asthma Education and Prevention Program

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

International Society of Exposure Analysis (ISEA)

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

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

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

For information on membership

Dr. Steven Colome
Integrated Environmental Services
University Tower, Suite 280
4199 Camus Drive
Irvine, CA 92715 USA
e-mail: s_colome@ies.iaq.com

For information on international committee

Dr. Michael Brauer
The University of British Columbia
Occupational Hygiene Program
2206 East Mall, Vancouver, BC V6T 1Z3 Canada
e-mail: brauer@unixg.ubc.ca

ISEA's World Wide Web Homepage: http://www.isea.rutgers.edu/isea/isea.html

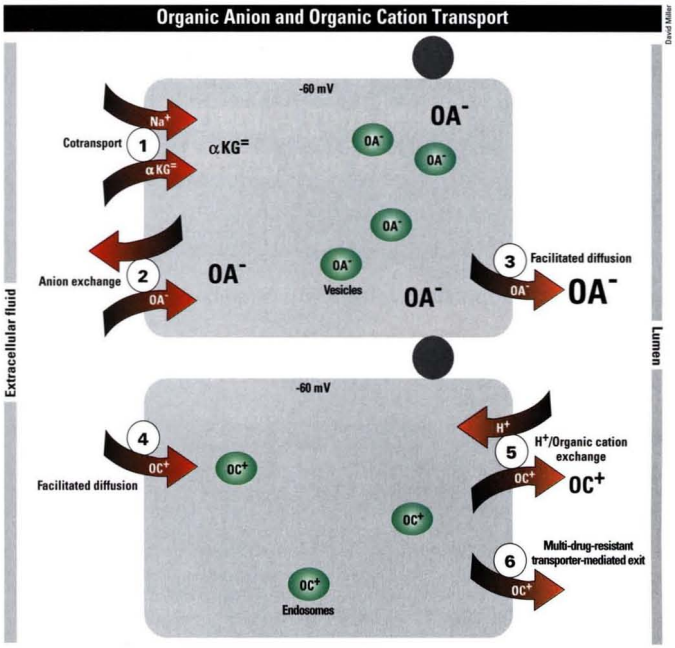
Getting Rid of What Ails Us

Each day, people are exposed to a number of potentially toxic organic materials. Metabolism of certain foods and drugs creates toxins within the body, while potentially harmful substances such as herbicides, pesticides, and exhaust fumes are taken into the body from the environment. One of the factors that determines how much damage these toxic substances can cause is how effectively they are eliminated from the body. The longer toxins remain in the body, the greater the potential for their accumulation and damage to bodily tissues. The members of the Comparative Membrane Pharmacology Section of the Laboratory of Cellular and Molecular Pharmacology at the NIEHS are using techniques in cell biology, physiology, and molecular biology to study the mechanisms responsible for excretion of xenobiotics from the body.

Physiology

The end products of metabolism are generally more polar than are the starting materials. In many cases, organic ions are produced. Organic ions destined for renal excretion are carried to the kidneys via the blood. The toxic ions diffuse out of blood vessels and into the extracellular fluid bathing the cells of the kidney tubule. Research physiologist John Pritchard has focused much of his research on the mechanisms by which negatively-charged organic ions, called organic anions (OA), enter the kidney epithelial cells from the extracellular fluid. To understand this problem, he first had to determine the energetics of OA entry into the cell.

Water-soluble OA are too large to pass easily through cellular membranes, and the negative charges of these compounds make passage even more difficult. The inside of kidney cells, like most cells, is negatively charged relative to the surrounding extracellular fluid. Negatively-charged chemicals must enter the cell against an electrochemical gradient; thus, OA uptake into the cell must be coupled with an energetically-favorable process. For some time, scientists noticed that the inflow of positively-charged sodium ions—a process driven by the out-to-in sodium gradient and, thus, energetically downhill—correlated with the entrance of OA into kidney epithelial cells. However, Pritchard and others studying the problem discovered that OA transport was not directly coupled with sodium-ion influx. An intermediary step was required. In 1987 Pritchard and German scientist Gerhardt Burkhardt determined independently that a ubiquitous cellular metabolite called α-ketoglutarate (α–KG) played this intermediary role. Their work showed that sodium-ion entry drives α–KG accumulation. In the next step, α–KG exits the cell, releasing energy, and OA enters in exchange. In this way, the energy derived from admitting sodium is indirectly used to admit OA.



* For both classes of solute, lumenal concentrations exceed cellular concentrations, which in turn exceed concentrations in the extracellular fluid.

① Divalent organic anions (α–KG=) enter the cell by coupling to the Na gradient through cotransport.

② Monovalent organic anions (OA-) also enter the cell by organic anion exchange. Within the cell, organic anions are partitioned between vesicles and the cytoplasm.

③ Organic anions may exit the cell by facilitated diffusion.

④ Organic cations enter the cell by voltage-dependent facilitated diffusion and are taken up by endosomes.

⑤ Organic cation exit is mediated by exchange for protons.

⑥ Organic cation exit is also mediated by multi-drug-resistant transporter proteins.

However, because the ions are still too large to pass through the membrane unaided, they must be transported by a protein embedded in the membrane. One of Pritchard's current interests is identifying the transporter protein, or proteins, that admit OA into kidney epithelial cells. Conversely, while the entrance of positively-charged ions, called organic cations (OC), is energetically favorable, special transporter proteins are also necessary for their entrance into the cell. Pritchard hopes to study these proteins as well.

While one side of the kidney epithelial cell is responsible for taking up toxins from the extracellular fluid, the other side mediates the secretion of these toxins into the tubular lumen, where urine is formed. The energetics of expelling organic ions is the mirror-image of admitting them. Eliminating OC requires the input of energy, while eliminating OA may not. In addition to the various transporter proteins required for these processes there may be other mechanisms at work as well. Pritchard and colleagues are also studying the proteins and auxiliary mechanisms of organic-ion expulsion.

Molecular Biology

Molecular biologist Doug Sweet is working to characterize the various transporters at the molecular level. He hopes to use molecular biology techniques to identify the genes for the various transporters. By determining the genetic sequence of the transporters, Sweet will be able to derive

the amino acid sequences and, thus, the structures of the various transport proteins.

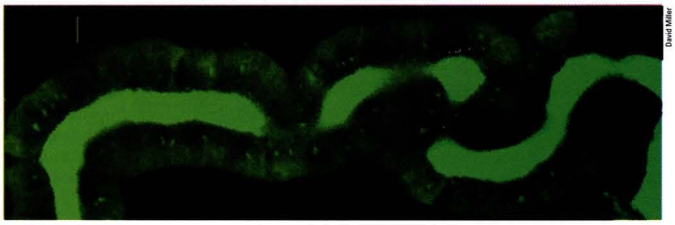
Sweet's research continues the work of his predecessors Natasha Wolff and Rosemary Walsh. Using the technique of expression cloning, Walsh searched for the transporter protein (or proteins) that mediates the excretion of OC by the kidney epithelium. To do this, she created a library of genes expressed in the kidney. These genes were then amplified on plasmids and inserted into bacteria. Sweet screens these bacteria to identify the group that contains the gene or genes of interest—in this case, the one encoding the OC transporters. The bacteria are then divided into groups and the plasmids containing the various kidney genes are purified. Using *in vitro* transcription, the DNA of each group of plasmids is made into messenger RNA, which is microinjected into the oocytes of *Xenopus laevis* (the African clawed toad). This mRNA directs production of the proteins encoded in each of the genes of the kidney library. Finally, the oocytes are assayed for their ability to support OC transport. With each subsequent round of screening, the number of bacteria in each group is narrowed, eventually leading to a single bacterial colony containing a single kidney gene—in this case the OC transporter gene.

Sweet is now in the final stages of that screening process and believes he is close to locating the gene. In the next phase of his work, Sweet plans to search the library for genes encoding the OA transporters. Identifying these genes will help the researchers to learn more about how ions are admitted into kidney cells.

Having a detailed understanding of the gene and protein sequences of the transporters may help the researchers learn about the precise nature of the interactions between these proteins and organic ions. It may also help decipher interactions between organic ions competing for the same binding sites on the transporters, which can contribute to the relative toxicity of a compound. Sweet also plans to study the genomic sequences for the transporter genes in order to understand how the genes respond transcriptionally to changes in the toxic load present in a cell's environment.

Translocation

Transporting toxins into a kidney cell is only the first step in their elimination. In fact, the presence of these toxic substances inside the kidney epithelial cells raises the question of how the kidney protects itself from these toxins. Studies performed by research physiologist David Miller may provide an answer. Miller has found that once organic ions have entered the kidney cell, some of the ions—both positive and negative—are sequestered into small membrane-bound vesicles. Miller has discovered that the vesicles serve not only to protect the cell, but may also ferry toxins from one side of the cell to the other. Miller has evidence that once the vesicles reach the exit site, they release their contents outside of the cell. This might be accomplished, he says, by a fusion between the vesicular and cellular membranes.



Killifish kidney tubules. Fluorescent organic anion (bright areas) is secreted into the tubular lumen and taken up by adjacent cells. (green bar=10 micrometers)

Miller can actually observe some of these processes as they occur by attaching a small TV camera to his microscope and projecting the images into his computer. By using organic ions that are linked to fluorescent dyes, Miller can watch the flow of ions through and out of kidney epithelial cells on his computer screen.

For some of this research, Miller uses the killifish, a type of minnow that he predicts could become the "laboratory rat of the fish world" because it is so hardy and adaptable to a wide range of environmental conditions. Furthermore, the killifish provides a model in which the entire excretion process can be studied in the intact kidney tubule.

Choroid Plexus

Whereas the killifish is good for studying gross features of renal organic-ion transport, the choroid plexus allows Miller, in collaboration with physiologist Alice Villalobos, to look at the passage of individual ion-transport vesicles across and out of cells.

One function of the choroid plexus is analogous to what the kidney does for the entire body: mediating removal of organic ions from the brain into the bloodstream for subsequent renal or hepatic excretion from the body. The choroid plexus is located within the brain's ventricles and forms the interface between the cerebrospinal fluid and the body's blood supply. The cells of the choroid plexus share much of their basic physiology with kidney epithelium. Both cell types have transporter proteins regulating the influx and outflow of organic ions, and both cells shuttle the ions across the cells in vesicles.

Villalobos has succeeded in culturing cells from the rat choroid plexus. In culture, these cells form a layer only one cell thick, making them easily visible under the microscope. Villalobos has observed that vesicles filled with fluorescent dye clearly cross the choroid plexus cell until they appear to burst, releasing their fluorescent contents outside of the cell.

Miller and Villalobos have performed several experiments whose results suggest that the cytoskeleton is crucial to vesicular transport. Specifically, they have shown that disrupting the filaments called microtubules halts transport. This, notes Miller, suggests that the microtubules provide a sort of "railroad track for the vesicles, where the end of the line is fusion of the membranes and release of vesicular contaminants."

Characterizing transport mechanisms—the transporter proteins and the vesicular transport system—is crucial to understanding xenobiotic excretion. Understanding the factors that determine whether or not a compound will be taken up and excreted by the kidney or the choroid plexus helps toxicologists to predict the compound's toxicity. In addition, these studies may provide insight into the factors that determine the use of drugs and other beneficial organic ions within the body.

Michelle Hoffman

BANKING ON TISSUES

Twenty years ago this July in Seveso, Italy, a reaction vessel in a chemical factory exploded, spewing a foul-smelling compound that caused birds to fall from the sky. Over the next month, 200 residents developed chloracne, indicating exposure to dioxin, says Neil Caporaso, a scientist in the Genetic Epidemiology Branch of the National Cancer Institute. All farm animals were slaughtered and the town was divided up into several zones based on level of exposure. In the most heavily exposed districts, the soil was dug up and carted away, and houses were razed. Fortunately, blood samples of residents had been banked, for although only the highest exposures could be measured at the time, now "we can classify the exposures that literally hundreds of people had, and tell what areas were hit," says James Pirkle, assistant director for science in the Division of Environmental Health Laboratory Sciences of the Centers for Disease Control (CDC). "Some areas were declared safe that actually were hit."

Studies based on these samples are beginning to reveal the relationship of dioxin exposure to various cancers, and researchers have been able to go back to exposed individuals and find out how strongly the compound has persisted in their bodies. "Much of our understanding about the persistence of dioxin in humans depends on this," says Caporaso.

"Banked specimens present a great resource for evaluating environmental and occupational health problems," says Paul A. Schulte, acting director of the Education and Information Division of the National Institute for Occupational Safety and Health. "Human tissue samples really [provide] a measure of dose," explains Ken Sexton, professor of environmental health at the University of Minnesota School of Public Health.

The Holy Grail of tissue banking is to relate levels of environmental contamination to health outcomes via doses—the main pieces of information one looks for in banked samples, says Jack A. Taylor, head of the Molecular and Genetic Epidemiology Group at the NIEHS. Unfortunately, samples are often banked without information on exposures, and cohorts are often not followed because it is difficult and expensive to do. Nonetheless, there is plenty of value in more narrowly designed studies.

Lately, a controversy over informed consent has flared, threatening to force researchers to obtain new consent from every individual in a cohort study every time something new is done with the banked materials. The issue "terrorizes me," says Taylor.

The controversy is particularly threatening to researchers who use tissue banks in one of their newest applications: research on the genetics of susceptibility to specific carcinogens. "If we don't solve this dilemma, no prospective cohort that we design now will be usable," says John Groopman, professor and chairperson of the Department of Environmental Health Sciences at the Johns Hopkins University School of Hygiene and Public Health of the issue of consent.

National Monitoring

The monitoring of exposures to dioxin at Seveso represents a quick response to an acute and obvious problem. There is also a need to track chemical contamination of human tissues on a more ongoing basis, according to the National Research Council (NRC). In the United States, more than 60,000 chemicals were

approved for commercial use in 1984. Of 3,400 pesticide ingredients, only about 10% had data available for a complete health hazard assessment, and more than 700 organic chemicals have been identified in U.S. drinking water.

Several federal programs have experimented with population-based monitoring of human tissue samples. The National Human Adipose Tissue Survey (NHATS), an EPA program, scrutinized mainly pesticide residues from 1967 to 1989, collecting about 12,000 tissue samples, mostly from cadavers. But NHATS was cited by the NRC for quality control and other problems and is virtually defunct. The National Health and Nutrition Examination Surveys (NHANES) of the National Center for Health Statistics collects blood periodically from a population-based sample of about 20,000 U.S. citizens, but the focus is mostly on nutrition.

Recently, several agencies including the EPA and the CDC have established a pilot population-based survey, the National Human Exposure Assessment Survey (NHEXAS), to monitor blood (50 ml per subject), urine, hair, and in some cases, fingernail, samples while also sampling the air, water, soil, food, and dust from inside the houses of subjects, says Sexton, who is helping to run the program. Subjects will answer a questionnaire concerning age, gender, occupation, type of housing, and indoor exposures to toxins from sources such as gas stoves and cigarettes. The survey will be conducted on 50 people in the Baltimore metropolitan area, 300–400 people in Arizona, and about 500 people in EPA Region 5 (which includes Indiana, Illinois, Michigan, Minnesota, Ohio, and Wisconsin), says Sexton. Each pilot area is run by a consortium of scientists who will choose what to sample from among metals, volatile organics, pesticides and PAHs. The same compounds will be sampled in human subjects and the environment.

According to Jerry Akland, project officer for NHEXAS, the project's success will be measured by how much the additional information reduces uncertainty in current exposure models. In other words, if the distribution of exposures is different from what current models predict, it will justify the cost of a national survey. Such a survey of about 5,000 people would be conducted with subjects selected through statistically-based sampling design. "This will give a pretty good sense of average exposures, and . . . over time, you can see the trends," says Sexton. "Almost without exception, we don't have that kind of information in this country."

What Tissue Banking Can Tell Us

Despite the lack of a direct link of exposures to adverse health outcomes, a national monitoring study could be critically important. The data could "help identify new or previously unrecognized hazards . . . [and] establish trends in body burdens of toxicants that result from changes in manufacture, use, and disposal patterns, and thus monitor the results of programs intended to control specific chemical hazards," according to the NRC's 1991 study, *Monitoring Human Tissues for Toxic Substances.*

For example, roughly five years after the pesticide Mirex was used to kill fire ants, retrospective analysis of NHATS tissues from the period when it was used found contamination in human tissues. "Evidence of Mirex exposure . . . led to a more intensive survey of the general population in treated counties of the southeastern United States," according to a 1985 article in the *Journal of Toxicology and Environmental Health.*

Nationwide surveys are too broad to furnish definitive data on health effects, says John C. Bailar, III, chairperson of the Department of Health Studies at the University of Chicago. But they are very important for estimating body burdens and, hence, population-wide risk, as well as for tracking the success of control programs, he said.

Furthermore, they provide a framework for designing epidemiological studies, once they have raised suspicions, says David Kalman, a professor in the Department of Environmental Health at the University of Washington in Seattle. "Knowing in advance the patterns in prevalence [of a problem] gives clues as to how to construct the study."

Studies where subjects are followed after specimens are banked can furnish a clearer picture of how specific compounds influence human health. Once disease has set in, researchers can search the premorbid materials for chemical, biochemical, immunological, and other changes that might predict development of cancer or other diseases.

Peterson Portraits

Human tissue samples provide a measure of dose. Ken Sexton

Kathy J. Helzlsouer, an associate professor of epidemiology in the Department of Epidemiology in the Johns Hopkins University School of Hygiene and Public Health has been conducting studies using banked samples for two decades. In 1974 and again in 1989, over 25,000 blood samples were banked at the Washington County (Maryland) blood bank. Helzlsouer is comparing the level of organochlorines in blood samples from 400 women who have since developed breast cancer with samples from matched controls, although there are no results yet. She is also planning a similar investigation of cadmium in the etiology of a similar number of cases of prostate cancer.

In similar case-controlled studies of serum and urine samples banked since the early 1980s from cohorts in China and Africa, Groopman and his collaborators discovered "a strong interactive effect between aflatoxin exposure and hepatitis B virus exposure in the development of liver cancer." Supporting the hypothesis that aflatoxin had influenced development of cancer, mutations found in the *p53* tumor suppressor gene in these samples were characteristic of types that form uniquely in response to aflatoxin.

Attention has been focused on recent discoveries of genes such as *BRCA1* and *HNPCC*—mutations that are associated with a very high risk of breast and colon cancer, respectively. Researchers have also begun to discover genes that have far more subtle relationships with cancer. For example, "smoking is associated with increased risk of lung, bladder, and other cancers," says Taylor. The protein product of the gene, glutathione-S-transferase MBA (*GSTM1*), binds certain carcinogens, rendering them easier to excrete into the urine. But, Taylor says, "50% of Caucasians, roughly, are walking around with no working copy of that gene. . . . Within two groups who smoke the same amount, the people without the detoxification pathway are at about twice the risk of developing cancer as those with a working copy." Taylor and his colleagues are looking at a whole series of additional genes to try to identify the genetic and environmental determinants of bladder cancer. "We continue to use banked samples and detailed exposure information from the same cases to try to put the genes and exposures together," he said.

In another similarly constructed study using blood samples from the prospective Harvard University Physicians Study, Frederica Perera, a professor of public health in the Division of Environmental Health Sciences at the Columbia University School of Public Health, is investigating the interaction of markers such as polyaromatic hydrocarbon adducts on albumin and DNA with genes controlling metabolism of carcinogens in the development of cancer.

In addition to determining causal agents for specific cancers, researchers can use banked tissues to investigate the molecular pathways by which specific pollutants cause cancer. Several decades ago, Geno Saccomanno, a pathologist at St. Mary's Hospital in Grand Junction, Colorado, noticed that lung cancer was common among uranium miners, who breathe large quantities of radon. Saccomanno began storing blocks of resected lung tumors. Taylor wondered whether the miners' tumors might have different patterns of genetic damage from those of smokers. Examining 52 large and squamous cell cancers from miners, he found that 31% had an identical mutation in the *p53* tumor suppressor gene, but that this mutation was extremely rare in smoking-associated tumors.

Asbestos-associated lung cancers have yet another pattern of mutation. Using a bank of asbestos-associated lung tumors from the Telemark Central Hospital in Norway, Taylor and his colleagues conducted studies, presented at the Meeting of the American Association of Cancer Research in April 1996, showing patterns of base-pair changes that are different from the guanine (G) to thymine (T) mutations that predominate in smoking-associated tumors. Identifying exposure-specific mutations could lead to development of screens for early detection of cancers, and—in the somewhat distant future—to developing chemotherapies, says Taylor.

Steve McCaw, Image Associates

The Holy Grail of tissue banking is to relate levels of environmental contamination to health outcomes via doses. Jack Taylor

Different tissues can provide a variety of other insights into the effects of pollution. The U.S. Public Health Service is banking lung specimens from relatively young accident victims in Los Angeles and two control cities, Miami and Seattle, to investigate the effects of ozone on tissue structure, to measure particulates, and possibly to perform other histochemistry and biochemistry studies, says Richard Everson, a medical officer with the PHS.

Taylor praised the study's thorough collection of exposure data, which includes full residential histories of subjects linked to zip code-related pollution measurements taken by the California Air Resources Board, as well as smoking, work-related exposures, and even physical activity levels of subjects.

What to Collect

Organ tissues are unusual in banking studies. Ninety-five percent of materials collected for storage are blood and urine. The reasons are simple. First, it is easy to do cohort studies, because people are used to having both types of samples taken under normal circumstances, says Helzlsouer. The question of what type of sample to use as a control becomes more complicated with, for example, surgical specimens of cancer. "Do you use benign disease such as breast lumps?" asks Helzlsouer.

"We recommend blood [as a standard specimen] because it is readily accessible, and not likely to be contaminated the way hair and nails are," says Bailar. "People wash their hair in all kinds of funny things, and they get all kinds of funny stuff on their hands." DNA can be obtained directly from blood and analyzed using PCR, or white blood cells can be immortalized and frozen in liquid nitrogen for future thawing and expanding.

Even fat can be obtained from blood for sampling of fat-soluble contaminants such as PCBs and pesticides. "There is a very small lipid fraction in the white blood cell that is in equilibrium with whatever is in the blood stream," says Bailar. "The fat fraction of blood should have about the same concentration as any other body fat. Our committee [Committee on National Monitoring of Human Tissues of the National Research Council] did recommend that EPA undertake studies to validate this." Furthermore, "new developments in analytic chemistry over maybe the last 15 years can take this tiny fraction and give you adequate readings of unbelievable sensitivity," says Bailar. Others say that sampling of fat-soluble contaminants can be done still more easily and just as accurately from blood lipids.

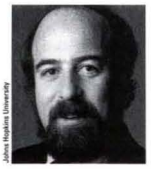
Easier than drawing blood is buccal swabs, says Taylor. "It's nothing but a sterile Q-tip. You have the person rub it on the inside of their cheek." In a study investigating contribution of environment and genotype to etiology of cleft lip and palate in Norway, Taylor's colleagues are using buccal swabs to collect samples from controls and from family members. "If we tried to stick a needle into every person that we wanted to get DNA samples from, we would never get close to the participation rates that we can get with buccal swabs," he says. Nonetheless, "blood is still the gold standard because it gives you a lot of DNA that's very high quality."

Urine is also a source of DNA, "because cells are sloughed off from the bladder," says Taylor. "In fact, for bladder cancer, there's increasing interest in using new techniques to look at the DNA of sloughed cells to see if we can detect cancer earlier than using traditional screening techniques."

Some materials are much harder to collect than blood and urine, but may offer unique advantages. By virtue of its biological function, the liver accumulates pollutants, but livers can be obtained only from cadavers or surgical specimens. The National Institute of Standards and Technology (NIST) examined how feasible it would be to collect livers from individuals who had been normal and healthy at death, and determined that it was feasible, especially from victims of accidents or gunshots, says Stephen Wise, a research chemist at NIST. Wise was able to collect 300 livers in a single year. According to Wise, lesson number one is that "you need the cooperation of the medical examiner. If the medical examiner is not interested in getting you samples, you won't get any." Of course, samples that are collected from cadavers are inherently biased. "Accident victims don't have the same range of ages and sex as the general population," says Bailar. "They may differ in other ways, too."

Whatever the tissue, researchers should collect it with an eye toward the needs of future investigators, says Taylor. He should know; he has spent years doing research with old samples of cancers from the pathology departments of hospitals. In one study, Taylor says, "I had to go to hospitals all over the country to track down tumors and contact individual pathologists and say, 'Can you find this 30-year-old block?'" Samples are normally fixed in formalin and then embedded in paraffin blocks for storage.

Old blocks in particular often get hidden in the deepest, darkest corners of a hospital, so retrieving them is no easy task. "We've had them all melted together in a giant blob, or destroyed by fire, so you can't always get what you want," Taylor said. "Getting the pathologist to give up the blocks is often tricky. You have to be careful to return the blocks to the pathologist, something I try hard to do as fast as I can."



Johns Hopkins University

If we don't solve the dilemma of consent, no prospective cohort we design now will be usable. John Groopman

Storing and Maintaining Collections

Storage is the key to longevity of samples, and there are many ways to do it. The best method of storage is to freeze them in liquid nitrogen at temperatures of -180°C to -196°C. "The main reason you would want to use liquid nitrogen is if you are working with any cellular component where you want all metabolic processes to stop dead, and be able to revive the cell," says Elaine Gunter, chief of the NHANES laboratory at the CDC. "Also, if you have a cell that has an infectious agent that you aren't aware of, it keeps the agent from killing the cell." Additionally, liquid nitrogen also prevents the condensation and desiccation that can plague a mechanical freezer, says Gunter. Kalman refers to freezing in liquid nitrogen as a "suspenders and belt" approach that offers the best protection in view of uncertainty about future investigations of stored samples.

On the other hand, if the goal is simply to be able to identify environmental pollutants in tissues at some later date, fairly high temperatures and even room temperatures can be adequate to preserve many of the more common pollutants, such as heavy metals and aromatic hydrocarbons, says Kalman.

For successful long-term freezing, liquid nitrogen is also more of a fail-safe than mechanical freezing, says Elio Riboli, chief of the Unit of Nutrition and Cancer at the International Agency for Research on Cancer (IARC). Pressure differences drive replenishment of the storage containers from a large reservoir of liquid nitrogen, which can stay cold enough to maintain the storage containers for two months without electricity. "I have had samples stored [in mechanical freezers] at -80°C, but we have all had the experience of the freezer breaking down, usually between Saturday night and Sunday," Riboli said.

In the course of a major project conducted by IARC, the European Prospective Investigation into Cancer and Nutrition, samples from 350,000 subjects are being banked. The program is hailed as a model of excellent storage techniques. To avoid having to thaw and refreeze samples every time an analysis is needed, "plasma, serum, buffy coat, and red blood cells will be stored in small plastic straws with a capacity of only 0.5 ml," according to IARC, with 30 ml being collected from each subject.

Pre-labeled jackets in a different color for each of the four blood fractions stored fit tightly around each straw. For insurance, the 28 straws for each subject are divided into identical series of 14, one stored at the center where the blood is collected, and the other at IARC. IARC has developed a system for organizing the samples for quick, easy access. "The large capacity [of liquid nitrogen systems lends itself] to a very organized storage system," says Gunter, "so you can set up a computerized inventory and track by rack, box, position, tray, or whatever."

The CDC has about 500 mechanical freezers storing materials at -70°C, says Gunter. "It's easier where you are going in and out every day." But the CDC is renovating a facility of 220 large-capacity, liquid-nitrogen freezers, large enough to store "literally millions of samples," says Gunter.

As for storage requirements of other materials, the 25,000 samples of sera from the Washington County Blood Bank are stored at -70°C in mechanical freezers over 20 years old, says Helzlsouer. "We still get levels of nutrients and hormones that we would expect, so we think they are fairly stable."

So far, for periods of at least three years, at temperatures of -20°C and -70°C and in alcohol at room temperature, buccal swabs are "pretty stable," says Taylor. "That's the longest we've looked." As for urine, longevity hasn't been established, says Taylor, who freezes most of the samples he collects at -20°C. "I'm just winging it."

NHATS provided some examples of how not to store samples, according to the NRC. Specimens stored in glass bottles sealed with metal caps with foil-lined cardboard inserts had rusty caps, and 10–15% of the specimens contained pieces of foil. Some caps were loose, and specimens had dried out. "Storage artifacts . . . become severe after five to six years of storage," the report concluded.

Informed Consent

Almost ten years have passed since the National Center for Environmental Health (NCEH) of the CDC decided to create a DNA bank from NHANES blood. At the time, says Karen Steinberg, chief of the Molecular Biology Branch of the NCEH, "there weren't a lot of things to test for. All the issues apparent today were not apparent then." But as new gene discoveries began to occur on practically a weekly basis, Steinberg began to worry. "We didn't feel our consent was sufficient to do linked testing," she says.

Groopman says that the debate two years ago over health care reform as it related to preexisting conditions further highlighted the issue of consent. Steinberg is more worried that subjects in research on one gene, who have said they want the results, might be presented by well-meaning researchers with information concerning genes not covered in consent forms, such as breast cancer or colon cancer genes.

These concerns led to a meeting sponsored jointly by the National Center for Human Genome Research and the CDC of medical ethicists, lawyers, and researchers from around the country, and to an article in the 13 December 1995 issue of the *Journal of the American Medical Association* (*JAMA*) by Steinberg and several co-authors. The article advocated policies that some researchers consider to be dangerously restrictive, creating quite a controversy. Such policies include a requirement that it be impossible for anyone, including the principal investigator,

Matthew Gibson

Nationwide surveys are important for estimating population-wide risks and for tracking the success of toxin control programs. John C. Bailar, III

Some Environmental Studies Using Banked Tissues

Name of study	No. of samples	Type of sample	Endpoint
National Human Adipose Tissue Survey (NHATS)	12,000	fat tissues	pesticide residues
National Health and Nutrition Examination Surveys (NHANES)	20,000	blood	mostly nutrition
National Human Exposure Assessment Survey (NHEXAS)	about 950	blood, urine, hair, fingernail	metals, volatile organics, pesticides, PAHs
Breast and prostate cancer studies (Helzlsouer)	25,000	blood	organochlorines and breast cancer, cadmium and prostate cancer
Harvard University Physicians Study	15,000	blood	PAH biomarkers, gene metabolism and carcinogens
Uranium miner studies (Taylor)	52	squamous cell cancers	mutation in the *p53* tumor suppressor gene
Lung cancer study (Taylor)	25	asbestos-associated lung tumors	mutations in smoking-associated tumors
Aflatoxin and liver cancer study (Groopman)	18,000	serum and urine	interaction between aflatoxin and hepatitis B in liver cancer

to link human specimens with individuals, or that a new informed consent must be obtained every time a researcher studies a new gene.

In their rush to protect research subjects, says Caporaso, Steinberg and others are confusing the big risks to individuals of having a *BRCA1* breast cancer gene or *HNPCC* colon cancer gene with risks from metabolic polymorphisms that are insignificant from the standpoint of the individual, yet important to public health. "It would be farfetched to think that [genes for carcinogen-metabolizing proteins] would have health implications for families," says Everson. But the proposed standards would hamstring this research, says Schulte.

The standard of anonymity is impossible to achieve without dumbing information on each individual down to uselessness, some researchers say. It only takes a few pieces of information such as grade and stage of tumor, age at resection, race, and smoking habit to make possible a definitive identity, even if all links in the database have been cut. "As an example of anonymity," says Steinberg, " a set of such parameters should identify at least three people.

"Requiring consent for every new gene to be studied from a sample is untenable," asserts Caporaso, "in that it does not appear to serve either the individual's interest or those of science. The cost is prohibitive. If it's a population-based cohort . . . and you want to write to every person and say we are going to test a genetic marker, this is what it means: with a cohort of 100,000 people, the cost is conservatively in the millions."

But in dealing with human subjects, it is important to err on the side of caution, Steinberg asserts, alluding to the medical establishment's past paternalism. "We are the servants of the public. We have to be very sensitive to issues of importance to the public . . . and we have to deal with their perception of risk."

The *JAMA* authors also recommended "enactment of more general legislation to ensure that no person or institution be able to obtain access, even by court order or subpoena, to either the samples used in research or the specific results of research performed on such samples." Their reasoning was that "although protection may already be provided by certificates of confidentiality, sources are entitled to this higher level of protection in exchange for allowing their samples to be used for research."

The Office for Protection from Research Risks at NIH might develop its own guidance, and the new National Bioethics Advisory Commission being established by President Clinton could issue guidance, says John Miller, the office's deputy director. For the moment at least, such guidance remains the province of individual institutional review boards.

The controversy over informed consent has left those who bank on tissues holding their breath. Tissue banking has proven its value as a versatile method of studying the impact of environmental contamination on human health, and for contributing information for environmental policy-making. Questions such as deciding how to conduct a study, which tissues to collect, and how best to store them can be difficult to answer, but usually yield to creativity, hard work, and money. Researchers fear that current issues surrounding informed consent might not be nearly so tractable. How this controversy plays out will have a profound impact on a crucial area of environmental research.

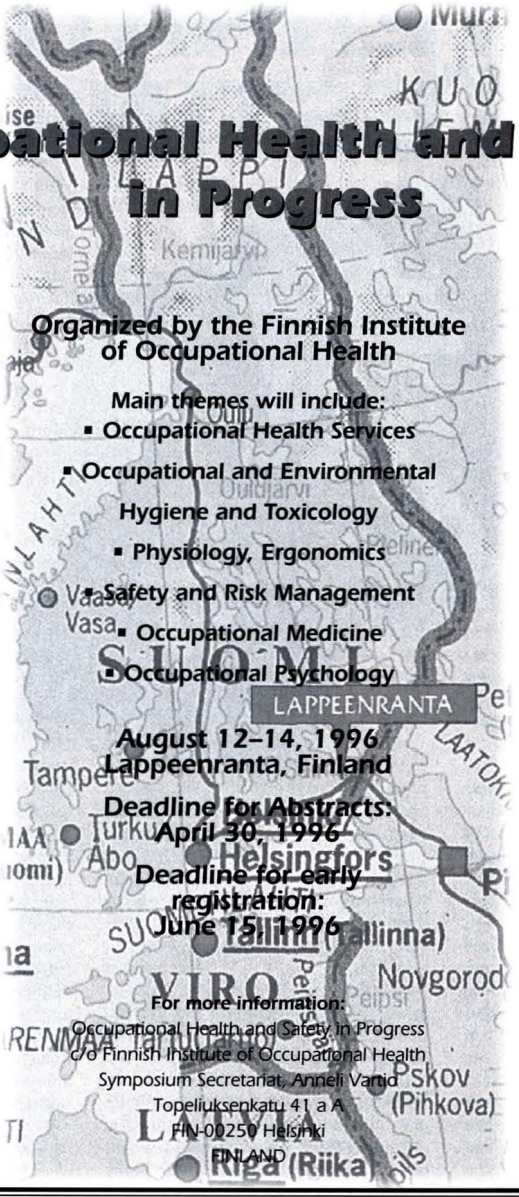
David Holzman

ERRATA

The second paragraph of the second column on page 23 of the article *The Attack of Asthma* in the January 1996 issue of *EHP* (104:1) mistakenly attributes statistics about Puerto Rican children and asthma to the *New York Times*. The information is taken from an article that appeared in the April 1993 issue of the *American Journal of Public Health* (83:4), entitled Reported Asthma among Puerto Rican, Mexican-American, and Cuban Children, 1982 through 1984. The statistics were also incorrectly identified as the rates of asthma among Puerto Rican, Mexican-American, Cuban, black, and white children between 1982 and1984. They should have been identified as the percentages of children who have ever had asthma. Also in this *EHP* article, the date at the bottom of the first column on page 23 mistakenly reads "between 1976 and 1908." The sentence should read "1980."

Northern-Baltic-Karelian Regional Symposium

Occupational Health and Safety in Progress



Organized by the Finnish Institute of Occupational Health

Main themes will include:

- Occupational Health Services
- Occupational and Environmental Hygiene and Toxicology
- Physiology, Ergonomics
- Safety and Risk Management
- Occupational Medicine
- Occupational Psychology

August 12–14, 1996
Lappeenranta, Finland

Deadline for Abstracts:
April 30, 1996

Deadline for early registration:
June 15, 1996

For more information:
Occupational Health and Safety in Progress
c/o Finnish Institute of Occupational Health
Symposium Secretariat, Anneli Vartio
Topeliuksenkatu 41 a A
FIN-00250 Helsinki
FINLAND

GLOBAL AGREEMENT ON ALTERNATIVE TESTING

In recent years, public pressure in the United States and abroad, coupled with advances in science and technology, have led to a radical reevaluation of the longtime and widespread use of animals in toxicological experiments. As a result, new testing methods designed to refine, reduce, and replace animal models have emerged in government, academic, and industry laboratories worldwide. But this sudden shift has left both researchers and regulators scrambling to determine when the results from alternative test methods are valid and acceptable and when they are not.

"It's a small world," says Neil Wilcox, special assistant to the associate commissioner for science at the Food and Drug Administration. "The scientific community and changes in toxicity testing are now international in scope. Everything we do has international implications and whatever we do impacts the international community. Harmonization will reduce the wasteful duplication of efforts by standardizing testing."

The search for a set of international standards began in earnest five years ago when the European Union (EU) created the European Center for the Validation of Alternative Methods (ECVAM). ECVAM's principal role is to coordinate the validation of alternative methods among the EU's 15 member states. As early as 1987, the global Organization for Economic Cooperation and Development (OECD) began to consider animal welfare issues in its international guidelines for the testing of chemicals. And in 1994 in the United States, the NIEHS established the Interagency Coordinating Committee of Criteria on the Validation of Alternative Methods (ICCVAM) with a goal of achieving domestic and international harmonization of criteria for the validation and acceptance of such methods. Today, the three groups are working together to bring scientific laboratories and regulatory agencies into agreement on alternative test guidelines.

"The challenge is to come up with useful criteria for scientists in any country who are developing new methods," says William Stokes, associate director for animal and alternative resources at the NIEHS and cochair of ICCVAM. "They must meet criteria that would be the same internationally. It wouldn't make sense for one country to consider a test method valid while another didn't. Each new method must be reliable, relevant, and reproducible in different laboratories. If you generate a new method, a company will be reluctant to use it if the data won't be acceptable to the regulatory agencies in the international marketplace. In the development and validation of alternative methods for acute toxicity, the Europeans have been pursuing a more focused effort and we're very interested in following the results of their work."

The EU issued a directive in 1986 discouraging scientists in its member states from using animals if they could reasonably and practicably achieve the same result without animals. "EU legislation requires that nonanimal test methods be used whenever possible, and that efforts are made to develop and validate such methods," says Michael Balls, head of ECVAM. "The main obstacles are scientific and administrative. [For example], the development and validation of relevant and reliable alternative methods is difficult [and] regulatory authorities tend to adopt a conservative stance and feel more comfortable with animal test data."

Because well-established animal tests have set the standard for decades, successfully replacing them will be a challenge. Alternative methods may feature the use of microbes, cells, tissues, and other *in vitro* methods, or rely instead on computer databases and mathematical models. Other new methods may involve use of genetically engineered animals or lower species. Revised methods may include modifications that require fewer animals or minimize or eliminate animal pain and distress. If international harmonization is achieved, each interested country's laboratories and regulatory agencies will accept the results of new or revised tests, including nonanimal tests.

For instance, as companies expand into overseas markets, their products must meet

the regulatory guidelines of each country to which they want to export. At the moment, many countries have their own guidelines, which may differ from those of other countries. With this *ad hoc* process, companies risk the loss of potential markets or the heavy expense of additional tests. For example, nonanimal test results of a product that might be acceptable in one country might be rejected in another for the same reason: because the test methods did not include animals.

"Harmonization helps us significantly," says Katherine Stitzel, associate director of the human safety department at Procter & Gamble. "In a global company that's working across borders, it saves us animals, time, and money. It's a drag on our resources to have to do things differently for different countries. It will make things so much easier when everyone is in agreement."

An International Platform

Founded in 1960 to stimulate economic progress and world trade and promote social welfare, OECD membership now includes 26 countries including most European countries, as well as the United States, Mexico, Canada, Australia, New Zealand, and Japan. With its global reach, the OECD has become a major player in the quest for international harmonization.

"The OECD is a unique forum for countries with advanced market economies," says Herman Koëter, principal administrator at the OECD's environmental health and safety division based in Paris. "Until the early 1990s, our role in alternative test methods was to accept applications and distribute them to our members for comments and approval. But we gradually began to realize that approaches to validation were not exactly the same in Europe and elsewhere. So in 1991, OECD decided to take a more active role in encouraging nonanimal testing by bringing members together to negotiate, discuss, and arrive at a consensus."

In 1987 and in 1992, the OECD issued guidelines for skin and eye testing that examined for the first time the possibility of using alternative, nonanimal methods for testing chemicals. At the time, however, the guidelines acknowledged that many tests were not ready to be conducted solely *in vitro*, especially those that assess the potential for skin sensitization. Accurate results, the guidelines said, could still only be achieved through standard *in vivo* testing.

In January 1996, the OECD organized a workshop in Stockholm, Sweden, on toxicological test alternatives to discuss international harmonization of validation and regulatory acceptance criteria for alternative tests. The ICCVAM draft report, ECVAM documents, and documents from other animal welfare centers were used as the basis for discussion. The draft ICCVAM report was developed by 15 U.S. federal scientific and regulatory agencies. At the workshop, participants agreed on test strategies for skin and eye tests "where schemes could be made mandatory," says Koëter. "When we discussed different approaches member-country-wide, there were no principal differences. Science and safety remain the key elements."

"One of the recommendations from the workshop was that developers of new methods should contact the appropriate regulatory agencies during the development and validation stages in order to optimize the usefulness of test methods," says Stokes. "This way, they can make changes in their method early on. You need a phase where you optimize the test protocol prior to formal validation studies."

Another workshop recommendation was to allow the use of patented methods, according to Stokes. Patented methods "may be necessary to stimulate creativity and innovation," he says. Currently, OECD guidelines do not permit patented methods because some countries may not be able to run the tests if such patented expertise and materials are difficult to acquire. In addition, Koëter said, adopting a patented test from one company as an OECD guideline may put the OECD in the position of providing that company with an unfair advantage over its competitors.

The OECD will present the workshop recommendations to the policy representatives or national coordinators of each of its member countries. The formal adoption of new methods, Koëter says, may take months because each proposed method will face legal, policy, and economic implications that must be addressed by each country's governing body. In order to be approved as an OECD guideline, each proposal must be unanimously approved by its member states.

After two years of discussions and negotiations, the OECD member states approved an alternative test method as an OECD guideline at the beginning of this year. Called the acute toxic class method, this alternative approach allows for a significant reduction in the number of animals required in acute oral toxicity tests. The test allows for the use of as few as three animals—"a fraction of the original amount," says Koëter. He also notes, "Sometimes it's easier to modify an existing guideline by adding new text in line with animal welfare. For example, an *in vitro* test could be recommended or even required before an animal test can be performed."

Modifying standard animal tests has opened many new questions about what constitutes an appropriate replacement. "While some *in vitro* methods appear to be useful for specific chemical classes, they are not generally useful for all chemicals," says Stokes. "You have to validate a method for a specific purpose, which is often more readily done in a stepwise fashion."

"Regarding the use of rodent models," adds Wilcox, "it's not always clear where it is adequate in different chemical classes. For example, in some classes, a rat might give 80% concordance or accuracy. But what do you do for the other 20%? Do you use a mouse model? Or should you use alternatives, such as a transgenic model? We still don't know if it is possible to take one rodent species and supplement it with an alternative. It's a very complex scenario, no question about it."

Harmony in Europe

Independent and neutral, ECVAM is part of the European Union Joint Research Center's Environment Institute in Ispra, Italy. The center's scientific advisory committee includes representatives from the member states of the EU as well as from the chemical, cosmetic, and pharmaceutical industries and from animal welfare groups. With no single EU-wide regulatory agency, harmonization must be approached industry by industry and country by country. But when agreements are reached in the form of EU directives, they are binding on all the member states, which must adapt their national legislation to comply with the directive's provisions.

Within the EU, there is "a communal commitment to the replacement of animal tests as validated nonanimal tests and testing strategies become available," says Balls. "However, the strength of feeling on this issue varies from country to country, as would be expected. Germany, the Netherlands, Sweden, and the UK are particularly committed to the reduction, refinement, and replacement of animal testing."

While ECVAM's principal role is to coordinate the validation of alternative methods at the EU level, its representatives are active in all worldwide discussions on harmonization. However, with 15 members, the EU is also in a position to make international progress on its own. In general, this could lead to tensions, says Balls, but as far as alternative methods are con-

cerned, "the existing good relations between partners such as ECVAM, ICCVAM, and the OECD should help to avoid that problem. There may be minor differences, but only because ECVAM's duty is to serve the European Commission and the EU, whereas ICCVAM's primary focus is the U.S. regulatory [and research] agencies."

Harmony in the United States

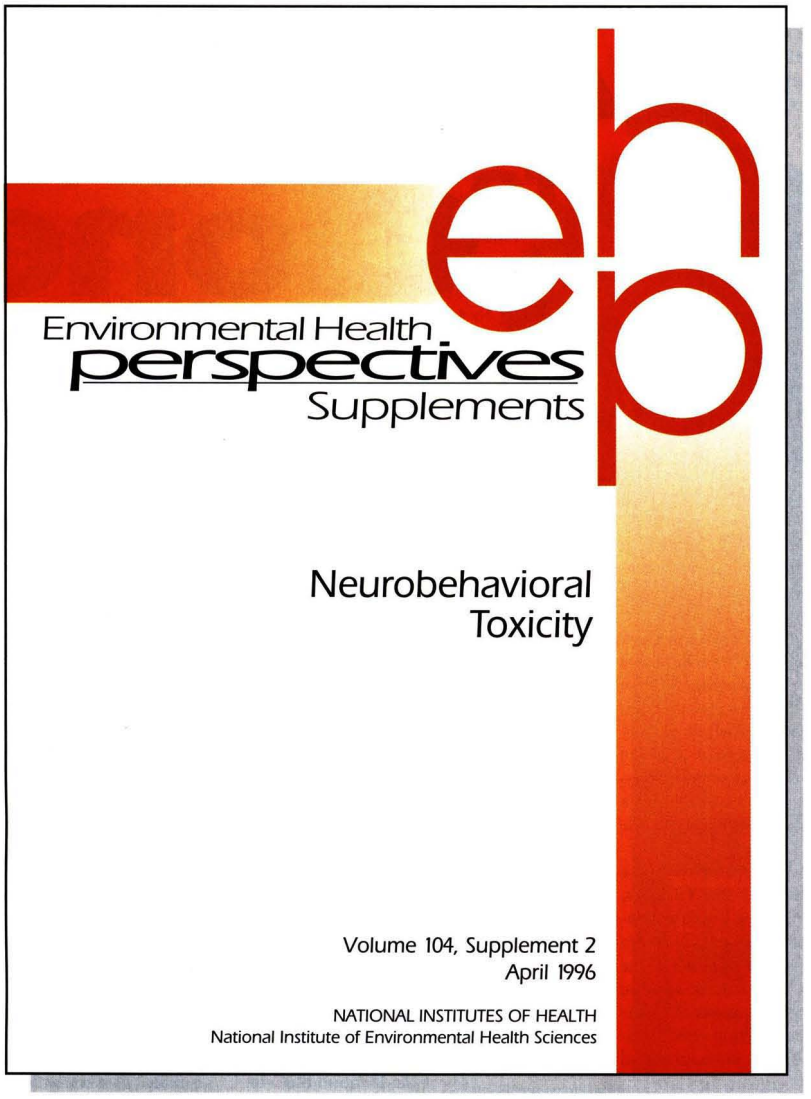
Similar in concept to ECVAM, ICCVAM also recommends processes acceptable to 15 U.S. scientific and regulatory agencies. Mandated by the National Institutes of Health Revitalization Act of 1993, ICCVAM consists of *ad hoc* representatives from those agencies. Though its principal mandate did not include international harmonization, ICCVAM approached the OECD to consider its criteria for validation and acceptance of new methods, knowing that all testing methods approved in the United States should also meet international criteria for approval.

"ICCVAM agreed OECD should be involved from the start to make it easier to get acceptance internationally," says Koëter. "Before finalizing its report, ICCVAM wanted OECD to take a look at it, not as a bible, but as a basis for discussion. As we determined at the Stockholm workshop there were no essential differences, OECD will now develop a document close to ICCVAM's. When it came to a way of thinking, there was widespread acceptance of alternative tests."

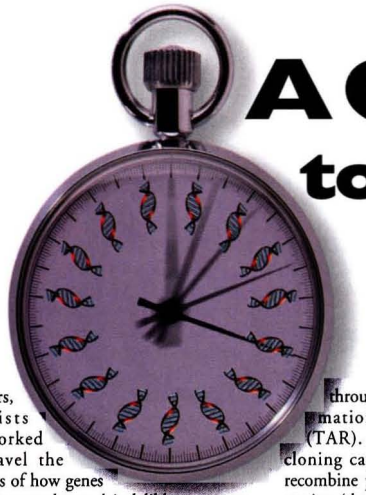
"We're in the business of developing and validating methods that would be more useful for predicting environmental health hazards," says Stokes. "As we learn more at the molecular and cellular levels about the mechanisms by which chemicals cause toxicity, we can transform our understanding into a mechanistic test method. As we develop better test methods, the hope is that we can better predict the toxicity of that chemical before it gets into the environment, the workplace, or the food supply, and before it can cause adverse effects on human health."

It is generally agreed that the process of harmonization will be lengthy, requiring ongoing meetings and workshops. It may take years to establish a universal set of guidelines agreed upon by scientists and regulators. But the results should dramatically reduce the need for animals in toxicological experiments while ensuring the validity and acceptance of such tests worldwide.

Rebecca Clay



For subscription information, see p. 664.
For a listing of published volumes of *EHP Supplements*, see p. 669.



A Quicker Way to Clone

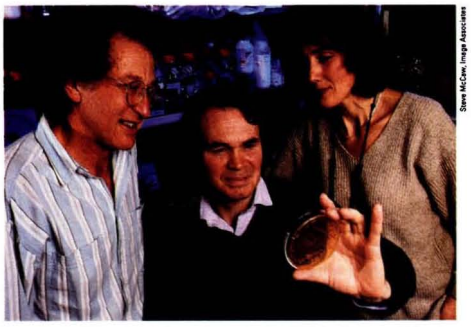
For years, scientists have worked to unravel the mysteries of how genes put their complex and indelible stamp on the lives of humans and other living organisms. In laboratories across the country, scientists scramble to identify the missing links in the chain of more than 100,000 estimated human genes. While the world awaits further advances that will bring the human genome into sharper focus, a group of scientists at the NIEHS has made an important genetic advance—an entirely new way to clone genes and parts of chromosomes—that may put the scientific community a few notches closer to understanding the role genes play in human disease.

Michael Resnick, a geneticist in the NIEHS Laboratory of Molecular Genetics, in collaboration with visiting Russian scientists Vladimir Larionov and Natalya Kouprina, has developed a new technique for swiftly isolating and cloning specific fragments of human genetic material directly into yeast cells. The resulting yeast artificial chromosomes (YACs) are engineered through a process known as transformation-associated recombination (TAR). According to Resnick, TAR cloning captures the ability of yeast to recombine pieces of DNA during transformation (the time during which DNA material is being introduced to a cell).

"This method will help us to focus on specific regions of human chromosomes in order to better understand origins of disease and to develop diagnosis of genetic diseases," says Francis S. Collins, director of the National Center for Human Genome Research, of the findings, which were published in the 9 January 1996 issue of the *Proceedings of the National Academy of Science.*

A Budding Collaboration

A mutual concern over the stability of human DNA in yeast played a part in bringing Resnick, Larionov, and Kouprina together to pursue this research. Larionov and Kouprina (both currently on leave from the Institute of Cytology in St. Petersburg, Russia) share Resnick's interest in this area, as the three discovered in 1988 when they met at a scientific meeting in Finland. Larionov, who heads the yeast genetics lab at the Institute of Cytology, has played a prominent role in yeast molecular biology in Russia, according to Resnick. A year later, Resnick invited Larionov to visit the NIEHS. They exchanged subsequent visits and a long-term transcontinental collaboration was born.



Steve McCaw, Image Associates

Yeast team. (left to right) Michael Resnick, Vladimir Larionov, and Natalya Kouprina examine some of their favorite subjects.

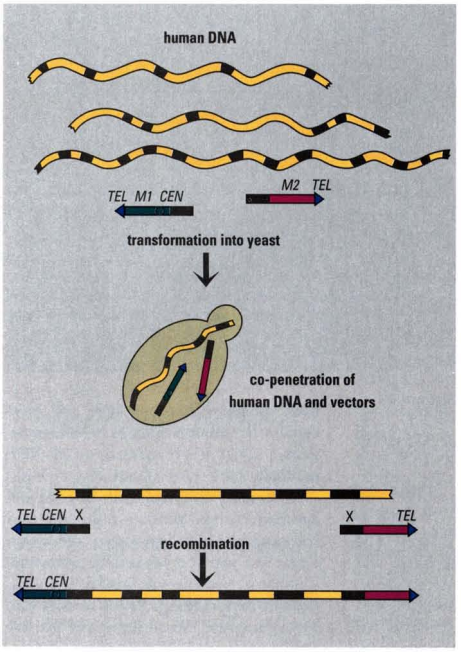
By the time the Russian-American team began its collaborative effort, human DNA's instability in yeast had begun to emerge as a serious problem in international human genetic research efforts. NIEHS researchers applied for and received funding from the Department of Energy and the National Center for Human Genome Research to study the underlying problem.

The traditional task of isolating and cloning DNA material involves randomly cutting DNA from cells into thousands and even millions of individual pieces. Each piece has to be treated so that it can be cloned into yeast or bacteria. Once cloning is complete, each fragment must be analyzed to find the desired fragment or gene. It's a random, hit-or-miss process defined mostly by endless hours of hair-splitting tedium.

With TAR cloning, however, scientists can rely on the yeast to find the precise sequence of interest. For this reason, Resnick refers to the new technique as a "smart" system. "Yeast has the ability to find the same or related molecules," explains Resnick, "and when it finds them, it also has the ability to join them to others." This uncanny ability to find and combine similar DNAs is independent of whether the DNAs originate in humans, animals, or plants. Human DNAs are introduced, or transformed, into yeast cells accompanied by a tiny piece of the gene or fragment that is ultimately desired. Only DNA that contains a region that matches that tiny piece is maintained, or cloned, as the yeast cells reproduce.

TAR Cloning

The team has been dealing primarily with large molecules, and is now exploring the maximum size that can be cloned. Currently the average size clone is approximately 250–300 kilobases (kb). Approximately 30% of human DNA contains short interspersed repeated sequences called SINEs. A specific subclass of SINEs called Alu sequences is used in TAR



Cloning chromosomes. Human DNA is isolated and transformed into yeast along with genetic markers (M1 and M2). YACs are created by recombination between Alu sequences (black blocks). Source: Larionov V et. al. Specific cloning of human DNA as yeast artificial chromosomes by transformation-associated recombination. *Proc Natl Acad Sci USA 93:1491–496 (1996).*

cloning. Alu sequences measure 300 bases in length; in the human genome, the average distance between Alu sequences is about 2–3 kb.

In the TAR cloning process, tiny plasmids of DNA, known as vectors, are transformed along with human DNA into yeast spheroplasts. At the end of each vector is a piece of DNA that corresponds to a repeat present on the human DNA and a genetic marker. According to Resnick, the end piece can be either precisely equal or simply related (i.e., diverged) by 10–20%. Once inside the cell, these vectors find their corresponding partner—either their relative or a precise match—on a chromosomal DNA fragment. At this point, a repeat in the chromosomal DNA fragment sequence joins by covalent linkage with a repeat on the vector through recombination, generating an artificial chromosome similar to the yeast cell's own chromosome.

Summing up one of the TAR method's greatest advantages, Marvin Stodolsky says, "It's quick and fast and there's no risky test-tube preparation of recombinant DNAs." Stodolsky is an administrator with the Department of Energy's Human Genome Task Group, which initially provided support for Resnick's team's work. "It provides a nice way to reclone economically," he adds, "and is thus a nice addition to the tableaux of genetic research done today."

Faster, Cleaner Cloning

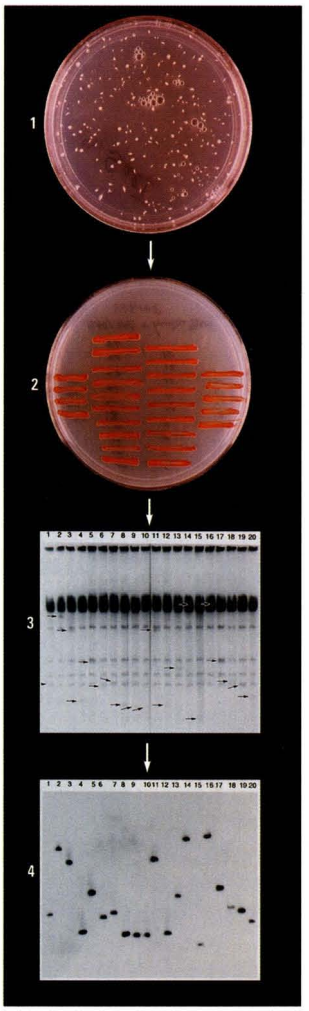
Maynard V. Olson, a professor of molecular biotechnology at the University of Washington in Seattle who has worked extensively in genome analysis, also praises the NIEHS findings. "It was unusually interesting," says Olson. "I think the results are somewhat surprising. They point the way toward a tremendous simplification in developing a clone library."

The original method for cloning genes was developed in Olson's laboratory 10 years ago. Since then, at least a thousand scientific papers have been written making use of these clones, with only three or four libraries carrying the weight of all this work, Olson says. Until now, libraries have been difficult to build, and Olson hopes this new technique will enable other scientists to develop their own libraries using their own material. "Though libraries have been widely used, it has stopped progressing," says Olson of the current cloning technique. "This is the first development in several years."

Besides saving time, the new cloning method sidesteps the land mines that typically litter the path toward traditional gene cloning. "One thing that's absolutely key is that the technique involves really a minimum handling of human DNA," says Resnick. Handling—or, more accurately, mishandling—may account for some of the chimeras (hybrid DNA molecules that arise by interactions between two or more noncontiguous DNA fragments) usually associated with cloning. With the old technique, the tricky process of managing DNA material includes extracting and isolating the DNA as gently as possible, restricting the DNA, and joining it to vector DNA, often followed by a reisolation—all before introducing the DNA into the yeast for cloning. "We think all those steps were sources of problems that could lead to instability," Resnick explains. "We know that if the DNA has lots of nicks in it, that could stimulate instability when the DNA goes into yeast."

Exploring the Gene–Disease Link

So far, the NIEHS team has isolated DNA regions from chromosomes 10, 16, and 22—regions that harbor several known disease genes. TAR cloning opens the door to



Treasure hunt. Fragments of human DNA are isolated from YACs created by TAR cloning (1 and 2) and analyzed by probing chromosomal gels (3) with a human DNA-specific probe. Dark spots (4) indicate human DNA.

cloning specific genes or other chromosome segments that are related to disease, Resnick says. So far, the promoter regions of genes that have been isolated and identified have been poorly understood. "The promoter region of the gene is what controls its expression," explains Resnick. "We think this might provide opportunities to more rapidly get many copies of promoters. We also foresee that this would be very useful in characterizing the genes that play a role in responses to genotoxins—either making us more susceptible or enabling us to deal with damage better," Resnick predicts.

Larionov, Kouprina, and Resnick are now joining others at the NIEHS, including Scientific Director J. Carl Barrett, in a quest to apply this new isolation and cloning method to further research in genetic diseases. Barrett's laboratory has played a key role in identifying breast cancer and prostate cancer suppressor genes.

In another experiment, the team successfully used the Alu repeat to clone human DNA that had been introduced into mouse cells. "Mouse cells do not have Alus," Resnick explains, "so there's no way to recombine them." This feature makes human/mouse hybrid cells useful for characterizing and isolating human genes. Once they completed the experiment, the team found a 30- to 60-fold enrichment of human DNA in the mouse cells. The amount of human DNA originally present in the mouse cells was only 2% of the total DNA. According to Resnick, chimeras formed by cloning unrelated human fragments or human and mouse fragments into a YAC confounded the scientists working on the Human Genome Project. With the TAR method, however, no chimeras were recovered. "We're going to be refining this," Resnick assures. The team has now developed a technique that enables them to get rid of excess background DNA plasmids and false positives.

The NIEHS team is also trying to determine just how efficiently they can extract DNA. "We've just done a set of experiments trying to isolate a family of genes—the ribosomal DNA family—and instead of using Alu, we're using a segment of ribosomal DNA." Of the YACs they extracted, between 20% and 50% contained ribosomal DNAs, which represents a major improvement over random methods.

Eventually the researchers hope to isolate entire single genes, a milestone they had originally hoped to accomplish within a year to a year and a half. "We now think it might be possible within a few months," Resnick says, adding, "That's being said very cautiously." Stodolsky agrees that the new cloning method may prove ideal for just that: "If you want to look at gene-sized clones, then the NIEHS process becomes very effective."

Jennifer Medlin

Suggested Reading

Ketner G et al. Efficient manipulation of the human adenovirus genome as an infectious yeast artificial chromosome clone. Proc Nat Acad Sci USA 91:6186–6190 (1994).

Larionov V et al. Specific cloning of human DNA as YACs by transformation-associated recombination. Proc Nat Acad Sci USA 93:491–496 (1996).

Burke DT et al. Cloning of large segments of DNA into yeast by means of artificial chromosome vectors. Science 226:806–812 (1987).

Nelson DL Brownstein BH, eds. YAC Libraries: A user's guide. New York:W.H. Freeman and Co., 1993.

Shizuya H et al. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. Proc Nat Acad Sci USA 89: 8794–8797 (1992).

1996 INTERSOCIETY MEETING

Integrative Biology of Exercise

A joint meeting of The American Physiological Society, The American College of Sports Medicine, and The Canadian Society for Exercise Physiology

Vancouver Convention and Trade Center
October 16-19, 1996—Vancouver, British Columbia, Canada

Wednesday, October 16, 1996

2:00–8:00 PM
Registration Opens

6:00–8:00 PM
Opening Reception *hors d'oeuvres and cash bar*

Thursday, October 17, 1996

8:00–11:00 AM
Comparative Exercise Physiology: Insights on Human Performance from Animals
Chair: **D.R. Jones**
Speakers: D.R. Jones, R.J. Full, R. Brill, W.K. Milsom, P.J. Butler, J.H. Jones, S.L. Lindstedt

8:00–11:00 AM
Central Neural Control of the Cardiorespiratory System During Exercise
Chair: **T.G. Waldrop**
Speakers: T.G. Waldrop, M.P. Kaufman, L.B. Wilson, G.A. Iwamoto, L. Adams, E. Garcia-Rill

11:00 AM–2:30 PM
Poster Sessions from Contributed Abstracts

2:30–5:30 PM
Plasticity of Muscle
Chair: **S. Kandarian**
Speakers: B. Russell, D.B. Thomason, K. Esser, J.A. Carson, R.W. Tsika

2:30–5:30 PM
Regulation of Glucose Utilization by Working Muscle
Chair: **J.L. Ivy**
Speakers: J.L. Ivy, L.J. Goodyear, A. Bonen, D.H. Wasserman, A.D. Baron

7:00–8:00 PM
Lecture
Hyperventilatory Response to Heavy Exercise: Causes and Consequences
Speaker: **J.L. Dempsey**

8:00–9:00 PM
Lecture
Magnetic Resonance Approaches in Exercise Physiology
Speaker: B. Balaban

Friday, October 18, 1996

8:00–11:00 AM
Linking Muscle Mechanics and Energetics: From Cross-Bridge to Locomotion
Chairs: **K.E. Conley, S.L. Lindstedt**
Speakers: K.E. Conley, E. Homsher, T.L. Daniel, M.J. Kushmerick, L.C. Rome, S.L. Lindstedt

8:00–11:00 AM
Cardiovascular Plasticity/Exercise
Chair: **M.H. Laughlin**
Speakers: R.L. Moore, C.A. Tate, L. Leinwand, J.M. Lash, T.H. Hintze

11:00 AM–2:30 PM
Poster Sessions from Contributed Abstracts

2:30–5:30 PM
Force Modulation in Skeletal Muscle: Molecules to Motor Units
Chairs: **B.R. MacIntosh, J.-M. Renaud**
Speakers: D.G. Allen, H.L. Sweeney, B.R. MacIntosh, J.-M. Renaud, C.J. de Luca, P.F. Gardiner

2:30–5:30 PM
Adaptations in Body Fluid Regulation to Physical Activity
Chair: **E.R. Nadel**
Speakers: S. Weinbaum, P. Watson, H. Nose, E.R. Nadel, G. Mack, P.D. Wagner

7:00–8:00 PM
Lecture
Molecular Approaches in Exercise Physiology
Speaker: **F. Booth**

8:00–9:00 PM
Graduate Student Poster Competition

Saturday, October 19, 1996

8:00–11:00 AM
Anabolic Effects of Exercise: A Systems Approach
Chair: **D. Cooper**
Speakers: D.M. Cooper, C. Roberts Jr., S. Mohan, G. Haddad, G. Attardi, F. Booth

8:00–11:00 AM
Fatigue
Chair: **R. Fitts**
Speakers: R. Enoka, J.K. Barclay, A. Wagenmaker, R. Fitts, R. Godt

11:00 AM–2:30 PM
Poster Sessions from Contributed Abstracts

2:30–5:30 PM
Vascular Remodeling: Angiogenic Growth Factors, Ischemia, and Exercise
Chair: **R. Terjung**
Speakers: O. Hudlicka, J. Abraham, E.F. Unger, J.F. Symes, R. Terjung

2:30–5:30
Muscle Use and Overuse
Chair: **B. Russell**
Speakers: R. Lieber, R. Fielding, M. Jackson, R. Armstrong, S. Kandarian

7:00–9:00 PM
Banquet and Awards Ceremony
Keynote Speaker: To be announced

Abstract Deadline:
July 1, 1996

Advance Registration Deadline:
September 4, 1996

Registrations will be taken on-site

For registration information/call for papers, send name, institution, address, and telephone to:
American Physiological Society
9650 Rockville Pike
Bethesda, MD 20814-3991
or for fastest service, Fax to: (301) 571-8313

EHP

Methylation Study of a Population Environmentally Exposed to Arsenic in Drinking Water

Claudia Hopenhayn-Rich,[1] Mary Lou Biggs,[1] Allan H. Smith,[1] David A. Kalman,[2] and Lee E. Moore[1]

[1]School of Public Health, University of California-Berkeley, Berkeley, CA 94720 USA; [2]School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195 USA

Methylation is considered the detoxification pathway for inorganic arsenic (InAs), an established human carcinogen. Urinary speciation analysis is used to assess the distribution of metabolites [monomethylarsonate (MMA), dimethylarsinate (DMA), and unmethylated arsenic (InAs)], as indicators of methylation capacity. We conducted a large biomarker study in northern Chile of a population chronically exposed to high levels of arsenic in drinking water. We report the results of the methylation study, which focused on the effects of exposure and other variables on the percent InAs, MMA, DMA, and the ratio of MMA to DMA in urine. The study consisted of 122 people in a town with arsenic water levels around 600 µg/l and 98 participants in a neighboring town with arsenic levels in water of about 15 µg/l. The corresponding mean urinary arsenic levels were 580 µg/l and 60 µg/l, of which 18.4% and 14.9% were InAs, respectively. The main differences were found for MMA:DMA; exposure, smoking, and being male were associated with higher MMA:DMA, while longer residence, Atacameño ethnicity, and being female were associated with lower MMA:DMA. Together, these variables explained about 30% of the variability in MMA:DMA. Overall, there was no evidence of a threshold for methylation capacity, even at very high exposures, and the interindividual differences were within a much wider range than those attributed to the variables investigated. The differences in percent InAs were small and within the ranges of other studies of background exposure levels. The biological significance of MMA:DMA, which was more than 1.5 times greater in the exposed group, and its relationship to sex, length of exposure, and ethnicity need further investigation because its relevance to health risk is not clear. *Key words*: arsenic, arsenic methylation, arsenic speciation, Chile, water pollution. *Environ Health Perspect* 104:620–628 (1996)

Inorganic arsenic (InAs) is an established human carcinogen. The main sources of human exposure are through inhalation of arsenic dust particles and ingestion from drinking water. Inhalation of arsenic, mainly from occupational exposures, increases the risk of lung cancer, and ingestion of arsenic causes skin cancer, in addition to other characteristic skin alterations such as keratosis and hyperpigmentation (*1*). More recent evidence indicates that ingested arsenic can also increase the risk of developing lung, bladder, kidney, and liver cancers (*2–4*). We have estimated that at the present EPA arsenic water standard of 50 µg/l, the internal cancer risks may be comparable to those of environmental tobacco smoke and radon in homes (*5*).

InAs can be ingested as either arsenite [As(III)] or arsenate [As(V)]. Although As(V) is less toxic, it is reduced biologically to As(III) before methylation, which is considered the detoxification mechanism for InAs (*6,7*). Two subsequent methylation steps take place: the first one produces monomethylarsonate (MMA), which is then further methylated to dimethylarsinate (DMA). Both MMA and DMA are considered less toxic and bind less to tissues than InAs (*6*).

It has been postulated that decrease or saturation of methylation capacity may lead to a threshold for the carcinogenicity of ingested InAs, given that methylation is considered a detoxifying mechanism for InAs (*6,8,9*). Under this assumption, as exposure increases, one would expect to see an increase in the proportion of InAs, with a corresponding decrease in MMA and DMA.

Several biological media have been used to assess arsenic exposure in humans. Because arsenic is cleared from the blood in a few hours, the arsenic level in blood is not considered a good indicator, especially for low-level exposures (*10,11*). Although it accumulates in hair and nails, surface arsenic contamination in the form of sorption from external sources also occurs, making arsenic concentrations measured in these samples less accurate for assessing dose (*12*). Urinary arsenic is generally regarded as the most reliable indicator of recent exposure to InAs and is used as the main biomarker of exposure (*11*). In the case of ingestion, studies show that around 60–75% of the dose is excreted in the urine (*6,7,13*).

Although total urinary arsenic has been used to assess InAs exposure, it is important to differentiate InAs and its metabolites from organic forms. In particular, certain types of seafood contain arsenobetaine, a much less toxic, organic form of arsenic that is quickly excreted in the urine. A recent meal of fish could lead to high measurements of total arsenic (due to arsenobetaine), resulting in an erroneously high evaluation of InAs exposure. Using methods of speciation analysis, InAs, MMA, and DMA can be separated from other arsenic compounds. Currently, the sum of these species in urine constitutes the preferred measure of exposure to InAs, and we henceforth refer to this sum as total arsenic (TotAs). The relative proportions of urinary InAs, MMA, and DMA have been used as a measure of methylation capacity (*14*).

Previously we presented the results of several studies reporting speciated urinary arsenic measures and showed that population studies do not support the methylation threshold hypothesis (*15*). The relative percentages of InAs, MMA, and DMA averaged approximately 15–20%, 10–15%, and 60–70%, respectively, across studies, and there was no systematic increase in percent InAs with increasing exposure. In contrast, a large interindividual variability was noted, independent of exposure level. However, the studies were of different populations, exposed to InAs from different sources, and urinary measurements were performed by different methods. In addition, urinary levels did not exceed 400 µg/l, so the question remained as to what happened in more highly exposed groups, such as the Taiwanese populations where high cancer risks have been described. For example, the medium exposure group in Taiwan had

Address correspondence to A. H. Smith, School of Public Health, University of California-Berkeley, 140 Warren Hall, Berkeley, CA 94720 USA.

Support for this work was provided by grants P30-ES01896 and P42-ES04705 from the National Institute of Environmental Health Sciences. Additional support was received from the Health Effects Component of the University of California Toxic Substances Program. We thank Nella Marchetti and the Instituto de Salud Pública de Chile for support and assistance in Chile; Martin Beeris, Verónica Moreno, Carmen Oyanguren, Fernando Toroco, Leda Mondaca, Mario Banchón, Brian Rich, Olivier Robert, Jill Dale, and many others who participated in the field work in Chile; and all study participants from San Pedro and Toconao. We also thank Irva Hertz-Picciotto, Michael Bates, and Mark van der Laan for helpful comments and Veronica Barroga for assisting with manuscript preparation.

Received 13 December 1995; accepted 15 March 1996.

used drinking water with a weighted average concentration of around 500 µg/l, which would result in urine levels above those published for previous occupational or environmental studies.

A more recent study in the state of Nevada identified individuals drinking well water containing arsenic levels >500 µg/l (mean = 1300 µg/l), with corresponding average urinary measurements of 750 µg/l (*16*). They were compared to an age- and sex-matched unexposed group in the same area, with mean arsenic water and urine levels of 16 µg/l and 68 µg/l, respectively. This small study (n = 18 matched pairs) did not find evidence of a methylation threshold at these high levels; percentages of InAs found for exposed and unexposed groups were similar (19.5% versus 18.6%).

Recently attention has focused on the relative distributions of MMA and DMA in the urine by exposure levels. A study in Mexico found a significant increase in the proportion of MMA relative to DMA among people drinking water with an arsenic concentration of 400 µg/l, compared to a control group at 19 µg/l. Although the proportions of InAs did not differ, the authors postulated that the second methylation step was impaired in the exposed individuals (*17*). Other investigators have proposed that the MMA:DMA ratio may be a better indicator of the saturation of the methylation pathway than the percent of InAs (*18*).

The purpose of the study reported here was to investigate arsenic methylation patterns as reflected in urinary metabolites among subjects chronically exposed to high levels of arsenic from drinking water in order to analyze the contribution of exposure, as well as other factors, to methylation capacity. The study was part of a large biomarker investigation conducted in two Chilean towns with different arsenic levels in their water supplies. This paper focuses on a cross-sectional comparison of the relative proportions of urinary arsenic metabolites (InAs, MMA, DMA, and MMA:DMA) with respect to arsenic exposure, as well as with respect to other variables such as sex, smoking, and ethnicity.

Methods

Study population. Study subjects were residents of two towns located in the high Atacama Desert of northern Chile (Fig. 1), and they were mainly of Atacameño ethnic origin. The towns were selected based on their levels of arsenic in drinking water, identified through the results of a previous study (*19*) and confirmed by subsequent analysis before and during biological sample collection. The water sources derive from rivers originating in the Andes Mountains and the arsenic content varies depending on the natural geological composition of the rivers' courses (*20*). San Pedro, with 1600 inhabitants, constituted the high-exposure town. San Pedro has two sources of water supply. Water from the Vilama River is piped to most homes and contains 600–680 µg As/l. The San Pedro River contains about 170 µg As/l and passes through some parts of town as a canal to irrigate nearby farms. Residents without public water supplies use this water for drinking purposes as well. Toconao, with a population of about 360, was selected as the low-exposure, control town, and is located about 40 km from San Pedro. The main source of water in Toconao is the Jerez River, which serves most houses and contains approximately 15 µg As/l.

We recruited study subjects through public announcements and meetings and by door-to-door invitations to participate. Each prospective participant was first interviewed by a local recruiter to ascertain age, smoking status, duration of residence, drinking water source, and interest in participation. They were asked to participate if they were at least 18 years of age and had lived in the town for the last 3 months. As the study progressed, participants from San Pedro and Toconao were selected to be frequency-matched on sex, age, and smoking status. In addition, an effort was made in San Pedro to enroll participants who reported drinking tap water because they were the most highly exposed.

The initial enrollment into the study consisted of 124 participants in San Pedro and 108 in Toconao. Each study subject was interviewed regarding general demographic characteristics, tobacco smoking, and alcohol drinking habits, dietary information including fluid intake, and medical, occupational, and residential histories. It was not possible for interviewers to be blinded with respect to town of residence or to arsenic exposure because interviews were done in each town and the contrasting arsenic levels were widely known. However, because biological samples were identified only by code, urinary arsenic speciation analyses were blinded.

Laboratory analysis. First morning urine samples were obtained by providing participants with precoded, sterilized propylene bottles and written and verbal instructions on how to obtain a clean sample. Samples were collected from participants the next morning and kept frozen in the field laboratory at -20°C until they were transported in dry ice to the University of Washington in Seattle, where they were analyzed for arsenic content.

Urine samples were assayed by hydride generation atomic absorption (HGAA) spectroscopy following a method detailed by Crecelius (*21*). Briefly, InAs [As(III) and As(V)], MMA, and DMA are reduced to the corresponding arsine in a batch reactor using sodium borohydride. The volatile reduction products (arsine, methyl arsine, and dimethyl arsine, respectively) are removed by sparging with helium. Entrained arsines are concentrated in a chromosorb-filled cryogenic trap at liquid nitrogen temperatures until all arsine-forming arsenic in the sample has reacted. The cryotrap is then allowed to warm, and the collected arsines are separated based on differential volatilization. The detection of the separated volatile arsenic species is accomplished by atomic absorption spectroscopy using a hydrogen microburner combustion cell to convert arsines to elemental arsenic.

Detection limits for InAs, MMA, and DMA were 0.5 µg/l, 1.0 µg/l, and 2.0 µg/l, respectively, with corresponding replicate precisions of 3%, 3%, and 10%. Creatinine meaurements were also performed to allow expression of TotAs in relation to urine concentration.

Nine samples had an unusually low proportion of methylated species. Replicates of these samples were tested by spiking in the laboratory with a mixture of known amounts of InAs, MMA, and DMA, which uncovered unusual and strikingly low recoveries of DMA and, to a lesser degree, MMA. It was concluded that for those samples the methylation assay suffered from interference by an as-yet unidentified substance. An alternative method was derived for these anomalous samples, which resulted in more complete recovery of the methylated species, although slightly lower recovery for InAs. Due to differences in methodology and in recovery rates, we chose to omit these samples from the methylation study. We also omitted one other sample from Toconao, an outlier with an unusually

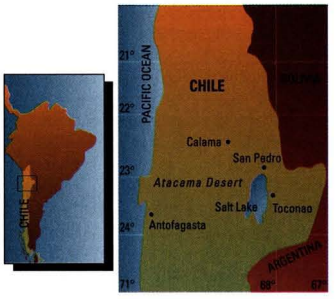


Figure 1. Map of the study area [adapted from Sancha et al. (*53*)].

high MMA:DMA ratio of 2.4 (several standard deviations away from the mean (0.15) for that town, possibly due to the fact that the TotAs was very low (4.4 µg/l) and species were close to their detection limits.

The recovery rates of urinary arsenic species were calculated by running control samples periodically with urine samples. Because the weighted average recovery rates (over 3 batches of 25, 120, and 19 control samples each) were so close to 100% (98.4%, 100.3%, and 100.1% for InAs, MMA, and DMA, respectively), we did not adjust the assay values of each metabolite.

To rule out any systematic differences between the study subjects whose urine samples were excluded and those that were included, the general characteristics of the two groups were compared. All but one of the excluded subjects were from the low-exposure town, and seven were women. Otherwise, the 10 excluded subjects were comparable in age, ethnicity, years of residence, years of education, smoking, and drinking of alcoholic beverages.

During the study, samples were taken from the various water supplies: tap water from San Pedro and Toconao and water from the San Pedro River, from which some participants drank as well. Water analyses were performed by HGAA according to a procedure similar to that used for urine samples and by a flow-injection analysis method for combined arsine-forming arsenic species (*22*).

Statistical analysis. We first compared general characteristics of study participants by town, including age, sex, ethnicity, education, length of residence, smoking, and drinking of alcoholic beverages. For the distribution analyses of urinary arsenic metabolites, TotAs (InAs + MMA + DMA), the percentages of InAs, MMA, and DMA, and the ratio MMA:DMA were first calculated by town. We also examined metabolite pattern distribution by reported water source: tap, canal, other, or mixed (e.g., tap water at home, canal water when working near San Pedro canal). The mean and median of each group were derived by first calculating the individual percentage or ratio and then averaging over each category.

TotAs was used as the measure of exposure and was expressed both as a direct measure (µg As/l urine) and as a creatinine-adjusted measure (µg As/g urinary creatinine). The mean urinary arsenic levels were slightly higher when expressed as a function of the urinary concentration of creatinine, a commonly used method to correct for differences in urinary concentrations. However, creatinine excretion can vary considerably by age, sex, lean body mass, diet, and other factors and is not considered a reliable indicator of urinary dilution, especially for spot samples (*23*). In some cases creatinine values are used to identify possible outliers (e.g., very dilute or very concentrated urine samples). We examined the distribution of creatinine in our study and found the mean concentration to be 998 mg/g, with 20 of the 220 participants' urine samples either <300 mg/g or >2000 mg/g (approximate values of the lower and upper 5%). We compared the distribution of metabolites including and excluding these samples and found no essential difference. For the regression analyses described below, we compared the results using the unadjusted and adjusted arsenic urine concentrations (µg/l and µg/g creatinine) and found them to be similar. In light of the problems associated with creatinine measurements and the similarities in the results using adjusted or unadjusted values, we decided to use the unadjusted urine measurements expressed as µg As/l urine.

We investigated various factors by univariate comparisons in relation to their possible association with metabolite distribution. Those considered relevant were then entered into multiple regression analyses to assess the contribution of each factor to the dependent variables: percent InAs, percent MMA, percent DMA, and MMA:DMA. Indicator (dummy) variables were used for dichotomous variables such as sex and town of residence. Statistical analyses were computed using Stata 4.0 software (*24*).

To examine the relationship of ethnicity to arsenic metabolite distribution patterns, we performed a separate analysis on a subsample of the study population restricted to participants of Atacameño and European descent. These two groups constituted most of the study subjects (80%) and excluded those reporting other (specified or unspecified) or unknown ethnicity.

Results

The final study sample consisted of 122 study subjects in San Pedro and 98 in Toconao; general characteristics of the subjects are shown in Table 1. Two subjects, one from each town, were excluded because of incomplete participation (no first morning urine sample). Ten were excluded from the final analysis because of irregularities in the urinary speciation assay as explained above (one from San Pedro, nine from Toconao).

Participants from both towns were generally quite similar, with comparable distributions by sex, age, and educational level (Table 1). The percentages of persons who smoked or drank alcohol were also quite similar. It was common for people to report smoking or drinking occasionally, so we also compared smokers who smoked at least one cigarette a day to nonsmokers and drinkers who consumed at least one drink a week to nondrinkers [one drink was defined as 12 oz. of beer, 4 oz. of wine, 1 oz. of liquor, or 8 oz. of *aloja* (*aloja* is an alcoholic beverage made from fermented pods of the Algarrobo tree)]. Although the overall proportion of smokers was similar in both towns (28% in San Pedro, 31% in Toconao), both men and women in San Pedro smoked almost 3 times as many cigarettes as those in Toconao (7.2 versus 2.6 cigarettes per day for men, 5.7 versus 2.0 for women). Similarly, the proportion reporting consumption of alcoholic beverages was almost identical in the two towns (57% and 56%); however, men drank about four times more drinks per week than women, and men in San Pedro drank about 75% more than those in Toconao.

The ethnic composition and length of residence varied somewhat between the two towns. Although the proportions of

Table 1. General characteristics of study subjects by town

Characteristic	San Pedro		Toconao	
No. of subjects (%)				
Total	122		98	
Male	69	(57)	52	(53)
Female	53	(43)	46	(47)
Mean age, years (range)				
Male	42.2	(20–74)	42.4	(19–75)
Female	40.1	(18–81)	40.1	(22–70)
Total	41.2	(18–81)	41.3	(19–75)
Mean residence, years (range)	20.0	(0.2–81)	27.9	(0.2–70)
<5	13%		12%	
≥5 to <15	29%		23%	
≥15	58%		65%	
Mean education, years (range)	7.2	(0–19)	7.7	(0–17)
Ethnicity (%)				
Atacameños	91	(75)	71	(72)
Aymara/Mapuche	5	(4)	4	(4)
European	13	(11)	3	(3)
Other	8	(6)	12	(12)
Unknown	5	(4)	8	(8)
Smokers (%)				
All	34	(28)	30	(31)
Male	22	(32)	22	(43)
Female	12	(23)	8	(17)
Smokers of ≥1 cigarette/day (mean cigarettes/day)				
Male	15	(7.2)	13	(2.6)
Female	9	(5.7)	3	(2.0)
Drink any alcohol (%)				
All	70	(57)	55	(56)
Male	50	(72)	43	(83)
Female	20	(38)	12	(26)
Drinkers of ≥1 drink/week (mean drinks/week)				
Male	47	(15.8)	33	(8.8)
Female	13	(2.8)	1	(2.0)

Atacameños and other indigenous groups (Aymara and Mapuche) were quite similar, in San Pedro 11% reported being of European descent, whereas in Toconao only 3% did. Similarly, 10% of San Pedro residents classified themselves as "other or unknown," compared to 20% in Toconao. The average length of residence was shorter in San Pedro (20 versus 28 years) but was quite long in both, with similar ranges.

The results of arsenic water analyses conducted during the study period for the three main water sources were within the ranges reported previously (*19,20*). The arsenic concentrations in San Pedro from tap water (from the Vilama River) and from canal water (San Pedro River) were 670 µg/l and 134 µg/l, respectively, while that from the tap water in Toconao was 13 µg/l. The arsenic of the different water sources was all inorganic, but the analytical method we used did not distinguish As(V) from As(III). However, previous analysis of water samples from the same sources found that practically all of it was As(V) (*19*).

TotAs and the metabolite distributions by town of residence are shown in Table 2. In San Pedro the mean TotAs was about 10 times that of Toconao (583 µg/l and 61 µg/l, respectively). Four people reported drinking only San Pedro canal water (mean TotAs = 238 µg/l), and 18 reported drinking from mixed sources, although their TotAs levels were similar to those using only tap water (545 µg/l).

The average TotAs concentration of people drinking water from Toconao was somewhat higher than the concentration found in the water, presumably due to the contribution from other sources (e.g., food

Table 2. Levels and relative distributions of urinary arsenic species, by town

	San Pedro (n = 122)				Toconao (n = 98)			
Measure	Mean	SD	Range	Median	Mean	SD	Range	Median
Total As (µg/l)	583	347	61–1,893	482	61	41	6–267	49
Adjusted total (µg As/g creatinine)	637	352	45–2,145	592	68	41	21–306	58
InAs (µg/l)	108	76	5–374	91	8.7	7.2	1.2–41.0	6.6
MMA (µg/l)	91	66	4–329	73	6.1	4.3	0.9–23.0	4.8
DMA (µg/l)	385	234	43–1,225	320	45.8	33.5	3.8–246	39.1
%InAs	18.4	6.1	5.6–38.8	18.2	14.9	5.7	3.6–31.4	14.2
%MMA	15.0	5.5	1.7–30.6	14.8	10.6	4.2	3.1–23.9	9.7
%DMA	66.6	9.9	42.0–92.6	67.1	74.5	8.6	51.2–92.4	75.8
MMA:DMA	0.24	0.12	0.019–0.68	0.23	0.15	0.08	0.034–0.47	0.13

Abbreviations: InAs, inorganic arsenic; MMA, monomethylarsonate; DMA, dimethylarsinate.

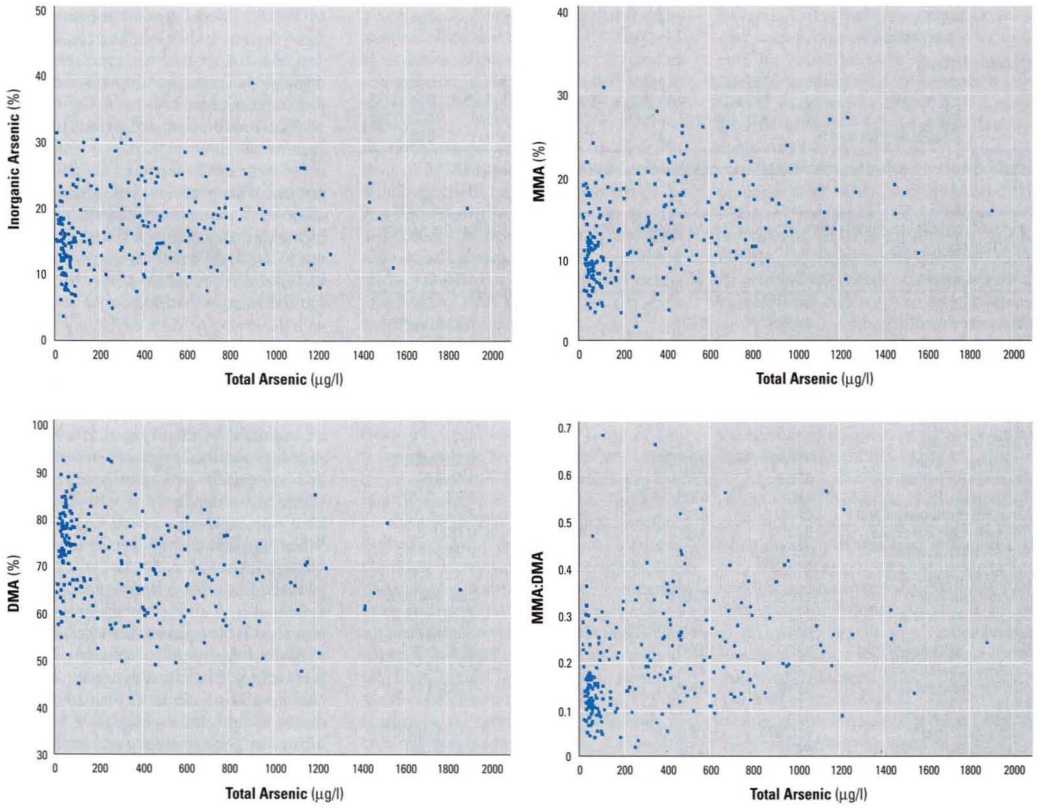


Figure 2. Distribution of percent inorganic arsenic, percent monomethylarsonate (MMA), percent dimethylarsinate (DMA), and MMA:DMA by total urinary arsenic levels for all the study participants from both towns.

Table 3. Urinary arsenic metabolite distribution by various factors

Factor		n	Mean (SD) Total (µg/l)	%InAs	%MMA	%DMA	MMA:DMA
Town	San Pedro	122	583 (347)	18.4 (6.1)	15.0 (5.5)	66.6 (9.9)	0.24 (0.12)
	Toconao	98	61 (41)	14.9 (5.7)	10.6 (4.2)	74.5 (8.6)	0.15 (0.08)
	p-value[a]			<0.001	<0.001	<0.001	<0.001
Sex							
	Male	121	381 (397)	17.1 (6.0)	14.4 (5.9)	68.5 (10.5)	0.23 (0.13)
	Female	99	314 (326)	16.4 (6.3)	11.5 (4.3)	72.0 (9.4)	0.17 (0.09)
	p-value[a]			0.43	<0.001	<0.001	<0.001
Ethnicity							
	Atacameño	162	371 (390)	16.8 (6.1)	12.6 (4.8)	70.6 (9.4)	0.19 (0.10)
	European	16	325 (210)	18.3 (5.6)	17.2 (6.9)	64.5 (11.0)	0.30 (0.16)
	p-value[a]			0.34	<0.001	0.015	<0.001
Smoking							
	No	156	354 (379)	16.3 (6.2)	12.4 (5.1)	71.3 (9.9)	0.18 (0.10)
	≥1/day	41	392 (370)	18.8 (5.4)	15.9 (5.9)	65.2 (10.1)	0.26 (0.14)
	p-value[a]			0.018	<0.001	<0.001	<0.001
Alcohol							
	No	95	341 (379)	15.4 (5.5)	12.5 (4.8)	72.0 (9.2)	0.18 (0.09)
	≥1/week	94	394 (364)	17.8 (6.2)	14.1 (6.0)	68.2 (10.4)	0.22 (0.13)
	p-value[a]			0.006	0.048	0.007	0.018
Length of residence (years)							
	<5	28	307 (306)	18.6 (5.9)	15.6 (5.9)	65.8 (9.7)	0.25 (0.13)
	≥5 to <15	57	411 (428)	17.6 (5.8)	14.3 (5.6)	68.1 (9.3)	0.22 (0.12)
	≥15	135	340 (370)	16.1 (6.3)	12.1 (5.0)	71.8 (10.2)	0.18 (0.10)
	p-value (test for trend)			0.01	<0.01	<0.01	<0.01
Age groups (years)							
	<30	56	355 (365)	18.0 (5.1)	14.4 (5.2)	67.6 (8.9)	0.23 (0.12)
	≥30 to <50	96	381 (401)	17.1 (6.9)	12.9 (5.5)	70.1 (10.7)	0.20 (0.12)
	≥50	68	303 (318)	15.4 (5.6)	12.3 (5.4)	72.2 (9.9)	0.18 (0.11)
	p-value (test for trend)			0.01	0.03	0.01	0.02

Abbreviations: InAs, inorganic arsenic; MMA, monomethylarsonate; DMA, dimethylarsinate.
[a]*t*-test.

Table 4. Multiple linear regression results using %InAs, %MMA, %DMA, and MMA:DMA as the dependent variables ($n = 217$)

Predictor variables	Coefficient	SE	*p*-value	95% CI
% InAs ($R^2 = 0.10$)				
Total arsenic (µg/l)	0.0029	0.0011	0.011	0.00067–0.0051
Sex (male vs. female)	0.61	0.85	0.474	-1.06–2.28
Age (years)	-0.45	0.032	0.169	-0.11–0.019
Length of residence (years)	-0.051	0.027	0.061	-0.10– -0.023
Smoking (cigarettes/day)	0.10	0.134	0.448	-0.16–0.37
Alcohol (drinks/week)	-0.017	0.030	0.56	-0.075–0.041
% MMA ($R^2 = 0.30$)				
Total arsenic (µg/l)	0.0039	0.00086	<0.001	0.022–0.0056
Sex (male vs. female)	2.76	0.66	<0.001	1.47–4.06
Age (years)	0.0042	0.025	0.867	-0.045–0.053
Length of residence (years)	-0.072	0.021	0.001	-0.11– -0.31
Smoking (cigarettes/day)	0.33	0.10	0.002	0.13–0.54
Alcohol (drinks/week)	-0.039	0.022	0.089	-0.084–0.0060
% DMA ($R^2 = 0.23$)				
Total arsenic (µg/l)	-0.0068	0.0017	<0.001	-0.010– -0.0034
Sex (male vs. female)	-3.38	1.30	0.010	-5.93– -0.82
Age (years)	0.040	0.049	0.415	-0.057–0.14
Length of residence (years)	0.12	0.041	0.003	0.041–0.20
Smoking (cigarettes/day)	-0.43	0.21	0.036	-0.84– -0.029
Alcohol (drinks/week)	0.056	0.045	0.219	-0.033–0.14
MMA:DMA ($R^2 = 0.28$)				
Total arsenic (µg/l)	0.000079	1.9 ($\times 10^{-5}$)	<0.001	4.1–11.3 ($\times 10^{-5}$)
Sex (male vs. female)	0.053	0.014	<0.001	0.024–0.081
Age (years)	4.91 ($\times 10^{-7}$)	0.00054	0.999	-0.0011–0.0011
Length of residence (years)	-0.0014	0.00045	<0.002	-0.0023– -0.00050
Smoking (cigarettes/day)	0.0081	0.0023	<0.001	0.0037–0.013
Alcohol (drinks/day)	0.00060	0.00050	0.226	-0.0016–0.00038

Abbreviations: InAs, inorganic arsenic; MMA, monomethylarsonate; DMA, dimethylarsinate.

and recent consumption of water in another town). Given the much lower arsenic levels in Toconao water, these factors would contribute a greater proportion of the total than in San Pedro, where water was the overriding arsenic source.

The proportions of InAs and MMA were slightly higher in San Pedro than Toconao (18% versus 15% and 15% versus 11%, respectively), with a corresponding inverse relationship for DMA (67% versus 75%). This resulted in a greater MMA:DMA ratio (0.24 versus 0.15). The association between the distribution of metabolites and TotAs levels across the entire study population is illustrated graphically in Figure 2. No clear, consistent pattern of increase in percent InAs is evident; there is a wide range of interindividual variation. The very slight positive trend for percent MMA and the negative relationship with percent DMA can be synthesized in the ratio of MMA:DMA.

The distribution of arsenic metabolites by factors other than exposure is presented in Table 3. We compared nonsmokers to those that smoked at least one cigarette per day, and for alcohol we compared nondrinkers to those that drank at least one drink (as defined above) per week.

The proportions of metabolites were significantly different (although the differences were small) for most variables presented. The greatest differences were observed in the ratio of MMA:DMA, with higher ratios for men (0.23) and for smokers (0.26), and lower ratios for women (0.17) and Atacameños (0.19). Increasing age and length of residence were associated with decreasing MMA:DMA, and a smaller but positive association was found for alcohol consumption.

Given the effects of these variables on the distribution of arsenic metabolites, they were all included in the regression analyses. Although age and length of residence were both associated with metabolite distribution (Table 3), as expected, they were also correlated with each other ($r = 0.5$, $p<0.0001$). When they were entered together in a regression model including TotAs, age was not a good predictor, but length of residence was associated with all the metabolite outcome measures. Table 4 shows the models for each of the four dependent variables assessed. For percent InAs, TotAs was significant, but the coefficient was quite small (0.0029%), predicting about a 1% increase for a 500 µg/l increase in TotAs. Smoking and gender were not statistically significant. Length of residence was associated with a decrease in percent InAs, although the magnitude of the coefficient was small (-0.05% per year).

With respect to the methylated metabo-

lites, TotAs, smoking, sex, and length of residence were all contributing factors, whereas alcohol and age showed no clear association. By focusing on the MMA:DMA ratio, the linear regression analysis predicted that a 500 µg/l increase in TotAs would result in a 0.04 change in the ratio, comparable but somewhat lower than the difference found between Toconao and San Pedro (0.15 versus 0.24; Table 2). However, when the regression analysis was restricted to TotAs levels <500 µg/l, the predicted change rose to 0.13 for a 500 µg/l increase, but the coefficient was small and not significant for the model including TotAs >500 µg/l (these models not shown). This suggests that there is an increasing trend in MMA:DMA with increasing exposure up to around 500 µg/l TotAs, which then levels off and is unaffected by further increases in TotAs.

Smoking 10 cigarettes a day had an effect on MMA:DMA (0.08 decrease), as did being male (0.05 decrease). For length of residence, the analysis predicted that a negative change in MMA:DMA of 0.07 would be expected for 50 years of residence (50 × -0.0014).

When location rather than TotAs was used as an exposure indicator (Table 5), the other variables remained quite similar, but location contributed to a 0.081 change in MMA:DMA (similar to the difference of 0.09 in the comparison of towns in Table 3). When the analysis was restricted to the 176 individuals of European or Atacameño descent, ethnicity (being Atacameño) proved to be the strongest predictor of the model, with a coefficient of 0.076, but there was little change in the rest of the variables.

Table 5. Multiple linear regression: additional models

Predictor variables	Coefficient	SE	*p*-value	95% CI
MMA:DMA, using location (San Pedro vs. Toconao) as exposure measure ($n = 217$; $R^2 = 0.33$)				
Location	0.081	0.014	<0.001	0.054 – 0.11
Sex (male vs. female)	0.060	0.014	<0.001	0.033 – 0.087
Age (years)	-0.00040	0.00053	0.455	-0.0014 – 0.00065
Length of residence (years)	-0.0010	0.00044	0.026	-0.0019 – -0.00012
Smoking (cigarettes/day)	0.0074	0.0022	0.001	0.0030 – 0.012
Alcohol (drinks/week)	-0.00087	0.00048	0.071	-0.0018 – 0.000077
MMA:DMA, restricting analysis to subjects of Atacameño and European ethnic origin ($n = 176$; $R^2 = 0.31$)				
Total arsenic (µg/l)	0.000068	0.000019	<0.001	0.000031 – 0.00010
Sex (male vs. female)	0.044	0.015	0.003	0.016 – 0.073
Age (years)	-0.000081	0.00058	0.891	-0.0012 – 0.0011
Length of residence (years)	-0.0014	0.00046	0.003	-0.0023 – -0.00050
Smoking (cigarettes/day)	0.0048	0.0024	0.049	0.000020 – 0.0094
Alcohol (drinks/week)	-0.00048	0.00046	0.297	-0.0014 – 0.00043
Ethnicity (European vs. Atacameño)	0.076	0.025	0.003	0.027 – 0.12

Abbreviations: InAs, inorganic arsenic; MMA, monomethylarsonate; DMA, dimethylarsinate.

Discussion

In this study, the mean percentages of urinary InAs were within the ranges described for two other populations chronically exposed to high levels of arsenic in drinking water, one in the state of Nevada, USA (*16*), and one in the Region Lagunera, Mexico (*17*). Percentages of urinary InAs were also within the range of a number of studies including arsenic exposures of background, occupational, environmental, and experimental groups (*15*).

Although the mean percent InAs was slightly higher in San Pedro than in Toconao, the 3.5% difference was quite small given the difference in mean TotAs of about 500 µg/l between the two towns, and its biological significance is unclear. In the regression analysis the percent InAs was associated with TotAs, but the small magnitude of the coefficient (1.4% increase in percent InAs for a 500 µg/l increase in TotAs) suggested only a weak effect, and lessened after adjustment for other factors. In contrast, the much larger interindividual variability of about 20% for any given exposure level suggests that factors other than arsenic exposure have an overall greater role in determining the distribution of metabolites. No evidence of a threshold or saturation phenomenon was observed in this population, which included several individuals having TotAs levels well over 1000 µg/l.

With respect to the relative distributions of MMA and DMA, the differences were larger, with an MMA:DMA ratio in San Pedro more than 1.5 times that found in Toconao (0.24 versus 0.15). This is consistent with the results of the only other study, that, to our knowledge, has examined MMA:DMA ratios in similarly contrasting exposure groups (*17*).

After controlling for exposure, other factors were associated with a higher MMA:DMA ratio, including smoking. The effect of smoking may be related to competition between arsenic and some of the many chemicals found in cigarette smoke for common detoxification pathways or factors. For example, glutathione (GSH) is involved in several steps of InAs metabolism (*25,26*) and is likely to play a part in the detoxification of several polycyclic aromatic hydrocarbon epoxides generated from cigarette smoke (*27*). Experimental studies have shown an elevation in GSH in response to arsenic insult (*28*), and levels of GSH are higher in the bladder tissue of smokers compared to nonsmokers (*29*). In both cases, the increase in GSH is likely to be a response caused by increased exposure to toxins. On the other hand, pretreatment with GSH depletors impairs arsenic methylation in animal tissue (*30–32*). While a higher MMA:DMA ratio suggests modulation of the second methylation step, and the animal studies have suggested that GSH depletion mainly affects the first methylation reaction, these inconsistencies may be due to differences in arsenic methylation that exist between animals and humans. Alternatively, the detoxification of arsenic and tobacco carcinogens may compete for other necessary substrates; for example, binding sites in nonspecific proteins as described for arsenic (*33*), and/or enzyme systems such as glutathione or methyl transferases.

The sex-related difference in the relative proportions of MMA and DMA was also reported in the recent study in Mexico (*34*), but there is no clear explanation for this finding. Although women appear to be better arsenic methylators than men, the relevance of this difference to arsenic-related health effects is unknown. Another example of a gender difference in the handling of arsenic comes from an *in vitro* study in the arsenical area of Mexico that found that impaired proliferation was greater in lymphocytes from arsenic-exposed women than from exposed men (*35*).

Length of residence showed a positive effect on the distribution of all the metabolites, but age did not affect distribution when duration of residence was controlled for. This suggests that an adaptation response may provide a slight improvement in methylation ability.

Ethnicity was a predictive factor: Atacameños had a lower MMA:DMA ratio compared to subjects of European descent. The level of exposure in San Pedro is similar to that of the medium exposure group in Taiwan, where overt signs of arsenicism such as keratosis, hyperpigmentation, and skin cancer were apparent. A previous study found that residents of San Pedro had an increased prevalence of white spots on their skin compared to other towns in the area

with lower water arsenic levels, but more serious skin alterations were not observed (*19*). The Atacameño people have lived in the region for 11,000 years (*36*), and studies of mummies buried in the area up to 3000 years ago have revealed high arsenic concentrations in their internal organs (*37*). All other known populations exposed to high arsenic drinking water have been exposed for much shorter time periods: the Taiwanese for about 70 years (*4*), the Chilean population of Antofagasta (mainly of Spanish descent) for about 15 years (*38*), the Argentine population for less than 150 years (*39*), and the more recently described residents of West Bengal in India for about 30 years (*40*). All but the Atacameños have shown characteristic keratosis and skin cancer. The question remains whether some characteristic of the Atacameño skin renders it inherently more resistant to these alterations, or whether some other evolutionary adaptation over thousands of years has made Atacameños less susceptible to arsenic, and if so, whether this protective mechanism is related to methylation capacity.

A possible hypothesis that could explain such an adaptation relates to Chagas disease, endemic in many parts of South America including the Atacama Desert. The infection is transmitted by the *vinchuca* bug, a vector of the parasite *Trypanosoma cruzi* (*41*), of the same family as the African trypanosome that causes sleeping sickness, which was traditionally treated with arsenical drugs. Arsenic inhibits trypanothionine disulfide reductase, a GSH equivalent in African trypanosomes necessary for their survival (*42*). If the South American trypanosome has a similar enzymatic system, it is plausible that over time the Atacameños developed a resistance to arsenic, as it protected against the consequences of Chagas infection [which includes reproductive effects such as congenital abnormalities, abortion, and stillbirths (*41*)], leading to a selective survival of those more capable of tolerating the toxic effects of arsenic.

Most of the findings in this study are related to variations in the MMA:DMA ratio, and the significance of these differences by factors such as arsenic exposure, sex, ethnicity, and smoking is not clear and needs further investigation. Experimental studies of rat liver cytosol indicate that for high exposures, InAs inhibits the second methylation step, leading to an accumulation of MMA (*32*), which could partly account for the MMA:DMA differences by exposure levels. One possible explanation is the binding of InAs to a dimethyltransferase involved in the second methylation step (*26*).

Given the known relative genotoxicities of InAs, MMA, and DMA (roughly 3000:2:1) (*43*) and the small change in percent InAs across exposure levels, it is unclear how an increased concentration of MMA relative to DMA would contribute significantly to an increased risk of arsenic-induced health effects. Although it has been suggested that during methylation a highly reactive intermediate form of MMA may be formed (*26*), the existence of this hypothetical chemical has not been demonstrated. Laboratory studies *in vitro* (*43,44*) and in animals (*45*) show that although MMA appears to be twice as potent as DMA in genotoxicity and cytotoxicity tests, they are both weak and far less toxic than the inorganic forms. On the other hand, it has also been postulated that an increase in DMA may be more deleterious than previously thought. *In vitro* studies found that DMA can cause chromosomal damage (*46,47*); similar studies were not reported for MMA. A recent report of the study in Mexico indicated that exposed individuals with skin alterations had a higher MMA:DMA ratio than exposed persons without skin effects (*34*). However, the exposure magnitude of the two groups was not reported, and the possibility that overall higher exposures to InAs or longer duration of exposure could account for the signs of arsenicism cannot be excluded.

A new hypothesis proposes that competition for methyl groups between arsenic metabolism and DNA methylation could lead to DNA hypomethylation, which has been associated with the changes in gene transcription found in epithelial cancers (*48*). For this competition to occur, there would have to be a limited availability of methyl groups. Animal studies show that the bioavailability of *S*-adenosylmethionine, the source of methyl groups for arsenic methylation, is not a limiting factor for methylation under normal *in vivo* conditions (*31*), but it has been suggested that high arsenic exposure may cause the demand for methyl groups to exceed the supply, particularly for individuals with a diet poor in methionine (*18*). However, it was estimated that exposures to an arsenic concentration of 1800 µg/l in drinking water would require only 1.5% of a person's daily dietary intake of methyl source for arsenic methylation (*49*), making it unlikely that the supply of methyl groups would be exceeded. Nevertheless, there may be a limited supply of methyltransferases or other chemicals involved in both arsenic and DNA methylation.

The large interindividual variability in methylation ability, as well as the possible ethnic differences, may be due to genetic polymorphisms associated with methylating enzyme activity. Inheritance is a major factor in individual variation in several methyltransferases involved in the biotransformation of many drugs (*50*), and the susceptibility to some occupational exposures appears to be associated with differences in detoxification enzymes such as *N*-acetyltransferase and aryl hydrocarbon hydroxylase (*51*). Genetic polymorphisms of the still-uncharacterized arsenic methylation enzymes may help explain the interindividual variation. Similar genetic differences may exist in arsenic-specific binding proteins, which are thought to decrease the toxicity of InAs by decreasing its tissue availability until it can be methylated (*33*).

Alternatively, because GSH is involved in arsenic metabolism, genotypic differences in the activity of glutathione transferases (GSTs), which catalyze the conjugation of GSH to a variety of carcinogens and are part of the protective mechanism against cancer caused by environmental carcinogens (*52*), may affect methylation ability. For example, the null genotype for one of these enzymes, GSTM1, varies from 30% to 70% depending on ethnicity (*27*).

In conclusion, the factors investigated did not contribute to biologically meaningful differences in the percentage of InAs in urine. The large biomarker study presented here in a chronically highly exposed population indicates that, at high levels of arsenic exposure, there is no evidence of a plateau saturation effect and that variations in methylation capacity, at least as reflected by urinary metabolites, can be quite large. Arsenic exposure level and duration, sex, smoking, and ethnicity were associated with differences in the MMA:DMA ratio, together explaining about 30% of the total variation. It is possible that several genetic polymorphisms operating in the mechanisms of arsenic detoxification play a role in determining methylation capacity, along with other exogenous factors such as diet and other concurrent exposures. The significance of the differences in MMA:DMA ratio in relation to arsenic exposure and to sex and smoking warrants further study, since at present there is no clear evidence of their relevance to health risks. In general, given the current gaps in understanding the mechanisms of arsenic detoxification, several lines of research need to be continued and pursued to confirm or reject some of the more recently proposed hypotheses.

References

1. IARC. Arsenic and arsenic compounds. In: IARC monographs on the evaluation of the carcinogenic risk of chemicals to man, vol 23. Some metals and metallic compounds. Lyon: International Agency for Research on Cancer, 1980;39–141.

2. Bates MN, Smith AH, Hopenhayn-Rich C. Arsenic ingestion and internal cancers: a review. Am J Epidemiol 135:462–476 (1992).
3. Wu M-M, Kuo T-L, Hwang Y-H, Chen C-J. Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. Am J Epidemiol 130:1123–1132 (1989).
4. Chen C-J, Chen CW, Wu M-M, Kuo T-L. Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. Br J Cancer 66:888–892 (1992).
5. Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan H, Wood R, Kosnett MJ, Smith MT. Cancer risks from arsenic in drinking water. Environ Health Perspect 97:259–267 (1992).
6. U.S. EPA. Special report on ingested inorganic arsenic: skin cancer; nutritional essentiality. EPA 625/3-87/013. Washington, DC:U.S. Environmental Protection Agency, 1988.
7. Vahter M. Species differences in the metabolism of arsenic compounds. Appl Organ Chem 8:175–182 (1994).
8. Marcus WL, Rispin AS. Threshold carcinogenicity using arsenic as an example. In: Advances in modern environmental toxicology: risk assessment and risk management of industrial and environmental chemicals (Cothern CR, Mehlman MA, Marcus WL, eds). Princeton, NJ:Princeton Scientific Publishing Company, 1988;133–158.
9. Carlson-Lynch H, Beck BD, Boardman PD. Arsenic risk assessment. Environ Health Perspect 102:354–356 (1994).
10. Vahter M. Metabolism of arsenic. In: Biological and environmental effects of arsenic (Fowler BA, ed). Amsterdam:Elsevier, 1983;171–198.
11. ATSDR. Toxicological profile for arsenic, update. Report no. TP-92/102. Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1993.
12. Hewitt DJ, Millner GC, Nye AC, Simmons HF. Investigation of arsenic exposure from soil at a Superfund site. Environ Res 68:73–81 (1995).
13. Tam GKH, Charbonneau SM, Bryce F, Pomroy C, Sandi E. Metabolism of inorganic arsenic (^{74}As) in humans following oral ingestion. Toxicol Appl Pharmacol 50:319–322 (1979).
14. Buchet JP, Lauwerys R, Roels H. Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int Arch Occup Environ Health 48:111–118 (1981).
15. Hopenhayn-Rich C, Smith AH, Goeden HM. Human studies do not support the methylation threshold hypothesis for the toxicity of inorganic arsenic. Environ Res 60:161–177 (1993).
16. Warner ML, Moore LE, Smith MT, Kalman DA, Fanning E, Smith AH. Increased micronuclei in exfoliated bladder cells of persons who chronically ingest arsenic-contaminated water in Nevada. Cancer Epidemiol Biomarkers Prev 3:583–590 (1994).
17. Del Razo LM, Hernández JL, García-Vargas GG, Ostrosky-Wegman P, Cortinas de Nava C, Cebrián ME. Urinary excretion of arsenic species in a human population chronically exposed to arsenic via drinking water. A pilot study. In: Arsenic exposure and health (Chappell WR, Abernathy CO, Cothern CR, eds). Northwood, UK:Science and Technology Letters, 1994;91–100.
18. Beck BD, Boardman PD, Hook GC, Rudel RA, Slayton TM, Carlson-Lynch H. Response to Smith et al. [letter]. Environ Health Perspect 103:15–17 (1995).
19. Sancha AM, Vega F, Venturino H, Fuentes S, Salazar AM, Moreno V, Barón AM, Rodríguez D. The arsenic health problem in Northern Chile. Evaluation and control. A case study preliminary report. In: International seminar proceedings arsenic in the environment and its incidence on health (Sancha AM, ed), 25–29 May 1992, Santiago, Chile. Santiago:Universidad de Chile, Facultad de Ciencias Físicas y Matemáticas, 1992;187–202.
20. Alonso, H. Arsenic enrichment in superficial waters. II. Region Northern Chile. In: International seminar proceedings arsenic in the environment and its incidence on health (Sancha AM, ed), 25–29 May 1992, Santiago, Chile. Santiago:Universidad de Chile, Facultad de Ciencias Físicas y Matemáticas, 1992;101–108.
21. Crecelius EA. Modification of the arsenic speciation technique using hybrid generation. Anal Chem 50:826–827 (1978).
22. Atallah R, Kalman DA. On-line photooxidation for the determination of organoarsenic compounds by atomic absorption spectrophotometry with continuous arsine generation. Talanta 38:167–173 (1991).
23. Boeniger MF, Lowry LK, Rosenerg J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. Am Ind Hyg Assoc J 54:615–627 (1993).
24. StataCorp. Stata statistical software: release 4.0. College Station, TX:Stata Corporation, 1995.
25. Georis B, Cardenas A, Buchet JP, Lauwerys R. Inorganic arsenic methylation by rat tissue slices. Toxicology 63:73–84 (1990).
26. Thompson DJ. A chemical hypothesis for arsenic methylation in mammals. Chem-Biol Interactions 88:89–114 (1993).
27. Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst 85:1159–1164 (1993).
28. Li W, Yang S, Cao Z, Andry CD, Chour IN. Effects of arsenite on the cytoskeleton and cellular GSH in cultured cells. Toxicologist 11:125 (1991).
29. Giralt M, Lafuente A, Pujol F, Mallol J. Enhanced glutathione S-transferase activity and glutathione content in human bladder cancer. Followup study: influence of smoking. J Urol 149:1452–1454 (1993).
30. Hirata M, Tanaka A, Hisanaga A, Ishinishi N. Effects of glutathione depletion on the acute nephrotoxic potential of arsenite and on arsenic metabolism in hamsters. Toxicol Appl Pharmacol 106:469–481 (1990).
31. Buchet JP, Lauwerys R. Study of factors influencing the in vivo methylation of inorganic. Toxicol Appl Pharmacol 91:65–74 (1987).
32. Buchet JP, Lauwerys R. Role of thiols in the *in-vitro* methylation of inorganic arsenic by rat liver cytosol. Biochem Pharmacol 37:3149–3153 (1988).
33. Bogdan GM, Sampayo-Reyes A, Aposhian HV. Arsenic binding proteins of mammalian systems: I. Isolation of three arsenite-binding proteins of rabbit liver. Toxicology 93:175–193 (1994).
34. Del Razo LM, García-Vargas G, Vargas H, Albores A, Cebrián M. Chronic high arsenic exposure alters the profile of urinary arsenic metabolites in humans. Toxicologist 15:262 (1995).
35. Gonsebatt ME, Vega L, Montero R, García-Vargas G, Del Razo LM, Albores A, Cebrián ME, Ostrosky-Wegman P. Lymphocyte replicating ability in individuals exposed to arsenic via drinking water. Mut Res 313:213–299 (1994).
36. Núñez Atencio LN, Hill HG, Martínez AL. Guía Museo Arqueológico, Universidad Católica del Norte Chile. San Pedro de Atacama (II Región): Instituto de Investigaciones Arqueológicas y Museo R.P. Gustavo Le Paige S.J., 1991.
37. Figueroa L, Razmilic B, González M. Corporal distribution of arsenic in mummified bodies owned to an arsenical habitat. In: International seminar proceedings arsenic in the environment and its incidence on health (Sancha AM, ed), 25–29 May 1992, Santiago, Chile. Santiago: Universidad de Chile, Facultad de Ciencias Físicas y Matemáticas, 1992;77–82.
38. Borgoño JM, Vicent P, Venturino H, Infante A. Arsenic in the drinking water of the city of Antofagasta: epidemiological and clinical study before and after the installation of a treatment plant. Environ Health Perspect 19:103–105 (1977).
39. Astolfi E, Besuschio SC. García-Fernández JC, Guerra C, Maccagno A. Hidroarsenicismo crónico regional endémico. Buenos Aires: Cooperativa General Belgrano, 1982.
40. Chatterjee A, Das D, Mandal BK, Chowdhury TR, Samanta G, Chakraborti D. Arsenic in ground water in six districts of West Bengal, India: the biggest arsenic calamity in the world. Part I. Arsenic species in drinking water and urine of the affected people. Analyst 120:643–650 (1995).
41. García-Zapata MT, McGreevy PB, Marsden PD. American trypanosomiasis. In: Hunter's tropical medicine, 7th ed. (Strickland GT, ed). Philadelphia:W.B. Saunders Company, 1991;628–637.
42. Aposhian HV, Aposhian MM. Newer developments in arsenic toxicity. J Am Coll Toxicol 8:1297–1305 (1989).
43. Moore MM, Harrington-Brock K, Doerr CL. Genotoxicity of arsenic and its methylated metabolites. In: Arsenic exposure and health (Chappell WR, Abernathy CO, Cothern CR, eds). Northwood, UK:Science and Technology Letters, 1994;191–198.
44. Tu S-H, Shirachi DY. Cytotoxicity of inorganic and organic arsenics in cell culture. Proc West Pharmacol 35:61–63 (1992).
45. Yamauchi H, Fowler BA. Toxicity and metabolism of inorganic and methylated arsenicals. In: Arsenic in the environment part II: human health and ecosystem effects (Nriagu JO, ed). New York:John Wiley and Sons, 1994;35–53.
46. Yamanaka K, Ohba H, Hasegawa A, Sawamura R, Okada S. Mutagenicity of dimethylated metabolites of inorganic arsenics. Chem Pharm Bull 37:2753–2756 (1989).
47. Endo G, Kuroda K, Okamoto A, Horiguchi S. Dimethylarsenic acid induces tetraploids in Chinese hamster cells. Bull Environ Contam Toxicol 48:131–137 (1992).
48. Mass MJ. Human carcinogenesis by arsenic. Environ Geochem Health 14:49–54 (1992).
49. Mushak P, Crocetti A. Risk and revisionism in

arsenic cancer risk assessment. Environ Health Perspect 103:684–689 (1995).
50. Weinshilboum R. Pharmacogenetics of methylation: relationship to drug metabolism. Clin Biochem 21:201–210 (1988).
51. Caporaso N, Landi MT, Vineis P. Relevance of metabolic polymorphisms to human carcinogenesis: evaluation of epidemiologic evidence. Pharmacogenetics 1:4–19 (1991).
52. Zhong S, Wyllie AH, Barnes D, Wolf CR, Spurr NK. Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis 14:1821–1824 (1993).
53. Sancha AM, Rodríguez D, Vega F, Fuentes S, Lecaros L. Arsenic removal by direct filtration, an example of appropriate technology. In: International seminar proceedings arsenic in the environment and its incidence on health (Sancha AM, ed), 25–29 May 1992, Santiago, Chile. Santiago:Universidad de Chile, Facultad de Ciencias Físicas y Matemáticas, 1992; 165–172.

ENVIRONMENTAL SCIENCES FACULTY POSITION

The University of Texas-Houston
Health Science Center
School of Public Health

MPH Program at San Antonio

The Environmental Sciences Discipline of The University of Texas-Houston School of Public Health is seeking candidates for a tenure-track position at the Assistant or Associate Professor level to join the faculty of the Satellite MPH program in San Antonio. The satellite program, an integral part of the UT-Houston Health Science Center School of Public Health, is located on the campus of The University of Texas Health Science Center at San Antonio.

Candidates must have a doctoral degree in the environmental sciences or an M.D., M.P.H. with board certification in preventive medicine; demonstrated competence and experience in teaching environmental and occupational health at the graduate level; and evidence of scholarly achievement as indicated by research projects and publications. Experience in the development and administration of environmental health programs at the community or state level is preferred. Responsibilities will include teaching, research, supervision of graduate students and community service. The incumbent ideally will have research interest pertinent to the particular problems of public health in South Texas and the United States-Mexico border region. An opportunity to assume administrative responsibilities may be available for a candidate hired at the Associate level.

Greater detail about this position, The University of Texas-Houston Health Science Center School of Public Health, and the University of Texas Health Science Center at San Antonio may be obtained through the Internet at the following address: *http://utsph.sph.uth.tmc.edu/*

The University of Texas is an Equal Opportunity Employer. Minorities and women are particularly encouraged to apply. The start date is flexible; review of applications will begin immediately and continue until a suitable candidate is selected.

To apply: Send your *curriculum vitae* to:

George L. Delclos, M.D., M.P.H., Search Committee Chair, School of Public Health,
The University of Texas-Houston, Health Science Center, PO Box 20186, Houston, Texas 77225

Metals in Lung Tissue from Autopsy Cases in Mexico City Residents: Comparison of Cases from the 1950s and the 1980s

Teresa Imelda Fortoul,[1,3] Liliana Saldívar Osorio,[2] Araceli Tovar Tovar,[2] Dolores Salazar,[2] Maria Elena Castilla,[2] Gustavo Olaiz-Fernández[3]

[1]Departamento de Biologia Celular y Tisular, Facultad de Medicina, UNAM, Mexico City, Mexico; [2]Departamento de Quimica Analitica, Facultad de Quimica ,UNAM, Mexico City, Mexico; [3]Dirección General de Salud Ambiental, SSA, Mexico City, Mexico

In autopsies performed on residents of Mexico City during the 1950s and 1980s (45 males and 24 females and 42 males and 42 females, respectively), concentrations of cadmium, copper, cobalt, nickel, and lead in the lungs were studied by atomic absorption spectrometry. Sharp increases were noted in samples taken in the 1980s compared to those from the 1950s. In samples from both time periods, the concentrations were influenced by gender. Smoking was not associated with higher levels of the metals. Only lead seemed to have a relation with age. The enormous differences by gender in the 1950s could be due to different patterns of exposure. The differences among samples from both periods appear to be associated with the increase of air pollutants in the metropolitan areas of Mexico City during the years under study. These results reinforce the importance of studying lung tissue to monitor air pollution by metals. *Key words:* atomic absorption spectrometry, cadmium, cobalt, copper, heavy metals pollution, lead, lung, nickel. *Environ Health Perspect* 104:630–632 (1996)

During the past 30 years, major Mexican cities and, in particular, the vast metropolitan area of Mexico City, have experienced drastic changes (*1*). Industry has boomed, urban population has more than doubled, and a dramatic increase in motor vehicles has brought on almost unmanageable congestion on streets and highways. Reports in 1972 indicated that the total number of motor vehicles in the entire country was 2.5 million, and in Mexico City alone 1 million were circulating. Today in Mexico City, there are almost 3 million vehicles (*1,2*). These and various other factors have produced a high concentration of air pollutants. Reports on air quality began in 1976, but they were produced at irregular intervals. Since 1986, SEDUE (Secretaría de Desarrollo Urbano y Ecología) has conducted monitoring programs of air quality in a more accurate and comprehensive way, and the only reported metal now or before 1986 is lead (*2*). During 1986 and 1987, SEDUE reported 3-month average concentrations of lead in air as high as 14 mg/m^3 in heavily industrialized areas of Mexico City (*1,3*).

In the affected areas, pollution control regulations have not been adequately enforced. This and other inadequacies in management have resulted in grave consequences. Investigations of the trend of toxic metal concentrations in lung tissue are of primary interest in research projects and in studies concerning health protection. Research in human exposure to the pollutants often uses tissue samples from repositories in pathology laboratories (*4*). We analyzed concentrations of metals in lung samples, currently preserved in a pathology laboratory in Mexico City, derived from autopsies performed during the 1950s and the 1980s .

Materials and Methods

We compared levels of cadmium, copper, cobalt, nickel, and lead in consecutive lung samples (69 taken during the 1950s and 84 from 1980s) at the Instituto Nacional de Cardiología in its Department of Pathology. The fixative was the same for all samples. Individual characteristics, such as age, gender, and place of residence, that could potentially be related to exposure to airborne pollutants were examined. However, data on cigarette smoking and the smokers' occupations were incomplete in the clinical records. Smoking was included when it was mentioned in the clinical records. All the cases had a medium to medium-high socioeconomic status. Cases of pregnancies or pulmonary lesions, other than light edema, which did not distort the lung morphology, were excluded. All the samples were dissected with the same knife and treated with the same formaldehyde solution. The same flasks with the same caps were used. Samples were taken from lungs previously sliced, but the samples did not include major bronchi or certain other types of tissue. Information concerning specific anatomical sites was lacking. In the 1980s all the lungs were perfused with the fixative before they were sliced. This procedure had not been used in the 1950s. Samples were treated according to a modification of a technique described by Locke (*5*): 0.5-g lung segments fixed in formaldehyde were dried at 150°C for 20 min to evaporate the formaldehyde. Dried samples were placed inside quartz beakers which had been washed with 1:1 nitric acid and sulfuric acid solutions (*6*).

Samples were digested with 2 ml of a mixture of pure nitric and sulfuric acids and heated for 20 min at 150°C. Beakers were capped and enclosed samples were boiled until a small volume was obtained. Digested samples were filtered and diluted with distilled, deionized water to 25-ml volume. The same water was used for the blanks (*7,8*). Samples were analyzed by atomic absorption spectrometry (model 2380, Perkin Elmer, Foster City, California). The light source came from a hollow cathode lamp, specific for each separate element, using an acetylene-air flame. Each metal was identified after substracting the results obtained. Formaldehyde and the blanks were also analyzed to exclude metals from this source. Accuracy was assured by three random determinations of seven different standard solutions, prepared with the same reagents used during the analysis. Wavelength, detection limit, sensitivity, slit and linear interval are summarized in Table 1. Each sample was analyzed in triplicate. Results were defined as microgram per gram of dry lung tissue (*9,10*).

To assure external quality controls, we analyzed 10 samples from each set of lung tissues by inductively coupled plasma emission spectrometry (*11*). In all the cases the differences within the samples was less than 15%. The averages for concentrations of the metals are summarized in Tables 2 and 3. A multiple regression analysis (Statgraphics, version 5.0) was performed to identify the association between the variables (gender, smoking habit, decade, and age) and metal concentrations in lung tissues (Table 4).

Results

Eighty-four samples from 85 cases (42 males and 42 females, ranging from 11 to 87 years of age) from the 1980s and 69 samples from the 1950s (45 males and 24

Address correspondence to T.I. Fortoul, Departamento de Biologia Celular y Tisular, Facultad de Medicina, UNAM, México DF, CP 04510.

We thank the Departamento de Patología, Instituto Nacional de Cardiología, for providing the lung samples. We also thank M. Shelby for editorial advice and encouragement, and we especially thank P. Ostrosky.

Recieved 28 July 1995; accepted 19 March 1996.

Table 1. Analytic parameters for atomic absorption spectrometry

Element	Wavelength (nm)	Width (nm)	Linear interval (mg/l)	Detection limit (ppm)
Cadmium	228.2	0.7	2.0	0.1
Cobalt	240.7	0.2	3.5	0.08
Copper	324.8	0.7	5.0	0.06
Nickel	232.0	0.2	2.0	0.35
Lead	217.0	0.7	20.0	0.37

Table 2. Mean concentrations of metals in lung tissue from the 1980s and the 1950s

Element	Mean ± SD (mg/g dry weight) 1980s (n = 84)	1950s (n = 69)
Cadmium	25.6 ± 6.5	1.2 ± 0.37
Cobalt	37.2 ± 8.67	3 ± 0.97
Copper	44.8 ± 15.7	10 ± 2.97
Nickel	57.6 ± 9.3	3 ± 0.96
Lead	134.3 ± 26.7	12 ± 4.97

Table 3. Mean concentration of metals by gender from the 1980s and the 1950s

Element	Mean ± SD (mg/g dry weight) Males 1980s	Males 1950s	Females 1980s	Females 1950s
Cadmium	23.3 ± 5	1.19 ± 0.3	27.8 ± 6.9	10.08 ± 1.3
Cobalt	38.1 ± 5.7	4.03 ± 0.9	36.4 ± 10.7	2.04 ± 0.02
Copper	32 ± 8	9.02 ± 2.5	57.4 ± 10	10.08 ± 2.3
Nickel	51.1 ± 5	3.09 ± 0.4	63.9 ± 7.9	3.54 ± 0.9
Lead	122.9 ± 13	10.6 ± 2.1	145.2 ± 31.4	13.3 ± 3.5

females, ages 7–79) were analyzed. All the 1980s samples were from consecutive autopsies; samples from the 1950s were from well-preserved cases. None of the cases had a history of lung diseases, and only one case from the 1980s was excluded because this individual was pregnant at the time of death. All samples were from individuals who had been permanent residents of Mexico City. The five metals analyzed in the fixative were below the detection limit in all samples. A high increment of metal concentrations in cases from the 1980s was observed compared to those from the 1950s (Table 2).

Differences by gender were evident in both time periods. Samples from the 1950s showed high levels of cadmium among women but not samples from the 1980s. This gender difference could be explained by different patterns of exposure, but not by smoking. Clinical records showed that none of the women from which the 1950s samples were taken smoked, and only three men were positive. On the other hand, in the 1980s, more women and men smoked, and this may explain why cadmium was not higher in women compared to men for these samples.

Samples from the 1980s showed increases in lead, copper, cadmium, and nickel among women compared to 1950s samples from women. Metal concentrations in the 1980s showed, with exception of cobalt, that the mean value of the other metals was slightly higher among women (Table 3).

There was no association between age and smoking and the concentrations of the metals (Table 4). Only lead seemed to be associated with age.

Table 4. Multiple regression analysis for metals in lungs: model fitting results for each metal

Metal	Variable	Coefficient	SE	t-value	Significance level
Cadmium	Constant	-56.35	4.78	-11.78	0.00
	Decade	0.02	0.002	12.10	0.00
	Gender	-0.46	0.043	-10.56	0.00
	Age	-0.00006	0.0011	-0.59	0.551
	Smoking	0.0272	0.0459	0.59	0.553
	Adjusted r^2	0.81			
Copper	Constant	-40.5	2.29	-17.7	0.00
	Decade	0.02	0.001	18.35	0.00
	Gender	-0.168	0.020	-8.08	0.00
	Age	0.0004	0.0005	0.77	0.44
	Smoking	-0.008	0.022	-0.37	0.70
	Adjusted r^2	0.88			
Cobalt	Constant	-70.89	3.057	-23.18	0.00
	Decade	0.036	0.001	23.40	0.00
	Gender	0.123	0.027	4.42	0.00
	Age	0.001	0.0007	1.58	0.11
	Smoking	-0.025	0.029	-0.86	0.38
	Adjusted r^2	0.9			
Nickel	Constant	-80.5	1.405	-57.2	0.00
	Decade	0.041	0.0007	58.04	0.00
	Gender	-0.071	0.0128	-5.58	0.00
	Age	0.0002	0.0003	0.80	0.424
	Smoking	-0.0053	0.013	-0.39	0.690
	Adjusted r^2	0.9			
Lead	Constant	-66.02	1.49	-44.19	0.00
	Decade	0.034	0.0007	45.27	0.00
	Gender	-0.076	0.013	-5.59	0.00
	Age	-0.0007	0.0003	-2.20	0.0286
	Smoking	0.0138	0.0143	0.96	0.33
	Adjusted r^2	0.9			

Discussion

Little information is available concerning lung lesions among individuals living at high altitudes exposed for an extended time to significant levels of pollutants (Mexico City's altitude is 7500 feet above sea level). Airborne pollutants enter the respiratory system via the extensive surface of the lungs (*12*). The toxic effects of the metals included in this research are of serious concern (*13–17*). The toxicity of lead on the central nervous system and on organs such as the kidneys has been well documented. Few studies, however, have related metals to structural damage or impaired function of the lungs (*16–22*). Changes in immunological function by lead and cadmium have been reported in a few studies (*23,24*). Cadmium exposure has been associated with emphysema, and it may also induce pulmonary fibrosis (*25*). The carcinogenic effect of cadmium exposure is still controversial (*26*). Cadmium, nickel, cobalt, and lead are found in tobacco (*27*). It has been reported that Mexican tobacco has a high concentration of cadmium (*28*). In the respiratory tract, nickel is carcinogenic (*29,30*). Although there is no substantial evidence that copper is carcinogenic, lung cancer is frequent among copper workers. In tumors induced by hydrocarbons, the copper concentration in tissue is higher than normal (*31,32*). Cobalt is usually present in human tissue. Its carcinogenic effect has been demonstrated experimentally. This effect is not as potent as that of nickel, but is higher than previous reports would indicate (*13,33*).

We found higher concentrations of metals in the lungs than previously reported. The fixative showed a concentration below the detection limit, similar to the concentration in water or other reactants used in the analysis of the samples. Thus, contamination from these sources can be ruled out (*34*). Metal concentrations differ according to the region of the lung (*35–37*) and, although it is assumed that more 1980s samples with higher levels of metals were included, the magnitude of differences noted between both periods of time under study cannot be explained solely by sampling bias. A possible explanation is the increase of air pollution

levels due to the increase of vehicles and the growing number of industries in Mexico City. This is supported by some studies that report that the concentration of lead, copper, and nickel in the air are from anthropogenic sources (*39*). In this study, smoking was not a factor in the increased concentrations of metals in the lung.

All of these factors, in addition to the limited information in the literature concerning metal concentrations in the lungs and smoking, are important in consideration of the total problem (*27*). The decreased deposition for cobalt and nickel during the 1950s could be a consequence of a lower exposure and possibly a consequence of a decreased oral intake of these two essential metals through the diet with age (*17*). It is possible that the concentrations in the lung could be the result of an equilibrium between metal intake by inhalation and tissue dissolution of each metal, followed by disposition throughout the organism (*17*).

Even though the data on individuals' occupations were incomplete, it was evident that 95% of the female subjects were homemakers in both sets. This may suggest that, among women, occupational exposure is not responsible for the sharp increases in the 1980s samples. Contrary to many reports that mention higher levels of metals among men, our results indicate similar or higher concentrations among women.

At present there is no explanation as to why women in this study would have higher exposures. It is probably the case that at home women are exposed to a great amount of not well-identified pollutants in Mexico. In Japan, there is also a report that in lung and other tissues, higher levels of metals among women may be explained by hormonal differences (*40*), which in some way could be part of the explanation for these variations. Further research on this topic is essential.

The only metal associated with age was lead. Lead is found in high concentrations in the blood of Mexican children, so it is possible that sources other than air explain this finding (*41*).

Our results call for more accurate analyses and more in-depth observations and conclusions. The focus in this research must be geared to the entire population of Mexico City, and the same attention must be given to all other urban areas in which pollution is a critical problem.

References

1. Rivero SO, Ponciano RG, Fortoul TI. Contaminación atmosférica y enfermedad respiratoria. Biblioteca de la Salud. Mexico City:UNAM y FCE, 1993.
2. Marquez ME. La contaminación atmosférica en Mexico. Memorias de la Reunion Nacional sobre Problemas de Contaminacion Ambiental P.N. Mexico City, 1983.
3. Albert L, Badillo F. Environmental lead in Mexico. Rev Environ Contam Toxicol 11:2–49 (1991).
4. WHO. Assesment of exposure. Guidelines on studies in environmental epidemiology. Environmental Health Criteria no 27. Geneva:World Health Organization, 1983.
5. Locke J. The determination of eight elements in human liver tissue by flame atomic absorption spectrometry in sulfuric acid solution. Anal Chem Acta 104:225–231(1979).
6. Laxen PH, Harrison RM. Cleaning methods for polyethylene containers prior the determination of trace metals in freshwater samples. Anal Chem 53 :345–350 (1981).
7. Nürenberg WH. Voltametric trace analysis in ecological chemistry of toxic metals. Pure Appl Chem 54:853–878 (1982).
8. Perkin-Elmer manual, model 403. Atomic absorption spectrophometer. Foster City, CA:Perkin Elmer, 1975.
9. U.S. EPA. Manual for the analysis of water. Washington, DC:Environmental Protection Agency, 1984.
10. Keith HL. Principles of envronmental analysis. Anal Chem 55:2210–2218 (1983).
11. Boumans PW. Inductively coupled plasma emission spectroscopy. New York:John Willey and Sons, 1987.
12. Comroe HJ. Physiology of respiration. Chicago: Year Book Medical Publishing, 1975.
13. Fishbein L. Environmental metalic carcinogens: an overview of exposure level. J Toxicol Environ Health 2:77–109 (1976).
14. Norseth T. Metal carcinogenesis. Ann NY Acad Sci 534:377–385 (1988)
15. Malavé Y, de Ruffino TD. Altered immune response during cadmium administration in mice. Toxicol Appl Pharmacol 74:46–56 (1984)
16. Jian Z, Ying-Han X, Hong-Fu Ch. The effects of lead ion on immune function of rabbit alveolar macrophages: quantification of immune phagocytosis and rosette formation by 51Cr in vitro. Toxicol Appl Pharmacol 78:484–487 (1984).
17. Tsalev DL, Zaprianov S. Atomic absorption spectrometry. In: Occupational and environmental health practice: analytical aspects and health significance. Boca Raton, FL:CRC Press,1983;105–150.
18. Schroeder AH. Abnormal trace elements in man: lead. J Chron Dis 14:408–425 (1961).
19. Schroeder AH, Brattleboro V, Tipton IH, Knoxville T. The human body burden of lead. Arch Environ Health 17:965–978 (1968).
20. Stringer AC, Zingaro RA, Creech B, Kolar FL. Lead concentrations in human lung samples. A statistical analysis. Arch Environ Health 29:268–270 (1974).
21. Lippmann M. Lead and human health: background and recent findings. Environ Res 51:1–24 (1990).
22. Kobayashi N, Okamoto T. Effects of lead oxide on the induction of lung tumors in Syrian hamsters. J Natl Cancer Inst 52:1605–1610 (1974).
23. Hillman PR, Nuri OA. Comparison of local and systemic immunity after intratracheal, intraperitoneal, and intravenous immunization of mice exposed to either aerosolized or ingested lead. Environ Res 39:265–277 (1986)
24. Cohen N. Increased concanavalin-A-induced supressor cell activity in humans with occupational lead exposure. Environ Res 48:1–6 (1989).
25. Fortoul TI. Mecanismos de lesión por algunos contaminantes. Cadmio y su acción patogénica sobre pulmón. Mexico City:Facultad de Medicina, UNAM, 1984.
26. Adees AE, Kaazantis G. Lung cancer in the nonferrous smelters:the role of cadmium. Br J Int Med 45:435–442 (1988).
27. Pääkko P, Kokkonen P, Anttila S, Kalliomäki PL. Cadmium and chromium as markers of smoking in human lung tissue. Environ Res 49:197–207 (1989).
28. Saldívar L, Luna M, Reyes E, Soto RI, Fortoul TI. Cadmium determination in Mexican produced tobacco. Environ Res 55:91–96 (1991).
29. Nielsen HF. Nickel toxicity. In: Advances in modern toxicology (Goyer RA, Mehlman MA, eds). New York:Hemisphere Publishing, 1977;129–146.
30. Raithel HJ, Schaller KH, Reither A, Svenes KB, Valentin H. Investigations on the quantitative determination of nickel and chromium in human lung tissue. Int Arch Occup Environ Health 60:55–66 (1988).
31. Raithel HJ. Analyses of chromium and nickel in human pulmonary tissue. Investigations in lung cancer patients and control population under special consideration of medical expertise aspects. Int Arch Occup Environ Health 61:507–512 (1989).
32. Gimenez ME, Martin MMC. Determinación de elementos traza, ceruloplasmina y proteínas plasmáticas en las neoplasias pulmonares. Rev Esp Oncol 32:633–640 (1985).
33. Furst A, Haro RT. A survey of metal carcinogenesis. Prog Exp Tumor Res 12:102–133 (1969).
34. Teraoka MH. Distribution of 24 elements in the internal organs of males and metallic workers in Japan. Arch Environ Health 36:155–165 (1981).
35. Kollmeier H, Seemann WJ, Rothe G, Müller KM, Wittig P. Age, sex, and region adjusted concentrations of chromium and nickel in lung tissue. Br J Ind Med 47:682–687 (1990).
36. Bartsh P, Collignon A., Weber G, Robaye G, Delbrouk JM, Roelandts, Yujie J. Distribution of metals in human lung: analysis by particle induced X-ray emission. Arch Environ Health 37:111–117 (1982).
37. Vanoerten CR. The regional distribution of trace elements in human lungs with differentiation of the traction present in the deposited dust. In: Trace elements. Analytical chemistry in medicine and biology. Boca Raton, FL:CRC Press, 1975.
38. Liebscher K, Smith H, Scotland G. Essential and nonessential trace elements. A method of determining whether an element is essential or nonessential in human tissue. Arch Environ Health 17:881–890 (1968).
39. Aldape F, Flores M, Diaz VR, Miranda J, Cahill TA, Morales R. Two year study of elemental composition of atmospheric aerosols in Mexico Clty. Int J PIXE 84:373–388 (1991).
40. Sumino K, Hayakawa K, Shibata T, Kitamura S. Heavy metals in normal Japanese tissues. Amount of 15 heavy metals in 50 subjects. Arch Environ Health 30:487–494 (1975).
41. Olaiz FG, Rojas MR, Fortoul TI, Doyer M, Palazuelos E, Tapia CR, Doyer M, Palazuelos E, Tapia CR. Risk factors for high levels of lead in blood in schoolchildren in Mexico City. Arch Environ Health (in press).

6th International Congress on Cell Biology and 36th American Society for Cell Biology Annual Meeting

December 7–11, 1996
San Francisco, California

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

PLENARY SYMPOSIA

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

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

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

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

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

CONCURRENT SYMPOSIA

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

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

Tuesday, December 10
The RNA World, H. Blau, C. Guthrie, G. Joyce, R. Lehmann, and R. Klausner
Cell Biology of Infectious Diseases, S. Falkow, R. Nussenzweig, B.B. Finlay, P. Sansonetti, and J. Theriot
Heat Shock and Chaperones, S. Lindquist, H. Nelson, C. Georgopoulos, and A. Horwich
Cellular Shape and Function, M. Driscoll, D. Ingber, S. Farmer, and P. Gunning
Adhesion Receptors, M. Hemler, D. Wagner, R. Assoian, E. Dejana, and M. Schwartz
Caveolae and GPI-Anchored Membrane Proteins, R. Anderson, D. Brown, M. Lisanti, and R. Parton

Wednesday, December 11
Molecular Mechanisms in Epithelial–Mesenchymal Interactions, S. Artavanis-Tsakonas, I. Thesleff, C. Birchmeier, P. Ekblom, and M. Kedinger
Signals to and from the Endoplasmic Reticulum, M.-J. Gething, P. Walter, N. Borgese, P. Cosson, and T. Kreis
Proteolysis and Biological Control, K. Anderson, A. Varshavsky, A. Ciechanover, S. Coughlin, and J. White
Growth Inhibition Signalling, C. Prives, J. Wang, R. Derynck, and A. Horwitz
Cell–Cell Interactions and Junctions, K. Miller, M. Takeichi, R. Moon, and W. J. Nelson
Silencing, D. Gottschling, J. Rine, J. Bender, A. Johnson, and E. Selker

J. Michael Bishop, 6th International Congress and ASCB President
Contact the ASCB, 9650 Rockville Pike, Bethesda, MD 20814; telephone (301) 530-7153, fax (301) 530-7139; congress@ascb.faseb.org, or http://www.faseb.org/ascb

Lipopolysaccharide-induced Hepatic Injury Is Enhanced by Polychlorinated Biphenyls

Alan P. Brown,[1] A. Eric Schultze,[2] Wendy L. Holdan,[1] John P. Buchweitz,[1] Robert A. Roth,[1] and Patricia E. Ganey[1,3]

[1]Department of Pharmacology/Toxicology, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824 USA; [2]Department of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901-1071 USA; [3]Department of Medicine, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824 USA

After intravenous administration of bacterial lipopolysaccharide (LPS) to rats, polymorphonuclear neutrophils (PMNs) rapidly accumulate in the liver, and midzonal hepatic necrosis is prominent by 6 hr. PMNs are required for the development of hepatic injury in rats. Certain polychlorinated biphenyls (PCBs) can activate PMNs, resulting in production of superoxide anion ($O_2^{\cdot-}$) and release of cytolytic factors from granules. This raises the possibility that PCB exposure might enhance PMN-mediated tissue injury, such as LPS-induced hepatotoxicity. We treated female Sprague-Dawley rats with a minimally toxic dose of LPS in saline (2 mg/kg, intravenous) and 90 min later exposed them to Aroclor 1248 (50 mg/kg, intraperitoneal), a mixture of PCBs. The animals were killed 6 hr after LPS administration, and hepatic injury was assessed. Neither LPS nor Aroclor 1248 alone produced liver injury. Co-treatment with LPS and Aroclor 1248 resulted in pronounced liver injury as demonstrated from increased activities of alanine aminotransferase and isocitrate dehydrogenase in plasma. Histological evaluation indicated increased severity of hepatic necrosis in rats receiving both LPS and Aroclor 1248. Hepatic accumulation of PMNs, normally observed after LPS, was not altered by co-exposure to PCBs. Aroclor 1248 stimulated rat PMNs *in vitro* to produce $O_2^{\cdot-}$ and to degranulate. In addition, PMN-mediated cytotoxicity to isolated rat hepatocytes in culture was increased upon addition of Aroclor 1248. PCBs activate PMNs *in vitro* and increase PMN-dependent hepatocellular damage *in vitro* and after LPS treatment *in vivo*. PCBs may act *in vivo* as an additional inflammatory stimulus to activate PMNs to become cytotoxic, resulting in increased tissue injury. *Key words*: Aroclor 1248, hepatotoxicity, liver, neutrophils, polychlorinated biphenyls. *Environ Health Perspect* 104:634–640 (1996)

The sequelae of gram-negative bacterial sepsis are a major cause of morbidity and a leading cause of death in the United States (*1*). Gram-negative bacteria are a normal constituent of the gastrointestinal tract, and pathogenesis from these organisms can arise during systemic exposure to either whole bacteria or their constituents. Such exposure can occur through increased translocation of bacteria from the gut to the portal circulation during surgery and during a wide range of disease states, including urinary tract infections, inflammatory bowel disease, obstructive jaundice, liver disease, and cirrhosis (*2–4*).

Lipopolysaccharide (LPS) is a major component of the cell wall of gram-negative bacteria and is likely responsible for many of the events that occur during sepsis (*5*). Exposure to LPS can produce an array of pathophysiological changes similar to those seen during sepsis, including hypotension and multi-organ failure involving damage to the liver, lungs, kidneys, heart, and gastrointestinal tract (*6*). Neutrophils (PMNs) have been implicated as playing a critical role in LPS-induced tissue damage (*7,8*). After an intravenous administration of LPS to rats, PMNs accumulate in the liver sinusoids within 1 hr, and multifocal, midzonal hepatic necrosis is prominent by 6 hr (*8,9*). Depletion of circulating PMNs before LPS exposure protects against hepatic injury, indicating a requirement for these cells (*8,10*). Activation of PMNs is likely a necessary step in producing PMN-mediated tissue injury, and studies *in vitro* have demonstrated that activated PMNs can kill both endothelial cells and hepatocytes (*11–13*). Although the exact mechanisms of PMN-mediated tissue injury are unknown, they probably involve production of reactive oxygen species such as the superoxide anion ($O_2^{\cdot-}$) and/or release of cytotoxic enzymes contained within granules (*14*).

Polychlorinated biphenyls (PCBs) are industrial chemicals that have been released into the environment. In experimental animals, these compounds produce an array of toxic responses including hepatotoxicity and alterations in the immune system (*15,16*). Humans accidentally exposed to high concentrations of PCBs expressed changes in immune system parameters such as alterations in lymphocyte subpopulations and suppression of cellular immunity (*17–19*). In addition, monocytes and PMNs obtained from individuals exposed to PCBs had lower percentages of cells bearing immunoglobulin and complement receptors (*20*). Primates exposed to PCBs have exhibited a wide range of adverse responses including inflammatory lesions in the liver (*21,22*), and abnormal liver function tests have been reported in exposed human populations (*19*).

Although immunotoxicity due to PCB exposure has been described in some detail, less is known about the interactions of these compounds with PMNs or inflammatory responses. PCBs rapidly activate PMNs *in vitro* to produce $O_2^{\cdot-}$ and to degranulate (*23*). PMN activation by PCBs occurs through signal transduction pathways involving phosphoinositide hydrolysis, inositol triphosphate production, phospholipase A_2 activation, and Ca^{2+} mobilization (*24–26*). Activation of PMNs results in production and release of cytotoxic mediators into the extracellular environment. Moreover, exposure to PCBs *in vitro* alters PMN responses to subsequent stimuli (*23*). Enhanced PMN activation or responsiveness by PCBs could lead to increased tissue injury where PMN involvement occurs, such as during exposure to LPS resulting from bacterial infections or increased translocation of bacteria from the gut. Accordingly, since LPS-induced hepatotoxicity is PMN dependent, we hypothesized that co-exposure to PCBs would enhance LPS-induced liver injury. This hypothesis was tested by exposing rats to a minimally toxic dose of LPS followed by the PCB mixture Aroclor 1248 and assessing hepatic injury. Aroclor 1248 was chosen because this mixture of PCBs is composed predominantly of tetrachlorinated biphenyls and *ortho*-substituted congeners (*27*), which activate PMNs *in vitro* (*26*). Furthermore, *ortho*-substituted PCB congeners have been identified in human biological samples, and their potential health effects have yet to be determined (*19*).

Address correspondence to P.E. Ganey, Department of Pharmacology/Toxicology, Life Sciences Building, Michigan State University, East Lansing, MI 48824 USA.

This research was supported by NIH grant ES04911. A.P.B. was supported in part by a National Research Service Award from the National Institute of Environmental Health Sciences, NIH grant ES05684. R.A.R. was supported in part by a Burroughs Welcome Toxicology Scholar Award. W.L.H. was supported by an American Society for Pharmacology and Experimental Therapeutics Undergraduate Student Fellowship.

Received 14 September 1995; accepted 29 February 1996.

Materials and Methods

Animals. We used female Sprague-Dawley rats [Crl:CD BR(SD) VAF/plus, 175–220 g] for studies of LPS toxicity. Male Sprague-Dawley retired breeder rats were used as donors of isolated PMNs. Hepatocytes were isolated from male Sprague-Dawley rats (175–300 g). All animals were obtained from Charles River Laboratories (Portage, Michigan). Animals were maintained on a 12-hr light/dark cycle for at least 1 week before use. Food (rat chow, Teklad, Madison, Wisconsin) and water were provided *ad libitum.*

Chemicals. Aroclor 1248 was purchased from ChemService (West Chester, Pennsylvania). Lipopolysaccharide (*E. coli* 0128:B12 serotype), glycogen (type II from oyster), phorbol myristate acetate (PMA), superoxide dismutase (SOD), ferricytochrome C, cytochalasin B, guaiacol, dimethylformamide (DMF), *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, Missouri). Histochoice fixative was purchased from Amresco (Solon, Ohio). Collagenase type A was purchased from Boehringer-Mannheim Biochemicals (Indianapolis, Indiana). Williams' medium E and gentamicin were purchased from Gibco (Grand Island, New York). Fetal calf serum was purchased from Intergen (Purchase, New York).

Treatment protocol. Female Sprague-Dawley rats received either LPS (2 mg/kg) or sterile saline vehicle (2 ml/kg) via a tail vein. The animals then received either Aroclor 1248 (50 mg/kg) or corn oil vehicle (2 ml/kg) 90 min later by intraperitoneal injection. We used this dosing regimen to minimize potential effects on hepatocytes by the PCBs alone and to maximize exposure of both circulating and tissue-adherent (in the liver) PMNs to PCBs. Rats were killed 6 or 9 hr after LPS/saline exposure. The animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal), the abdominal cavity was exposed, and blood was drawn from the inferior vena cava into a syringe containing heparin. Livers were removed, and sections were prepared and immediately placed in fixative. We used an aliquot of plasma on the same day to determine enzyme activities.

Assessment of hepatic injury. We assessed hepatic injury by measuring activities of alanine aminotransferase (ALT) and isocitrate dehydrogenase (ICDH) in plasma by kinetic determination using a Gilford Spectrophotometer (Sigma kits no. 59-UV and 153-UV, respectively) (*28*). Liver sections were fixed, embedded in paraffin, cut at 6 µm, stained with hematoxylin and eosin, and coded and evaluated without bias by a veterinary pathologist. The severity of hepatic injury was graded on a scale from 0 (no significant lesions) to 5 (severe, coalescing necrosis).

Quantification of hepatic PMN accumulation. PMNs in liver sections were visualized using an immunohistochemical technique (*9*). Liver sections were fixed, embedded in paraffin, and sectioned at 6 µm. Paraffin was removed from the tissue sections with xylene before staining. PMNs within the liver tissue were stained using a rabbit anti-rat PMN immunoglobulin. This anti-PMN immunoglobulin was isolated from serum of rabbits immunized with rat PMNs as described previously (*8*). After incubation with the primary antibody, the tissue sections were incubated with biotinylated goat anti-rabbit IgG, avidin-conjugated alkaline phosphatase, and Vector Red substrate to stain the PMNs within the tissue. We assessed hepatic PMN accumulation by averaging the number of PMNs counted in 20 midzonal, 400× fields throughout 3 separate lobes using light microscopy. PMNs were identified by positive staining and morphology.

Isolation of PMNs. PMNs were isolated from the peritoneum of male Sprague-Dawley retired breeder rats by glycogen elicitation as described previously (*29*). Isolated PMNs were resuspended in Hanks' balanced salt solution (HBSS), pH 7.4. The percentage of PMNs in the cell preparations was routinely >95%, and the viability was ≥95% as determined by the ability to exclude trypan blue. The PMNs were suspended in borosilicate glass test tubes, 12 × 75 mm (VWR, Chicago, Illinois), at a final concentration of 2×10^6 cells/ml. The isolation procedure was performed at room temperature.

Detection of superoxide anion production. PMNs (2×10^6 cells) were prepared in HBSS in a final volume of 1 ml and exposed to either vehicle (1 µl DMF) or to 1 or 10 µg/ml Aroclor 1248 for 30 min at 37°C. These concentrations of PCBs were chosen for their ability to affect PMN function in the absence of cytotoxicity (*23,26*). PMA (0 or 20 ng/ml) was then added for an additional 10 min at 37°C. We measured $O_2^{\cdot-}$ generated during this 40-min incubation period by the SOD-sensitive reduction of ferricytochrome C (*30*). In a separate series of experiments, PMNs (2×10^6 cells) were incubated with LPS (10 or 100 µg/ml) or vehicle (HBSS) for 10 min, followed by exposure to Aroclor 1248 (1 or 10 µg/ml) or vehicle (DMF) for an additional 30 min. We detetected $O_2^{\cdot-}$ produced during this 40-min period as described above.

Assessment of PMN degranulation. Degranulation was assessed by the release of the enzyme myeloperoxidase (MPO), which is contained within azurophilic granules. PMNs were prepared in HBSS and pretreated with 5 µg/ml cytochalasin B for 10 min at room temperature to facilitate release of the granules into the incubation medium (*31,32*). PMNs (2×10^6 cells) were then exposed to either vehicle (1 µl DMF) or to 1 or 10 µg/ml Aroclor 1248 for 15 min at 37°C. We exposed PMNs to fMLP (10 nM) for 15 min at 37°C as a positive control. fMLP is a chemotactic peptide derived from gram-negative bacteria that binds to a G-protein–coupled receptor, leading to PMN activation (*33,34*). The incubation was terminated by placing the cells in an ice-water bath followed by centrifugation at 4°C. The cell-free supernatant was collected, and MPO activity was measured by the H_2O_2-dependent oxidation of guaiacol (*35,36*). We measured the change in absorbance at 470 nm over 2 min at 25°C in a spectrophotometer and calculated MPO activity (U/l) using a molar extinction coefficient of 26,600 (*36*). Lactate dehydrogenase activity (LDH) present in the cell-free supernatant was measured according to the method of Bergmeyer and Bernt (*37*) as a marker of cytotoxicity.

Hepatocyte–neutrophil co-cultures. Hepatocytes were isolated according to the method of Seglen (*38*) as modified by Klaunig et al. (*39*). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg), and the portal vein was cannulated. The liver was perfused with approximately 150 ml of Mg^{2+}-free, Ca^{2+}-free HBSS followed by perfusion with 250 ml collagenase type A (0.5 mg/ml). The resulting liver digest was filtered through gauze and spun in a centrifuge at 50*g* for 2 min. The hepatocytes were resuspended in Williams' medium E containing 10% fetal calf serum and 0.1% gentamicin. Using this isolation procedure, 98% of the cells in the final preparation were hepatic parenchymal cells with viability routinely >90% (*13*).

The hepatocytes were plated in six-well plates at a density of 5×10^5 hepatocytes/well. After an initial 3-hr attachment period, the medium and unattached cells were removed, and either Williams' medium E containing 0.1% gentamicin or medium containing isolated PMNs was added. PMNs were isolated as previously described and plated at a density of 5×10^6 PMNs/well, resulting in a ratio of 10 PMNs/hepatocyte. PMNs were allowed to attach for 30 min, then vehicle (2 µl DMF) or Aroclor 1248 (1 or 10 µg/ml)

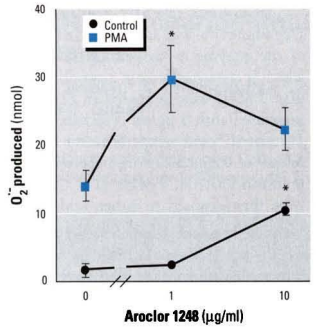


Figure 1. Superoxide anion (O_2^-) production by neutrophils during exposure to Aroclor 1248 and phorbol myristate acetate (PMA). Rat PMNs were exposed to either 0, 1, or 10 μg/ml Aroclor 1248 for 30 min at 37°C. The PMNs were then exposed to either 0 or 20 ng/ml PMA for an additional 10 min at 37°C. O_2^- produced during the incubation period was determined as described in Materials and Methods; N = 3–4. *Significantly different from respective value in the absence of Aroclor 1248, one-way ANOVA (p<0.05).

Table 1. Neutrophil degranulation in the presence of Aroclor 1248[a]

Treatment	MPO activity (U/l)
Vehicle control	5.6 ± 3.2
Aroclor 1248 (1 μg/ml)	29.2 ± 19.1
Aroclor 1248 (10 μg/ml)	87.6 ± 42.9*
fMLP (10 nM)	291.8 ± 84.6*

Abbreviations: MPO, myeloperoxidase; fMLP, formyl-methionyl-leucyl-phenylalanine.
[a]Rat neutrophils were pretreated with 5 μg/ml cytochalasin B for 10 min at room temperature. Neutrophils were then exposed to the various stimuli for 15 min at 37°C. Degranulation was determined by measuring the activity of MPO in the medium as described in Materials and Methods; N = 4.
*Significantly different from vehicle control, one-way ANOVA (p < 0.05).

was added to wells containing either hepatocytes alone or hepatocytes plus PMNs. After a 16-hr incubation at 37°C in 92.5% O_2/7.5% CO_2, the medium was collected. Previous studies have demonstrated that PMN-mediated hepatocellular toxicity in co-culture occurs by 16 hr after stimulation of PMNs (*13*). The cells remaining on the plate were lysed with 1% Triton X-100, followed by sonication. Both the medium and the cell lysates were spun in a centrifuge at 600*g* for 10 min. The activity of ALT in the cell-free supernatant fluids was determined (using Sigma kit no. 59-UV), and the activity in the medium was expressed as a percentage of total activity (activity in the medium plus activity in the cell lysates). ALT activity in PMNs is relatively small and is not changed upon exposure to Aroclor 1248 (data not shown).

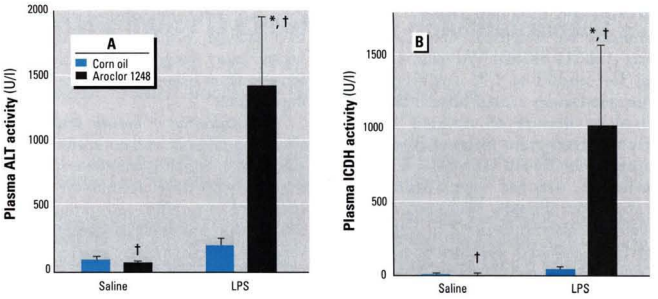


Figure 2. Aroclor 1248 enhances hepatic injury 6 hr after lipopolysaccharide (LPS) exposure. Animals received either LPS (2 mg/kg, intraperitoneal) or saline followed 90 min later by treatment with either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. They were killed 6 hr after LPS or saline exposure, and plasma activities of (A) alanine aminotransferase (ALT) and (B) isocitrate dehydrogenase (ICDH) were determined; N = 5–11. *Significantly different from respective value in the absence of LPS (p<0.05); †significantly different from respective value in the absence of Aroclor 1248 (p<0.05).

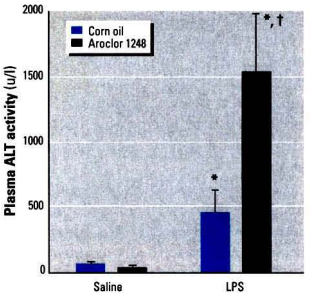


Figure 3. Aroclor 1248 enhances hepatic injury 9 hr after lipopolysaccharide (LPS) exposure. Animals received either LPS (2 mg/kg, intravenous) or saline followed 90 min later by treatment with either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. They were killed 9 hr after LPS or saline exposure, and plasma alanine aminotransferase (ALT) activity was determined; N = 4–11. *Significantly different from respective value in the absence of LPS (p< 0.05); †significantly different from respective value in the absence of Aroclor 1248 (p< 0.05).

ALT is a sensitive and specific indicator of hepatocellular damage in the rat, and in the co-culture system release of ALT from hepatocytes correlates with cell death measured by uptake of trypan blue and electron microscopy (*13*). Therefore, we used ALT activity in the medium as an index of injury to the hepatocytes. To examine the mechanism by which Aroclor 1248 interacts with PMNs and kills hepatocytes, hepatocytes were cultured with cell-free conditioned medium from PMNs exposed to Aroclor 1248. PMNs in suspension (2.5 × 10^6 cells/ml Williams' medium E containing 0.1% gentamicin) were pretreated with cytochalasin B (5 μg/ml) for 10 min at room temperature and then exposed to either vehicle (1 μl DMF/ml cells) or Aroclor 1248 (10 μg/ml) for 30 min at 37°C. The cells were removed by centrifugation, the cell-free supernatant was collected and added to the hepatocytes (2 ml/well), and release of ALT was determined 16 hr later as described above (*13*).

Statistical analysis. All results are presented as means ± standard error of the mean (SEM). For all results presented, N represents the number of individual animals and *in vitro* repetitions. Unless otherwise stated, data were analyzed by a two-way analysis of variance (ANOVA), and individual comparisons were performed using the least significant difference test. When variances were not homogenous, data were log-transformed before analysis. When appropriate, an outlier test (test for detection of extreme means) was applied (*40*). The criterion for statistical significance was p<0.05.

Results

Neither exposure of PMNs to vehicle nor to 1 μg/ml Aroclor 1248 resulted in generation of O_2^- (Fig. 1). However, PMNs exposed to 10 μg/ml Aroclor 1248 produced a significant amount of O_2^-. PMA alone caused an increase in O_2^- production. Exposure to 1 μg/ml Aroclor 1248 before stimulation with PMA caused significant generation of O_2^-. The amount of O_2^- produced in the presence of 1 μg/ml Aroclor 1248 and PMA was greater than the sum of O_2^- produced individually by these two agents. Pretreatment of PMNs with LPS did not alter O_2^- production in response to Aroclor 1248 (data not shown).

Exposure to 10 μg/ml, but not 1 μg/ml, Aroclor 1248 elicited a significant release of MPO from PMNs compared to vehicle control (Table 1). Release of MPO into the

Table 2. Aroclor 1248 potentiation of LPS-induced hepatotoxicity: histopathologic evaluation[a]

		% Rats with histopathologic score					
Treatment	*N*	0	1	2	3	4	5
Saline/corn oil	5	100	—	—	—	—	—
Saline/Aroclor	5	100	—	—	—	—	—
LPS/corn oil	11	27	18	36	18	—	—
LPS/Aroclor	9	11	—	11	33	22	22

LPS, lipopolysaccharide.
[a]Rats received either LPS (2 mg/kg, intravenous) or saline and 90 min later received either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. Animals were killed 6 hr after administration of LPS or saline, and liver sections were prepared from three different lobes. Severity of hepatic injury was graded on a scale of 0–5 reflecting the frequency and size of the hepatic lesions: 0 = no significant lesions; 1 = extremely mild necrosis; 2 = mild necrosis; 3 = moderate necrosis; 4 = marked necrosis; 5 = severe, coalescing necrosis.

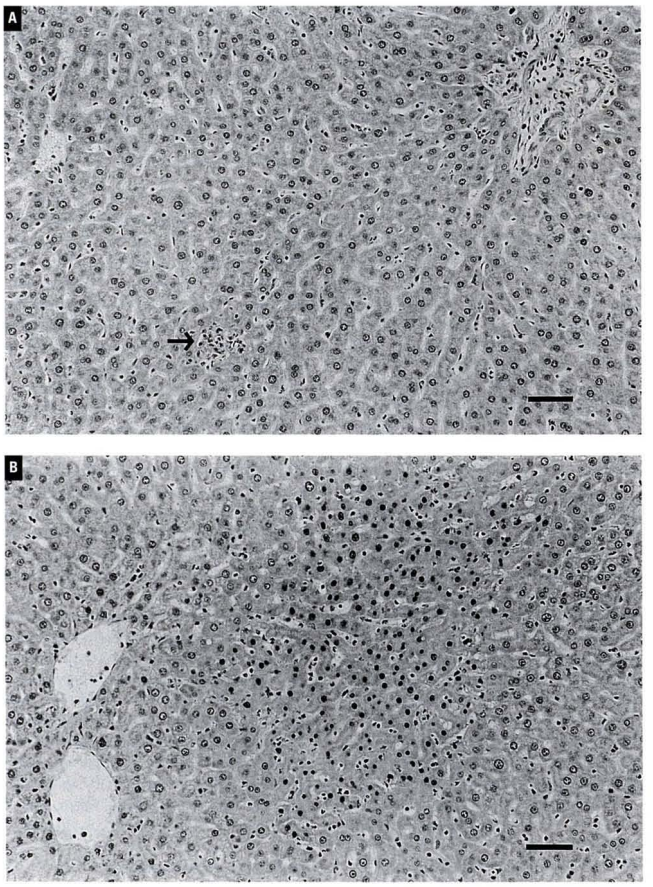


Figure 4. Photomicrographs of liver sections taken 6 hr after lipopolysaccharide (LPS) administration. (A) Liver from corn oil-treated animal given LPS had sinusoidal neutrophilia and a small lesion (arrow). (B) Liver from animal treated with Aroclor 1248 and LPS had sinusoidal neutrophilia and marked midzonal necrosis. Bar = 50 µm.

medium occurred in the absence of cytotoxicity as determined by release of LDH (data not shown). PMN degranulation in response to fMLP was included as a positive control.

Six hours after exposure to LPS alone, activities of ALT and ICDH in plasma were not significantly elevated (Fig. 2). Administration of Aroclor 1248 (50 mg/kg) 90 min after LPS exposure resulted in significant hepatic injury, as demonstrated from increased activities of ALT and ICDH (Fig. 2). Exposure to Aroclor 1248 in the absence of LPS had no effect on ALT or ICDH activities in plasma. Nine hours after administration of LPS alone, plasma ALT activity was increased compared to controls (Fig. 3). Activity of ALT in plasma of rats treated with Aroclor following LPS was significantly greater than in rats receiving LPS alone. Co-administration of Aroclor 1248 with LPS resulted in lethality in 10% of the animals. Lethality was not seen in animals treated with LPS or Aroclor 1248 alone. In a separate study, administration of a smaller dose of Aroclor 1248 (10 mg/kg) did not affect LPS-induced hepatic injury as determined by activities of ALT and ICDH in plasma (data not shown). Exposure to a larger dose of Aroclor 1248 alone (100 mg/kg) did not produce hepatic injury, as evidenced from activities of ALT and ICDH in plasma (74 ± 9 and 6 ± 1 U/l, respectively; not significantly different from vehicle controls) and histologic evaluation.

There were no significant lesions in the livers of vehicle-treated rats or rats exposed to Aroclor 1248 in the absence of LPS (Table 2). Six hours after LPS administration, livers from animals co-treated with either Aroclor 1248 or corn oil vehicle had lesions of varying degrees of severity (Fig. 4). The hepatic sinusoids of LPS-treated rats contained many PMNs, plump Kupffer cells, and small amounts of an eosinophilic, proteinaceous material. There were multifocal, irregularly shaped areas of midzonal hepatocellular necrosis. These lesions were characterized by hypereosinophilic parenchymal cells with small, pyknotic nuclei or by swollen, pale parenchymal cells with indistinct to absent nuclei and indistinct cytoplasmic borders. The necrotic foci contained degenerate PMNs. There were also foci of single-cell necrosis scattered throughout the tissue. Aroclor 1248 administration increased the severity of the hepatic lesions, but the nature of the lesions did not change.

PMNs were observed infrequently throughout the liver tissue from animals exposed to saline, regardless of co-treatment with Aroclor 1248 (Fig. 5). A marked

accumulation of PMNs was observed at 6 hr in livers from animals exposed to LPS, and this was not affected by co-administration of Aroclor 1248.

Isolated hepatocytes, either in the presence or absence of PMNs, were exposed to various concentrations of Aroclor 1248 for 16 hr (Fig. 6). Neither 1 µg/ml nor 10 µg/ml Aroclor 1248 was toxic to the hepatocytes. Addition of PMNs to the hepatocyte cultures did not produce cytotoxicity in the absence of Aroclor 1248. However, stimulation of the PMNs with either 1 µg/ml or 10 µg/ml Aroclor 1248 resulted in a significant release of ALT into the culture medium. In a similar experiment in which hepatocytes were incubated with cell-free conditioned medium from PMNs exposed to Aroclor 1248 or its vehicle, no significant differences were observed in these two groups (data not shown).

Discussion

Under normal circumstances, the liver is exposed to small amounts of gut-derived LPS originating from gram-negative bacteria residing in the gastrointestinal tract. However, during sepsis or numerous disease states exposure may increase, and LPS may initiate a systemic inflammatory response leading to significant morbidity and mortality. PMNs have been demonstrated to play a key role in the development of organ injury during sepsis (*6*). In such situations, enhancement of PMN activation or responsiveness by xenobiotic agents could lead to increased tissue injury. The ability of PCBs to enhance organ injury during an inflammatory event was examined in a model of LPS-induced hepatic injury. In this model, PMNs rapidly accumulate in large numbers in the hepatic sinusoids after administration of LPS and are required for development of hepatic injury (*8,10*). At the dose used in this study, rats exposed to LPS alone and killed 6 or 9 hr later developed mild hepatic necrosis. Administration of Aroclor 1248 increased the severity of the hepatic injury as evidenced by elevated ALT and ICDH activities in plasma and increased severity of hepatic lesions determined histologically. The pathological changes observed are consistent with previous studies describing hepatic injury in rats treated with LPS (*8,41*). In addition, co-treatment with Aroclor 1248 produced lethality, whereas no mortality occurred in animals exposed to LPS alone. Exposure to Aroclor 1248 alone up to 100 mg/kg did not produce hepatotoxicity.

These results are consistent with previous studies describing sensitization to LPS-induced lethality by pretreatment with PCBs. Mortality after administration of

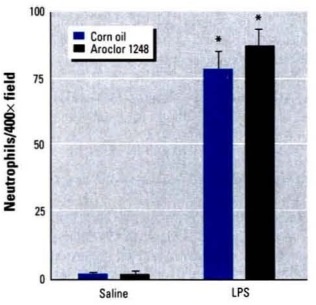


Figure 5. Hepatic neutrophil accumulation after lipopolysaccharide (LPS) exposure. Animals received either LPS (2 mg/kg, intravenous) or saline followed 90 min later by treatment with either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. They were killed 6 hr after LPS or saline exposure, the liver was removed, and sections prepared for immunohistochemistry as described in Materials and Methods. PMNs were counted in 20 400× midzonal fields throughout 3 separate lobes and averaged for each individual animal; $N = 5–11$. *Significantly different from respective value in the absence of LPS ($p < 0.05$).

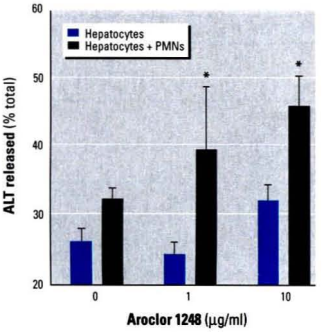


Figure 6. Aroclor 1248 stimulates PMN-mediated hepatocellular cytotoxicity in co-culture. Hepatocytes were cultured in the presence or absence of PMNs and exposed to either 0, 1, or 10 µg/ml Aroclor 1248. The activity of alanine aminotransferase (ALT) was determined in the cell-free medium and the cell lysates 16 hr later, and the percentage of total ALT released was calculated as described in Materials and Methods; $N = 5$. *Significantly different from respective value in the absence of PMNs ($p < 0.05$).

LPS was greater in mice fed Aroclor 1248 for 5 weeks compared to mice fed a control diet (*42*). Similarly, mice fed Aroclor 1242 for 6 weeks were more responsive to the lethal effects of LPS (*43*). Neither of these studies investigated damage to the liver or other organs. Other investigators have described a potentiation of LPS-mediated hepatotoxicity after exposure to compounds structurally related to some PCB congeners, i.e., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and polybrominated biphenyls (*44–46*). These studies involved treatment with halogenated biphenyls before administration of LPS so that effects on hepatic parenchymal cells, such as enzyme induction and alterations in gene expression, were likely to have occurred before LPS exposure and may have contributed to potentiation of toxicity. These effects may be related to Ah receptor-mediated events. In the present study, the direct effects of Aroclor 1248 on hepatic parenchymal cells cannot be ruled out; however, the treatment paradigm involved a short time (4.5 hr) between PCB exposure and evaluation of liver injury, so effects such as enzyme induction would be minimized.

PMNs begin accumulating in the hepatic sinusoids of rats within 30 min after LPS administration, and this event is a prerequisite for development of liver injury (*9*). Exposure to Aroclor 1248 did not affect hepatic PMN accumulation in LPS-treated rats; thus, potentiation of injury was not caused by increased numbers of PMNs in the liver. After absorption into the circulation, PCBs initially distribute in high concentration in the liver, which is a target organ for toxicity (*15,16,47*). Therefore, the presence of PCBs in the liver concurrent with PMN accumulation allows the possibility that PCBs affected the PMNs locally and that this contributed to increased hepatocellular damage. Indeed, isolated PMNs produced O_2^- and underwent degranulation upon exposure *in vitro* to Aroclor 1248, and Aroclor 1248 potentiated the production of O_2^- in response to PMA stimulation. PMA directly stimulates protein kinase C, leading to activation of the NADPH oxidase system and O_2^- production (*48*). These data with Aroclor 1248 are consistent with previous studies involving Aroclor 1242 and individual congeners of PCB (*23,26*).

PCBs initiate PMN responses within 15 min by activating the cellular signal transduction pathways responsible for these functions (*24,26*), thus providing a time-course for PMN activation that is consonant with the development of hepatic necrosis *in vivo*. This proposed mechanism for the enhancement of LPS-induced hepatic injury by Aroclor 1248 was supported by the results obtained using an *in vitro* co-culture system composed of isolated hepatocytes and PMNs. Aroclor 1248 was not toxic to the hepatocytes in the absence of PMNs; however, hepatocellular injury, as evidenced by increased release of ALT into the medium, occurred when hepatocytes were co-cultured with PMNs activated upon the addition of Aroclor 1248. The co-culture system is a

simplified model of events occurring *in vivo*, where PMN accumulation and adherence occurs before administration of Aroclor 1248. Our experiments involving cell-free conditioned medium from Aroclor 1248-treated PMNs suggested that either cell–cell contact between PMNs and hepatocytes is required for the mechanism by which Aroclor 1248 elicits PMN-mediated hepatocellular injury and/or the factor responsible for cytotoxicity is not stable enough to survive the time and process involved in preparing cell-free conditioned medium. This is not altogether surprising because Aroclor 1248 stimulates production of O_2^- by PMNs, which is highly reactive and short-lived. A combination of both reactive oxygen species and degranulation products may be involved in PMN-mediated killing of hepatocytes upon PCB exposure.

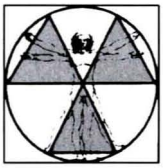
The mechanisms by which PMNs injure hepatic tissue are not fully understood. Recent studies suggest that proteases released from PMN granules can kill both endothelial cells and hepatocytes *in vitro* (*11,13*). Reactive oxygen species, such as O_2^-, may also play a contributing role in PMN-mediated tissue injury (*14*). Because Aroclor 1248 can elicit PMN O_2^- production and degranulation, both mechanisms may contribute to potentiating PMN-mediated tissue injury. It is also possible that PCBs may adversely affect hepatic parenchymal cells in a manner resulting in greater susceptibility to injury upon exposure to PMN-derived cytotoxic mediators.

In summary, Aroclor 1248 increased the hepatic injury that follows LPS administration. Because PMNs play a critical role in liver injury from LPS, these findings are consistent with previous studies demonstrating that PCBs can activate PMNs *in vitro* and sensitize animals to LPS-induced lethality. PCBs may act in a fashion analogous to inflammatory stimuli by activating PMNs to become cytotoxic, resulting in enhancement of PMN-mediated tissue injury during an inflammatory event. Studies with individual PCB congeners suggest that *ortho*-substituted PCBs can activate PMNs *in vitro*, whereas coplanar PCBs are inactive (*23*). Because Aroclor 1248 is a mixture of PCB congeners, the respective roles for both *ortho*-substituted and coplanar congeners in the mechanism for enhanced liver injury upon LPS exposure is uncertain at this time.

References

1. Parrillo JE. Pathogenetic mechanisms of septic shock. N Engl J Med 328:1471–1477 (1993).
2. Matuschak GM. Liver-lung interactions in critical illness. N Horizons 2:488–504 (1994).
3. Deitch EA, Sittig K, Li M, Berg R, Specian RD. Obstructive jaundice promotes bacterial translocation from the gut. Am J Surg 159:79–84 (1990).
4. Sedman PC, Macfie J, Sagar P, Mitchell CJ, May J, Mancey-Jones B, Johnstone D. The prevalence of gut translocation in humans. Gastroenterology 107:643–649 (1994).
5. Rietschel ET, Kirikae T, Ulrich Schade F, Mamat U, Schmidt G, Loppnow H, Ulmar AJ, Zahringer U, Seydel U, di Padova F, Schreier M, Brade H. Bacterial endotoxin: molecular relationships of structure to activity and function. FASEB J 8:217–225 (1994).
6. Hewett JA, Roth RA. Hepatic and extrahepatic pathobiology of bacterial lipopolysaccharides. Pharmacol Rev 45:381–411 (1993).
7. Windsor ACJ, Mullen PG, Fowler AA, Sugerman HJ. Role of the neutrophil in adult respiratory distress syndrome. Br J Surg 80:10–17 (1993).
8. Hewett JA, Schultze AE, VanCise S, Roth RA. Neutrophil depletion protects against liver injury from bacterial endotoxin. Lab Invest 66:347–361 (1992).
9. Pearson JM, Schultze AE, Jean PA, Roth RA. Platelet participation in liver injury from gram-negative bacterial lipopolysaccharide in the rat. Shock 4:178–186 (1995).
10. Jaeschke H, Farhood A, Smith CW. Neutrophil-induced liver cell injury in endotoxin shock is a CD11b/CD18-dependent mechanism. Am J Physiol 21:G1051–G1056 (1991).
11. Westlin WF, Gimbrone MA Jr. Neutrophil-mediated damage to human vascular endothelium. Role of cytokine activation. Am J Pathol 142:117–128 (1993).
12. Mavier P, Preaux A, Guigui B, Lescs M, Zafrani ES, Dhumeaux D. *In vitro* toxicity of polymorphonuclear neutrophils to rat hepatocytes: evidence for a proteinase-mediated mechanism. Hepatology 8:254–258 (1988).
13. Ganey PE, Bailie MB, VanCise S, Colligan ME, Madhukar BV, Robinson JP, Roth RA. Activated neutrophils from rat injured isolated hepatocytes. Lab Invest 70:53–60 (1994).
14. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 320:365–376 (1989).
15. Safe S. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. CRC Crit Rev Toxicol 13:319–395 (1984).
16. Safe S. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit Rev Toxicol 24:87–149 (1994).
17. Chang K, Hsieh K, Lee T, Tang S, Tung T. Immunologic evaluation of patients with polychlorinated biphenyl poisoning: determination of lymphocyte subpopulations. Toxicol Appl Pharmacol 61:58–63 (1981).
18. Chang K, Hsieh K, Tang S, Tung T, Lee T. Immunologic evaluation of patients with polychlorinated biphenyl poisoning: evaluation of delayed-type skin hypersensitive response and its relation to clinical studies. J Toxicol Environ Health 9:217–223 (1982).
19. Kimbrough RD. Polychlorinated biphenyls (PCBs) and human health: an update. CRC Crit Rev Toxicol 25:133–163 (1995).
20. Chang K, Hsieh K, Lee T, Tung T. Immunologic evaluation of patients with polychlorinated biphenyl poisoning: determination of phagocyte Fc and complement receptors. Environ Res 28:329–334 (1982).
21. Tryphonas H, Luster MI, Schiffman G, Dawson LL, Hodgen M, Germolec D, Hayward S, Bryce F, Loo JCK, Mandy F, Arnold DL. Effect of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the Rhesus (*Macaca mulatta*) monkey. Fundam Appl Toxicol 16:773–786 (1991).
22. Hori S, Obana H, Kashimoto T, Otake T, Nishimura H, Ikegami N, Kunita N, Uda H. Effect of polychlorinated biphenyls and polychlorinated quaterphenyls in cynomolgus monkey (*Macaca fascicularis*). Toxicology 24:123–139 (1982).
23. Ganey PE, Sirois JE, Denison M, Robinson JP, Roth RA. Neutrophil function after exposure to polychlorinated biphenyls *in vitro*. Environ Health Perspect 101:430–434 (1993).
24. Tithof PK, Contreras ML, Ganey PE. Aroclor 1242 stimulates the production of inositol phosphates in polymorphonuclear neutrophils. Toxicol Appl Pharmacol 131:136–143 (1995).
25. Tithof PK, Schiamberg E, Peters-Golden M, Ganey PE. Phospholipase A_2 is involved in the mechanism of activation of neutrophils by polychlorinated biphenyls. Environ Health Perspect 104:52–58 (1996).
26. Brown AP, Ganey PE. Neutrophil degranulation and superoxide production induced by polychlorinated biphenyls are calcium dependent. Toxicol Appl Pharmacol 131:198–205 (1995).
27. Albro PW, Corbett JT, Schroeder JL. Quantitative characterization of polychlorinated biphenyl mixtures (Aroclors® 1248, 1254 and 1260) by gas chromatography using capillary columns. J Chromatogr 205:103–111 (1981).
28. Bergmeyer HU, Scheibe P, Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin Chem 24:58–73 (1978).
29. Hewett JA, Roth RA. Dieldrin activates rat neutrophils *in vitro*. Toxicol Appl Pharmacol 96:269–278 (1988).
30. Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. J Clin Invest 52:741–744 (1973).
31. Hoffstein ST. Intra- and extracellular secretion from polymorphonuclear leukocytes. In: The cell biology of inflammation. Handbook of inflammation (Weissman G, ed). Amsterdam:North Holland Biomedical Press, 1980;387–430.
32. Smolen JE. Characteristics and mechanisms of secretion by neutrophils. In: The neutrophil: cellular biochemistry and physiology (Hallett MB, ed). Boca Raton, FL:CRC Press, 1989;23–61.
33. Marasco WA, Phan SH, Krutzsch H, Showell HJ, Feltner DE, Nairn R, Becker EL, Ward PA. Purification and identification of formyl-methionyl-leucyl-phenylalanine as the major peptide neutrophil chemotactic factor produced by *Escherichia coli*. J Biol Chem 259:5430–5439 (1994).
34. Tennenberg SD, Zemlan FP, Solomkin JS. Characterization of *N*-formyl-methionyl-leucyl-phenylalanine receptors on human neutrophils. Effects of isolation and temperature on receptor expression and functional activity. J Immunol 141:3937–3944 (1988).
35. Grisham MB, Everse J, Janssen HF. Endotoxemia and neutrophil activation *in vivo*. Am J Physiol 254:H1017–H1022 (1988).

36. Klebanoff SJ, Waltersdorph AM, Rosen H. Antimicrobial activity of myleoperoxidase. Meth Enzymol 105:399–403 (1984).
37. Bergmeyer HV, Bernt E. Lactate dehydrogenase UV assay with pyruvate and NADH. In: Methods of enzymatic analysis (Bergmeyer HV, ed). New York:Academic Press, 1974;524–579.
38. Seglen PO. Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. Exp Cell Res 82:391–398 (1973).
39. Klaunig JE, Goldblatt PJ, Hinton DE, Lipsky MM, Chacko J, Trump BF. Mouse liver cell culture. I. Hepatocyte isolation. In Vitro 17:913–925 (1981).
40. Dixon WJ, Massey FJ Jr. Introduction to statistical analysis. New York:McGraw-Hill, 1969;328.
41. Hewett JA, Roth RA. The coagulation system, but not circulating fibrinogen, contributes to liver injury in rats exposed to lipopolysaccharide from gram-negative bacteria. J Pharmacol Exp Ther 272:53–62 (1995).
42. Thomas PT, Hinsdill RD. Effect of polychlorinated biphenyls on the immune responses of rhesus monkeys and mice. Toxicol Appl Pharmacol 44:41–51 (1978).
43. Loose LD, Silkworth JB, Pittman KA, Benitz KF, Mueller W. Impaired host resistance to endotoxin and malaria in polychlorinated biphenyl and hexachlorobenzene-treated mice. Infect Immunol 20:30–35 (1978).
44. Rosenthal GJ, Lebetkin E, Thigpen JE, Wilson R, Tucker AN, Luster MI. Characteristics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced endotoxin hypersensitivity: association with hepatotoxicity. Toxicology 56:239–251 (1989).
45. Clark GC, Taylor MJ, Tritscher AM, Lucier GW. Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated endotoxin hypersensitivity in C57BL/6J mice congenic at the Ah locus. Toxicol Appl Pharmacol 111:422–431 (1991).
46. Shedlofsky SI, Hoglen NC, Rodman LE, Honchel R, Robinson FR, Swim AT, McClain CJ, Robertson LW. 3,3′,4,4′-Tetrabromobiphenyl sensitizes rats to the hepatotoxic effects of endotoxin by a mechanism that involves more than tumor necrosis factor. Hepatology 14:1201–1208 (1991).
47. Matthews HB, Anderson MW. Effect of chlorination on the distribution and excretion of polychlorinated biphenyls. Drug Metab Dispos 3:371–380 (1975).
48. Tauber AI. Protein kinase C and the activation of the human neutrophil NADPH-oxidase. Blood 69:711–720 (1987).



International Conference on Radiation and Health

sponsored by Ben Gurion University of the Negev, Beer Sheva, Israel

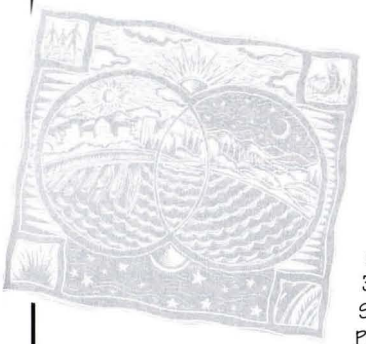
November 3–7, 1996

Subjects of the meeting will include exposure of human populations to both ionizing and nonionizing radiations, physical and biological methods of dosimetry, molecular radiation biology, metabolism of radioisotopes at cell, tissue and whole-body level, epidemiological procedures and results of ongoing studies of Chernobyl exposure, monitoring programs for evaluating population impact which would be important in assessing intervention, problems of occupational exposures, detection of long term-effects (carcinogenic, genetic, and nonneoplastic), risk assessment, and psycho-social considerations including counseling and the evaluation of radiation-associated postraumatic stress disorders.

For further information:
Conference Secretariat, Ortra Ltd., 2 Kaufman Street, PO Box 50432, Tel Aviv 61500, Israel
972-3-517-7888 FAX: 972-3-517-4433 E-mail: ortra@trendline.co.il

The Coastal Society

Seeking Balance: Conflict, Resolution and Partnership



15th International Conference
July 14–17, 1996
Seattle, Washington

Megan D. Bailiff, Conference Chair
The Coastal Society 15th International Conference
c/o Washington Sea Grant Program
3716 Brooklyn Ave. NE
Seattle, WA 98105
Phone: (206) 685-1108 Fax: (206) 685-0380
mbailiff@u.washington.edu

A Nested Case–Control Study of Kidney Cancer among Refinery/Petrochemical Workers

John F. Gamble, Eileen D. Pearlman, and Mark J. Nicolich

Exxon Biomedical Sciences, Inc., East Millstone, NJ 08875-2350 USA

A nested case–control study was designed to evaluate whether a nearly twofold excess of kidney cancer among workers at a refinery/petrochemical plant was associated with cumulative exposure to C_2–C_5 saturated, C_2–C_5 unsaturated, C_6–C_{10} aliphatic saturated, C_6–C_{10} aliphatic unsaturated, and C_6–C_{10} aromatic process streams. Nonoccupational risk factors were body mass index (BMI), blood pressure (both measured at about age 28), and smoking. There was no significant association with cumulative exposure or tenure as estimated by conditional logistic regression and adjusted for nonoccupational risk factors. Categorical analysis showed increased odds ratios only in the second (low) and fourth (high) quartiles compared to the first quartile reference group of lowest exposed workers, and a three-quarter–fold increased odds ratio for >32 years' tenure compared to the <25-year reference group. The number of cases was small with wide confidence intervals around estimate of risk, so the possibility of an exposure–response trend cannot be ruled out. Multivariate analysis identified overweight (high BMI; $p<0.01$) as the most important risk factor in this data set, followed by tenure and increased blood pressure. There was a weak association with current smoking, but not with pack-years smoked. The risk of kidney cancer for a nonsmoker with normal blood pressure but 25% overweight was increased about 2.6-fold (95% CI = 1.2–5.4). The risk of kidney cancer for a nonsmoker of normal weight with high blood pressure (e.g., 150/110), was increased about 4.5 (95% CI, 0.8–26). *Key words*: blood pressure, case–control study, kidney cancer, petrochemical workers, petroleum hydrocarbons, refinery workers, smoking. *Environ Health Perspect* 104:642–650 (1996)

Mortality studies of workers at a refinery and petrochemical plant have shown about a 55% kidney cancer excess (*1*) and a nearly twofold excess after 8 more years of follow-up (*2*). There is little evidence from cohort studies (with the possible exception of distribution workers) to suggest that workers in the petroleum industry are at increased risk of kidney cancer (*3,4*). However, there is also no strong evidence to the contrary. The evidence from case–control studies is contradictory. Nested case–control studies within petroleum worker cohorts show no apparent association with exposure (*5,6*). However, several population-based case–control studies show an increased risk of kidney cancer related to exposure to petroleum products (*7*). Overall, the studies of humans are inconclusive regarding potential risk.

Beginning in the early 1980s, there is an extensive body of experimental data showing that a wide variety of chemicals (primarily C_6–C_{10} saturated aliphatics) produce renal tumors in the male rat. The predominant hypothesis regarding the mechanism ($\alpha 2_\mu$-globulin nephropathy) (*8,9,10*) has been considered irrelevant in evaluating carcinogenicity to humans of petroleum hydrocarbons. A recent review points out inconsistencies in the hypothesis and suggests the possibility that these hydrocarbons may be carcinogenic in themselves (*11,12*). Experimental data are valuable for assessing the potential for human carcinogenicity by providing, or not providing, a biologically plausible mechanism. Experimental data appear to support the evaluation of the Environmental Protection Agency (*13*) that the response of male rats to these chemicals is probably not relevant to humans. There is not at present a plausible mechanism to explain why there might be an association between exposure to petroleum products and kidney cancer.

To further test the hypothesis that hydrocarbons cause kidney cancer, we selected the refinery/petrochemical plant cohort studied by Shallenberger et al. (*2*) to conduct a nested case–control study. The major objectives of the study were to estimate the relative risk of cumulative exposure to the following process streams: C_2–C_5 saturated hydrocarbons, C_2–C_5 unsaturated hydrocarbons, C_6–C_{10} aliphatic saturated hydrocarbons, C_6–C_{10} aliphatic unsaturated hydrocarbons, and C_6–C_{10} aromatics.

Although the etiology of kidney cancer is little understood, there are several nonoccupational risk factors consistently associated with kidney cancer and for which data in this study are available. The odds ratios for obesity (generally measured as body mass index, or BMI) in six studies ranged from 1.2 to 3.3 (*14–20*). One study showed trends for the risk in males to increase as BMI increased, with greater than a twofold increased risk in the fourth quartile compared to first quartile for three measures of BMI: at age 20, most recent BMI, and highest BMI (*19,20*). Kadamani et al. (*21*) suggested there was a positive interaction between high BMI and hydrocarbon exposure.

The odds ratios associated with high blood pressure ranged from 1.5 to 2.9 in three of four case–control studies (*15,16,22*), and was below 1.0 in the fourth (*23*). Raynor et al. (*24*) found that only kidney cancer cases (out of 10 other sites) had systolic blood pressure values above the average of the total cohort (162 mm Hg versus 134 mm Hg).

There are 11 case–control studies and 4 prospective studies that evaluated the risk of kidney cancer by a quantitative measure of cigarettes smoked. Ten of the 11 case–control studies had odds ratios ranging from 1.3 to 4.7 for the heaviest smoking categories. The risk ratios in the four prospective studies ranged from 1.2 to 3.0. Thus, there was a consistent pattern showing smokers at increased risk of kidney cancer (*14–17,19,20,23,25–33*).

Secondary objectives in this study were to evaluate the risks that obesity (measured as BMI), blood pressure, and smoking pose for kidney cancer in this study population and to control for them while evaluating hydrocarbon exposure.

Methods

Definition of Cases and Controls

All cases and controls were part of the cohort studied by Shallenberger et al. (*2*), with at least 1 month of service at the refinery and chemical plant sites between 1 January 1970 and 31 December 1992. Current employees and employees retired before 1970 and still

Address correspondence to J. F. Gamble, Exxon Biomedical Sciences, Inc., Mettlers Road, CN 2350, East Millstone, NJ 08875-2350 USA.

We thank all those persons at the refinery and petrochemical plant who through their knowledge and experience helped make it possible to classify jobs and departments by exposure, particularly the members of the industrial hygiene departments. We appreciate the assistance in collecting risk factor information, particularly Pat Dupont for her work on the annuitant questionnaires. Micki Vodarsik, Fran Merlino, and Gail Jorgensen were instrumental in data collection, entry, and editing. Linda Smart, Lauri Mackenzie, and Vicki Fowler did the typing. This project was funded by Exxon Chemical Americas and Exxon Company, USA.

Received 18 May 1995; accepted 7 February 1996.

alive as of 1 January 1970 are included. Eligible cohort members were determined from company personnel and payroll records. Vital status and death certificates were obtained from company files and from states where decedents were identified through the National Death Index and the Social Security Administration. Follow-up was through 1990. Cause of death was coded by a trained nosologist according to the eighth revision of the International Classification of Diseases.

Cases were defined as anyone diagnosed with renal cell carcinoma or renal cell adenocarcinoma (ICD8 189; hereafter referred to as kidney cancer). Confirmation of kidney cancer is from the death certificate (either primary or secondary cause of death) or from pathology or bioassay reports from the company tumor registry. Date of diagnosis or date of death were determined from the death certificate or tumor registry report and were used to estimate time at risk. There were 32 cases with only date of death and this was the time used to estimate latency; 5 cases were not dead, for a total of 37 cases.

Four controls per case were selected at random from the set of persons in the cohort who were at risk when the case was at risk, and frequency matched on sex (all males), race, date of birth (DOB) (±1 year), date of hire (DOH) (±3 years), and at-risk status (control must be alive and free of disease at the date of diagnosis or date of death of the cases.) A control also had to have in the records a work history to be eligible. A control could only be selected once and could not be a control for more than one case. The work history of the controls was stopped at the date of death or date of diagnosis of the case. Out of a total cohort of 9894, there were 2473 eligible controls, with a range of 6–253 eligible controls per case.

Abstraction of Records

Information from questionnaires and clinical medical forms were abstracted from each employee's personnel file without knowledge of case or control status. Height, weight, blood pressure, and cholesterol were recorded when available from initial exam, and for the years closest to ages 40 and 55. Information on tobacco and alcohol use were recorded for initial and last entries and when there were any changes in between. Any reports on history (such as whether parents had kidney cancer, whether subjects had kidney stones, cysts, and exposure to X-rays) were also recorded from the physicians' reports. Since only weight, height, blood pressure, and smoking history were systematically recorded, these were the only demographic data used in the analyses.

All sources were used to estimate an average tobacco consumption. For cigarette smokers, an average of cigarettes smoked per day and total pack-years (packs smoked/day × years smoked) were calculated. The major sources of smoking history were a survey conducted in 1959, annuitant surveys in 1986 and 1991, and physicians' notes. Four cases and 10 controls had no smoking history.

Exposure History

There are several sources of work history data. Jobs held before 1977–1978 were summarized from personnel records and include all unique job locations. From 1978 to 1979, jobs had been computerized on an annual basis, and after 1988 on a monthly basis. For controls, the work history ended at the date of diagnosis or death of the case, whichever came first. For cases, the work history ended at either the date of termination or date of death (or diagnosis), whichever came first.

A four-step process was used by an industrial hygienist to rate the relative potential for exposure for each job title and job department/location combination. A "high" rating indicates a greater potential for significant exposure compared to ratings of "medium" or "low": 1) rate each job title: L = background level, office work only; ML = minimal exposure to fugitive emissions only, time is spent in the plant but no hands-on work, personnel loaned to other locations; MH = plant jobs but primarily in control rooms or in activities where exposure is intermittent and/or less intense than the high category; H = primary activities are in the plant with the highest potential for exposure (e.g., assistant operators, laborers, certain maintenance positions); 2) rate each job department/location for each process stream: L = unlikely to be present or present at low levels as a contaminant or extreme end of the process or product stream; M = present as part of process stream but not as a major constituent; H = major or primary component of the process feed/product stream; 3) develop an exposure matrix of job title ratings and job department/location ratings and assign numerical values: H/H = 4; H/MH and MH/MH = 3; ML/ML = 2; and H/L, MH/L, ML/L, and L/L = 1; 4) apply the matrix to each combination for each stream.

The primary factor determining the level of exposure was the job title. The primary determinant of the process stream or hydrocarbon stream was the department or location. A low rating in either category automatically resulted in a low rating for the combination.

Mechanical-related jobs were the most problematic for exposure scoring. It was not always clear whether maintenance personnel rotated through various locations or whether in the past they were assigned long term to specific process areas/units. Before 1966 this function made no distinction between refinery and chemical plant operations. Use of individual job histories helped in the identification of particular locations and process stream exposures. Most mechanical locations were rated high exposure, except for construction and/or planning, which were rated medium exposure.

There were two major time periods when significant changes occurred. The first was in the early 1940s with the implementation of a process engineering staff. This led to better designs and implementation of process controls. The second major period was the early 1970s with the advent of computerized controls, environmental regulation, and upgrading of job classifications. This resulted in better exposure control as well as the addition/elimination/change of job titles and tasks. The late 1960s/early 1970s also saw major changes in pumps and compressors: packing was replaced with mechanical seals and reciprocating equipment was replaced with centrifugal equipment. All of this led to a decrease in leakage and maintenance.

Another aspect of changes over time is the introduction/elimination of specific process units. As units change, the types and relative degree of exposure will change. This may also lead to changes in task activities and subsequent exposures of a particular job title. However, there was not enough historical information to estimate the magnitude of any change. The historical changes were, therefore, assumed to affect absolute exposure levels but not relative exposures between jobs. Cases and controls should be equally affected by these changes because they were matched on DOH.

Primary process stream composition is relatively consistent and traceable over time. The hypothesis being tested relates to petroleum hydrocarbons. Five primary process streams were identified, based on process operations and the ability to differentiate between streams at the various process units. The five streams identified were C_2–C_5 saturated (alkanes/paraffins, e.g., propane); C_2–C_5 unsaturated (alkenes/olefins, e.g., propene, 1,3-butadiene); C_6–C_{10} aliphatic saturated (paraffins/cycloparaffins, e.g., octane, cyclohexane); C_6–C_{10} aliphatic unsaturated hydrocarbons (olefins/diolefins, e.g., octene, decahydronaphthalene or decalin); and C_6–C_{10} aromatics (e.g., benzene, toluene).

Process streams in the refinery were considered to consist primarily of saturated

streams. Process streams in the chemical plant were considered to be primarily unsaturated or aromatic streams. A major exception was steam cracking units in the chemical plant where saturated refining feed stocks were used to generate unsaturated streams for downstream chemical plant feed stocks.

Exposure Classification

The characterization of exposure–response relationships is an objective of epidemiologic investigation and is a major criterion by which to evaluate causality. It was the primary purpose of this study to evaluate exposure–response relationships using hydrocarbon streams as the major exposure variable. Because the effect is a chronic one, cumulative exposure received over time is considered the most relevant estimate of exposure.

Cumulative exposure in this study is defined as score-years. Score-years is the summation of job exposure rating × tenure in that job. For example, score-years for an individual exposure to aromatics is calculated as follows: job title/locations ratings for helper/mechanic and foreman/mechanic are 4 and 2, respectively. If a person worked 8 and 15 years in each job, their score-years is $\Sigma(4\times8)+(2\times15)=62$, and tenure = 23 years.

Tenure or employment duration is a surrogate marker of total exposure. Tenure can be a reliable surrogate when there is one predominant hazardous exposure and when the following conditions are met (*34*): relatively constant concentrations throughout the plant, relatively constant concentrations over time, assignment to jobs with high and low exposure concentrations is unrelated to tenure, and nonoccupational risk factors do not vary according to tenure.

In this study population, probably none of the conditions are met with consistency. There is not a constant concentration throughout the plant, and persons with greater seniority and training tend to move into jobs with decreasing intensity of exposure. The condition of a relatively constant concentration is not met in the total cohort, but matching cases and controls on DOH makes this condition less relevant. Nonoccupational risk factors are indirectly related to tenure in that increasing weight and higher blood pressure tend to increase with age, as does increasing tenure. When there are multiple potentially hazardous exposures (e.g., process streams, possibly asbestos), analysis of tenure effects has a nonspecific interpretation.

Misclassification of exposure is less likely when intensity is included in estimates of exposure. Cumulative exposure is preferred over tenure because it attempts to take into account varying concentrations throughout the plant, job assignment, and multiple exposures.

Different jobs have different intensities of exposure associated with them, but for tenure, all jobs assume a similar intensity. The ratio of score-years to tenure indicates a range of values from 1/1 (lowest-exposed job) to 4/1 (highest-exposed job).

Statistical Analysis

The odds ratios were estimated based on both a continuous score and a grouped (discrete) score. For example, for the continuous analysis, the observed BMI was the variable, and for the discrete analysis, the BMI scores were categorized into four classes of approximately equal size (>21, 21–23, 23–25, and ≥25). For the discrete case, the odds ratio is the ratio of the odds for a given class relative to the base (reference) class. For the continuous score, the odds ratio is the change in the ratio of the odds when the risk variable is increased by one unit. The advantage of the continuous method is that it does not depend on the groupings chosen for the classes; the disadvantage is that it assumes the log of the odds ratio is a straight line function of the risk factor. Tables 2–6 present the odds ratios for the grouped and continuous analysis, each with and without an adjustment for lifestyle factors.

Both unadjusted and adjusted coefficients for trend are calculated. The unadjusted coefficient has only a single variable in the conditional logistic regression. The adjusted coefficient represents the effect of × adjusted for the effects of the other variables. For the three nonoccupational risk factors, the adjustment is only for the other two nonoccupational risk factors and without consideration of workplace exposure variables. For the exposure variables (tenure, score-years), the adjusted exposure coefficient adjusts for the effects of BMI, blood pressure, and pack-years smoked. The three nonoccupational risk factors included in these analyses are 1) BMI as a measure of obesity = [pounds/(height in inches)^2] × 703.1; 2) mean arterial pressure (MAP) as a measure of blood pressure = (systolic BP − diastolic BP)/3 + diastolic BP; and 3) smoking: pack-years smoked = Σ(packs of cigarettes smoked/day) (years smoked); nonsmokers, never smoked cigarettes; ex-smokers, stopped smoking >1 year before end of at-risk status; smokers, >100 cigarettes in a lifetime. Pack-years is the variable used to estimate adjusted ORs and coefficients for the other variables, but grouped analyses by smoking category is also provided.

Results

Table 1 summarizes the characteristics of the 37 kidney cancer cases and 148 controls. Age and year of hire were quite similar between cases and controls, with DOB ranging from 1893 to 1944 and DOH from 1916 to 1980. The number of pack-years smoked by cases and controls was similar, but the proportion of ever-smokers was dissimilar. About one-fourth of the cases and controls were never-smokers. Among controls, the proportion of smokers and former smokers was about 50/50, but among cases was about 3/1. On average, the

Table 1. Characteristics of kidney cancer cases and controls

Characteristic	Case	Control
n	37	148
Mean year of birth (range)	1913.4 (1894–1944)	1913.5 (1893–1943)
Mean year of hire (range)	1941.4 (1919–1979)	1941.05 (1916–1980)
Nonsmokers, *n* (%)[a]	9 (27)	32 (23)
Former smokers, *n* (%)	6 (18)	55 (40)
Mean pack-years (SD)[b]	18.3 (14.1)	15.2 (13.4)
Smokers, *n* (%)	18 (55)	52 (37)
Mean pack-years (SD)	25.7 (17.2)	26.5 (16.1)
Mean tenure (SD; range)	30.7 (8.9; 1.4–50)	29.7 (8.5; 0.1–44)
Cumulative exposure scores, mean (SD; range)		
C_2–C_5 saturated	96.92 (36.1; 15–165)	88.58 (34.1; 0.5–154)
C_2–C_5 unsaturated	90.40 (33.7; 15–162)	84.44 (34.0; 0.5–154)
C_6–C_{10} aliphatic saturated	93.25 (38.0; 15–162)	86.64 (34.3; 0.5–154)
C_6–C_{10} aliphatic unsaturated	87.70 (34.75; 15–162)	82.99 (33.44; 0.5–154)
C_6–C_{10} aromatics	90.19 (37.2; 15–162)	84.27 (34.5; 0.5–162)
Body mass index, initial exam, mean (SD; range)[c]	24.5 (3.0; 18.6–31.4)	22.9 (2.8; 16.6–35.3)
Mean arterial pressure, initial exam, mean (SD)[d]	97.5 (7.8)	94.5 (7.6)
Blood pressure, initial exam, systolic/diastolic (SD)	129/82 (10.6/8.3)	125/79 (10.6/8.1)
Age at initial exam, mean years (SD)[e]	28.6 (6.7)	27.6 (6.5)

[a] *n* = 33 cases and 139 controls with known smoking history.
[b] Pack-years for 52 controls.
[c] Cases, *n* = 34; control, *n* = 141.
[d] Control, *n* = 145.
[e] Control, *n* = 145.

cases tended to have slightly increased BMI, blood pressure, and cumulative exposure scores compared to controls.

Tables 2–4 summarize the data showing the association of BMI, MAP, and smoking with risk of kidney cancer. Both adjusted and unadjusted risk ratios increase with increasing BMIs above the third quartile category. The adjusted regression coefficient indicates the risk ratio increases 0.17 for each unit increase in BMI (Table 2). That is, compared to a nonsmoker with normal blood pressure (120/80 with a MAP of 93.3) and an ideal BMI of 22.1 (*35*), a person 125% overweight (BMI = 27.6) has a calculated risk ratio of 2.58 (95% CI, 1.20–5.41).

The risk of kidney cancer tends to increase as arterial blood pressure increases, both with and without adjustment for BMI and smoking (Table 3). That is, a nonsmoker with ideal BMI but with hypertension (blood pressure = 150/110, MAP = 123.3) has a risk ratio of 4.48 (95% CI, 0.77–26.2) compared to a person with normal blood pressure (120/80). Although the point estimate shows a nearly fivefold increased risk, the 95% CI is quite wide.

There is no strong association of kidney cancer with smoking in this study population, either by smoking category or by pack-years smoked (Table 4). The calculated risk ratio is 1.32 (95% CI, 0.44–4.0) for a smoker with 40 pack-years but normal blood pressure and ideal BMI. Nevertheless, the pack-years variable is included (as well as BMI and MAP) in the calculation of adjusted values estimating the association with cumulative exposure.

When all three of the nonoccupational potential risk factors (high BMI, hypertension, heavy smoker) are present, the calculated risk ratio based on the equation ln OR = 0.172 (BMI) + 0.052 (MAP) + 0.007 (pack-years) is 14.4 with a 95% CI of 0.44–605.

Both adjusted and nonadjusted odds ratios tend to increase as tenure increases (Table 5). The odds ratios are increased three- and fourfold for the two groups with the longest tenure compared to the reference group with <25 years' tenure. The trend is close to significance in the conditional logistic regression model.

Table 6 summarizes the data showing the association of cumulative exposure with the risk of kidney cancer. None of the process streams show a statistically significant trend for the risk to increase as estimated cumulative exposure increases. The patterns in the grouped process stream data are similar, with the second and fourth quartiles showing higher risk ratios than the third quartile, which has point estimates around 1. That is, the risk ratio does not show a linear increase as cumulative exposure increases. The adjusted risk ratios are generally larger and have wider confidence intervals than unadjusted risk ratios. When a linear increase is assumed and the logistic regression models are used to estimate risk ratios (high exposed with a cumulative exposure score of 140 compared to low exposure with a cumulative exposure score of 30), the adjusted odds ratios and 95% CIs are as follows: stream 1, C_2–C_5 saturated, 4.18 (0.60–29.1); stream 2, C_2–C_5 unsaturated, 4.18 (0.74–23.5); stream 3, C_6–C_{10} aliphatic saturated, 3.74 (0.54–26.1); stream 4, C_6–C_{10} aliphatic unsaturated, 3.35 (0.48–23.3); stream 5, C_6–C_{10} aromatic, 2.41 (0.35–16.8).

The categorical analysis suggested a nonmonotonic increase, although the odds

Table 2. Odds ratios (OR) and coefficients from logistic regression analyses of the association of kidney cancer and body mass index (BMI)[a]

BMI	Cases	Controls	Adjusted[b] OR	Adjusted[b] 95% CI	Unadjusted OR	Unadjusted 95% CI
≥25	13	28	3.29	0.93–11.62	3.18	1.00–10.13
23–25	10	30	2.47	0.68–8.98	2.41	0.73–8.02
21–23	6	44	0.93	0.22–3.84	1.16	0.31–4.34
<21	5	39	1.0		1.0	

[a]Coefficient (SE) for trend in BMI adjusted for mean arterial pressure (MAP) and pack-years: 0.17 (0.07), $p<0.01$; coefficient (SE) for trend in BMI, unadjusted: 0.15 (0.06), $p<0.02$. Adjusted logistic regression equation: ln OR = 0.172 (BMI) + 0.052 (MAP) + 0.007 (pack-years).
[b]Adjusted for MAP and pack-years smoked.

Table 3. Odds ratios (OR) and coefficients from logistic regression analyses of the association of kidney cancer and mean arterial pressure (MAP)[a]

MAP	Cases	Controls	Adjusted[b] OR	Adjusted[b] 95% CI	Unadjusted OR	Unadjusted 95% CI
≥103	10	20	5.76	0.94–35.28	4.47	1.06–18.58
88–103	24	100	3.27	0.58–18.65	2.01	0.55–7.32
<88	3	25	1.0		1.0	

[a]Coefficient (SE) for trend in MAP adjusted for body mass index (BMI) and pack-years: 0.05 (0.03), $p<0.08$; coefficient (SE) for trend in MAP, unadjusted: 0.06 (0.03), $p<0.03$. See Table 2 for adjusted logistic regression with all nonoccupational variables included.
[b]Adjusted for BMI and pack-years smoked.

Table 4. Odds ratios (OR) and coefficients from logistic regression analyses of the association of kidney cancer and smoking cigarettes[a]

Smoking category	Cases	Controls	Adjusted[b] OR	Adjusted[b] 95% CI	Unadjusted OR	Unadjusted 95% CI
Smoker	18	52	1.36	0.44–4.19	1.21	0.44–3.35
Ex-smoker	6	55	0.58	0.17–1.97	0.37	0.12–1.18
Nonsmoker	9	32	1.0		1.0	

[a]Coefficient (SE) for trend in pack-years adjusted for body mass index (BMI) and mean arterial pressure (MAP): 0.007 (0.014), $p<0.62$; coefficient (SE) for trend in pack-years, unadjusted: 0.005 (0.012), $p<0.66$. See Table 2 for adjusted logistic regression using pack-years as the smoking variable. Adjusted logistic regression equation: ln OR = 0.139 (BMI) + 0.049 (MAP) – 0.552 (ex-smoker) + 0.309 (smoker).
[b]Adjusted for BMI and MAP.

Table 5. Odds ratios (OR) and coefficients from logistic regression analyses of the association of kidney cancer and tenure (years employed)[a]

Tenure (years)	Cases	Controls	Adjusted[b] OR	Adjusted[b] 95% CI	Unadjusted OR	Unadjusted 95% CI
≥38	9	27	4.08	0.24–68.72	6.68	0.47–94.74
32–38	9	39	3.26	0.27–39.72	3.01	0.27–33.47
25–32	9	39	1.34	0.23–7.77	1.37	0.29–6.44
<25	10	43	1.0		1.0	

[a]Coefficient (SE) for trend in tenure adjusted for body mass index (BMI), mean arterial pressure (MAP), and pack-years: 0.16 (0.09), $p<0.07$; coefficient (SE) for trend in tenure, unadjusted: 0.16 (0.08) $p<0.04$. Adjusted logistic regression equation for tenure: ln OR = 0.160 (BMI) + 0.055 (MAP) + 0.006 (pack-years) + 0.16 (tenure).
[b]Adjusted for BMI, MAP, and pack-years smoked.

Table 6. Summary of odds ratios (OR) and coefficients from logistic regression analyses of the association of kidney cancer and exposure to petroleum hydrocarbon measured as cumulative exposure (score-years)

Process stream	Adjusted OR (95% CI) by quartile of cumulative exposure				Trend coefficients (SE)	
	<65	65–90	90–115	≥115	Adjusted	Unadjusted
C_2–C_5 saturated	1	4.85 (1.0–23.6)	1.34 (0.25–7.1)	4.94 (0.88– 27.4)	0.013 (0.009)	0.012 (0.007)
C_2–C_5 unsaturated	1	4.28 (1.05–17.5)	0.52 (0.07–3.7)	5.09 (0.79–32.8)	0.013 (0.008)	0.009 (0.007)
C_6–C_{10} aliphatic saturated	1	4.45 (0.94–21.0)	0.92 (0.17–5.0)	4.17 (0.73–23.7)	0.012 (0.009)	0.010 (0.007)
C_6–C_{10} aliphatic unsaturated	1	2.86 (0.71–11.5)	0.56 (0.10–3.3)	3.93 (0.65–23.7)	0.011 (0.009)	0.007 (0.007)
C_6–C_{10} aromatic	1	2.97 (0.72–12.2)	1.01 (0.21–4.9)	3.73 (0.66–20.9)	0.008 (0.009)	0.009 (0.007)

ratio for each quartile was generally not significant and sometimes <1. The logistic regression did not show a monotonic response. Three possible reasons no monotonic increase was shown are: there is no relationship with exposure, there is no monotonic increase in risk with increasing exposure, and there is insufficient power to detect an effect.

Regression diagnostics showed no unusual behavior or undue influence of data points to suggest an exposure–response trend. The quartile analysis did not appear to be monotonic, so the lack of statistical power may not be a sufficient answer. However, the point estimates of the odds ratios are elevated but unstable, as shown by the very wide confidence intervals.

Based on this reasoning, the evidence is most suggestive of no relationship. Table 7 summarizes the adjusted and unadjusted odds ratios for all risk factors, for the highest quartile.

Figure 1 summarizes the cumulative frequency of renal cancer death by time since hire and time since terminating work. Latency since time of hire ranged from 24 to 62 years. Latency since termination was less than 22 years. When plotted on a cumulative log-normal plot, latency since termination does not approximate a log-normal distribution, whereas latency since hire does appear to show a log-normal distribution with a median of about 45 years (data not shown).

Table 7. Summary of adjusted and unadjusted odds ratios (OR) from occupational and nonoccupational variables[a]

Variable	Unadjusted OR	Adjusted OR	Unadjusted *p*-value	Adjusted *p*-value
BMI ≥25	3.18	3.29	<0.02	<0.01
MAP ≥103	4.47	5.76	<0.03	<0.08
Smoking	1.21	1.36	<0.66	<0.62
Tenure ≥38 years	6.68	4.08	<0.04	<0.07
C_2–C_5 saturated ≥115	3.08	4.94	<0.10	<0.15
C_2–C_5 unsaturated ≥115	2.55	5.09	<0.22	<0.14
C_6–C_{10} aliphatic saturated ≥115	2.46	4.17	<0.18	<0.17
C_6–C_{10} aliphatic unsaturated ≥115	2.23	3.93	<0.32	<0.22
C_6–C_{10} aromatic ≥115	3.25	3.73	<0.23	<0.32

Abbreviations: BMI, body mass index; MAP, mean arterial pressure.
[a]The adjusted analyses for BMI, MAP, and smoking contain the other two variables.
For the remaining dependent variables, all the adjusted analyses contain BMI, MAP, and smoking. The unadjusted analyses were adjusted only for age.

Discussion

This nested case–control study was composed of 37 male workers with kidney cancer and 148 controls matched on DOB, DOH, gender, and race. The *a priori* questions were the associations of kidney cancer with cumulative hydrocarbon exposure at work and three individual risk factors. In these workers there was a significant risk associated with BMI. The risk increased linearly with BMI and was increased about 2.6-fold for 125% overweight compared to normal BMI. There was a tendency for the kidney cancer cases to have higher blood pressure, longer tenure, and higher exposure to hydrocarbons (estimated as cumulative exposure to C_2–C_5 saturated, C_2–C_5 unsaturated, C_6–C_{10} aliphatic saturated, C_6–C_{10} aliphatic unsaturated, and C_6–C_{10} aromatics). The trend was linear for BMI, blood pressure, and tenure, but was not linear for the estimates of cumulative hydrocarbon exposure; none was statistically significant except the trend for BMI. Where no statistical significance was shown, the role of chance could not be discounted, as the confidence intervals around the odds ratios were quite wide. There was no association of kidney cancer with either smoking category or pack-years smoked.

Does this study test the light-hydrocarbon nephropathy hypothesis developed from the toxicology studies? The components of gasoline primarily responsible for the nephrotoxic activity of unleaded gasoline in the male rat are the branched saturated alkanes, C_6–C_9 (stream 3). These are light alkylate naphthas in the 145–280°F boiling range (*36*) and include 2-methylpentane, 2-methylhexane, 2,3-dimethylbutane, 2,2,4-trimethylpentane, and 2,2,5-trimethylhexane. Normal alkanes, alkenes, olefins, and aromatic compounds (i.e., process streams 1, 2, 4, and 5, respectively) did not show nephrotoxic effects. Thus, if the $\alpha 2_\mu$-globulin rat model hypothesis is relevant to humans, process stream 3 would be the most relevant exposure in this study.

Because of the high correlation (r >0.9 for any pair) between process streams, the observations are not independent and there is little difference in their exposure–response relationships in this study. Therefore, this part of the hypothesis cannot be tested in this data set. The "see-saw" nonlinear relationship of kidney cancer and process stream exposure is not characteristic of an exposure– response trend and contrary to what is expected when there is a causal association.

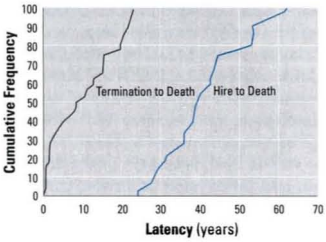


Figure 1. Cumulative frequency for latency.

Bias

Systematic errors are of particular concern in case–control studies. Potential biases due to selective recall and misclassification of disease and exposure are considered below.

A common problem in population-based case–control studies is recall bias. This occurs when cases remember more than controls about significant events or exposures that occurred in their past. As records were recorded prior to disease status, the possibility of recall bias is excluded.

Misclassification of kidney cancer is considered to be low. Percy et al. (*37*), examined 984 cases of kidney cancer to determine agreement between hospital and death certificate diagnoses. The detection rate (proportion of hospital diagnoses reflected as cause of death on death certificates) for kidney cancer was 87.9% and the confirmation rate (proportion of death certificate diagnoses confirmed by hospital diagnoses) was 93%. Thus, there were about 7% false positives with respect to kidney cancer as the underlying cause of death, or perhaps two misdiagnoses of the cases in this study.

The male rat model suggests an exposure–response curve showing a continous increase in tumors above the threshold exposure. The point estimates of the odds ratios in this epidemiologic study do not increase in a linear fashion, as the point estimates relative to the first quartile are elevated for the second and fourth quartiles, but the third quartile is generally <1. However, the confidence intervals are quite wide, so that drawing a curve through the lower confidence interval of the medium-low exposure category and through the upper confidence interval of the medium-high exposure category would result in the familiar exposure–response curve, i.e., continual increase response as exposure increases. The regression analysis suggests this is an unlikely event (about 1 chance in 6). The point estimates would move in the direction of a linear increase if there was nondifferential misclassification between medium-high to high exposure categories and medium-low to medium-high exposure categories.

On the other hand, these curves are similar in shape to those describing the relationship with aromatics in the Poole et al. (*5*) study of petroleum refinery workers, except their odds ratios are <2 in the medium-low and high categories, rather than the three- to fivefold increases seen in this study.

Thus, the point estimate for the odds of exposure are not like an exposure–response relationship, but the point estimates are unstable with very wide confidence intervals. More subjects are needed to more clearly define the shape of the curve.

Misclassification of exposure in case–control studies is a major concern as exposure–response relationships are a major measure of effect. For dichotomous exposures (exposure classified as yes or no), nondifferential misclassification tends to reduce risk ratios toward the null and decrease the power of statistical tests (*38*). Nondifferential misclassification occurs when the bias is independent of disease status or is random. In this study any misclassification of exposure is presumed to be nondifferential, as classification of exposure was made on the basis of job title/job location without knowledge of case or control status.

Marshall et al. (*39*) examined the potential effects of misclassifications on assessing exposure–response relationships, assuming misclassification only between adjacent exposure categories. They showed that if the pattern of errors is random, the bias will generally not mask an exposure–response trend. If the error rate is less than about 35%, the null hypothesis of no trend would be consistently rejected at the 5% level of significance for a sample size smaller than this study. A 50% misclassification rate would reduce a true odds ratio of 4 to an odds ratio between 2 and 3.

We have no way of determining if, or to what degree, misclassification may have occurred in this study. The blinded assessment of exposure intensity and the collection of work and medical history for both cases and controls from prerecorded personnel records suggest that any misclassifications are nondifferential. Given the apparent robustness suggested by Marshall et al. (*39*), bias is considered unlikely to significantly affect the exposure–response relationship shown in this study.

Selection bias could occur if kidney cancer retirees not dying before 1970 had nonrepresentative exposures. Ten of the 37 cases were retirees who were alive as of 1 January 1970.

Criteria for Causality

A number of criteria are regularly used in epidemiology to evaluate whether an association between an exposure and a disease is causal. Some of these criteria are considered in the context of the question posed in this study of whether hydrocarbon exposure increases the risk of kidney cancer.

The minimum period of time since DOH is 24 years among these cases, which should be sufficient time for the disease to develop if related to work exposure.

The presence of a trend of increasing risk with increasing exposure is strong evidence of a causal association. Risk tended to increase as tenure exceeded 30 years. Tenure is a surrogate and nonspecific measure of exposure and undoubtedly misclassifies exposure to process streams. Misclassification bias is considered less likely for estimates of cumulative exposure. However, the number of kidney cancer cases is relatively small, and the confidence intervals around both the slope of the exposure–response regression line and the odds ratios by exposure group are so wide that the possibility of a trend cannot be conclusively ruled out. Thus, the occurrence of an exposure–response trend is considered indeterminate.

Confounding by nonoccupational risk factors (BMI, MAP, smoking) is unlikely as they were adjusted for in the analysis, and are probably not related to exposure. There is potential confounding from asbestos exposure, where high exposure in asbestos-exposed cohorts showing a twofold or greater increased risk of lung cancer also show about a twofold increased risk of kidney cancer (*40,41*). We were not able to assess asbestos exposure, but it seems unlikely that the medium-low and high exposure categories of cases but not controls would be exposed to high enough levels of asbestos to increase the risk of kidney cancer. The risk of kidney cancer from asbestos exposure is also probably much less than the twofold risk observed in heavily exposed asbestos workers (*42*). Therefore, asbestos is not considered to be a likely confounder because differential exposure between cases and controls is unlikely, and the risk of kidney cancer from low-level exposure is probably too small to materially affect the odds ratios.

There are two other nested case–control studies within the petroleum industry. Both are of refinery workers (*5,6*) and do not show any apparent exposure–response trend. Poole et al. (*5*) identified 102 kidney cancer cases among five petroleum companies. There was no apparent increased risk associated with cumulative exposure to nonaromatic liquid gasoline distillates, aromatic hydrocarbons, and volatile hydrocarbons. There was about a twofold increased risk when the job held longest was laborer; jobs in receipt, storage, and movements; and unit cleaners. Wen (*6*) identified 22 kidney cancer cases in a Gulf oil refinery cohort. Odds ratios tended to decrease with increasing length of exposure to gasoline.

Occupational risk factors in population-based case–control studies are rarely suitable for establishing causality as exposure classification is generally inadequate because of too few exposed cases, exposure being too broadly defined and encompassing disparate jobs, industries, chemicals, and exposure–response usually being dichotomous. Population-based case–control studies provide suggestive evidence of a possible association of kidney cancer risk with various hydrocarbon-related jobs and industries including gasoline, kerosene, aviation fuel, petroleum refining and distribution, gasoline attendants, petroleum products, organic solvents, chemical manufacturing, and hydrocarbons (*3,7*). There are a number of other nonhydrocarbon-related job/industries that also show increased odds ratios. However, they have obscure, if

any, association with exposure to petroleum hydrocarbons and no known or suspected etiologic agent. These associations indicate the problem of multiple comparisons, i.e., if enough comparisons are made, something is bound to be significant.

Cohort studies of petroleum industry workers provide little evidence of possible increased risk, even when more highly exposed workers are analyzed separately (*3,4*). These cohort studies are often limited in evaluating risk and causality for several reasons, including exposure to multiple substances, inclusion of a proportion of workers with little or no exposure (which dilutes or obscures any work-related effect), some workers with too-short latency unless stratified by time since hire, and no evaluation of exposure–response trends.

None of the three available nested case–control studies directly addressing the question of whether petroleum hydrocarbons increase the risk of kidney cancer is clearly positive. The population-based case–control studies suggest there are a variety of chemicals and jobs that may be associated with increased risk of kidney cancer. The most specific substances identified are fuels (e.g., gasoline, kerosene, aviation fuel). Two cohort studies of distribution workers are consistent with the findings from the population-based case–control studies, but two are not, and the latter do not suggest an association with exposure.

The $\alpha 2_u$-globulin hydrocarbon hypothesis describes a possible mechanism whereby hydrocarbons might cause kidney tumors in the male rat. This hypothesis does not appear to be a plausible mechanism in humans (*9*).

Latent periods for a number of cancers show a log-normal distribution from time of exposure to the etiologic agent. Armenian and Lilienfeld (*43,44*) provided several examples of this relationship including thyroid cancer and childhood radiation exposure, leukemia following exposure to radiotherapy, intrauterine X-ray exposure, and radiation from the atomic bomb. Three examples were given where the onset of exposure was less precise; namely, lung cancer in asbestos workers and two instances of bladder cancer following occupational exposures to dyes. An exception to the log-normal distribution was noted for onset of acute lymphatic leukemia in children not exposed to intrauterine X-rays.

Similar distributions are seen for the incubation period of infectious diseases (*44*) and age of onset for genetic diseases (*45*). They suggest that observing a log-normal distribution of incubation periods in these diseases, as well as cancers caused by some environmental exposure, is suggestive that a specific etiologic agent initiates a chain of events that leads to the disease.

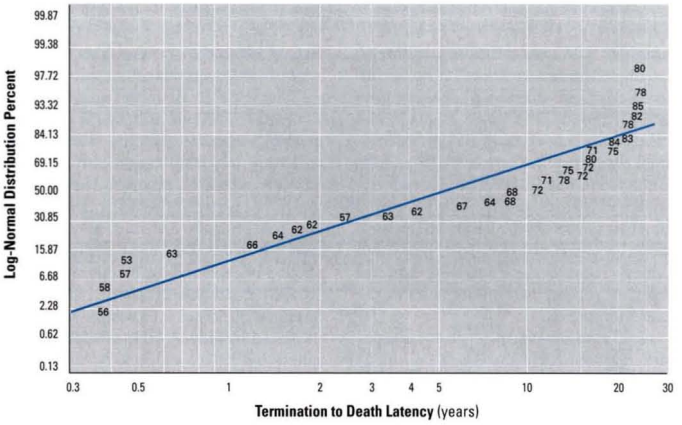


Figure 2. Cumulative log-normal plot for latency.

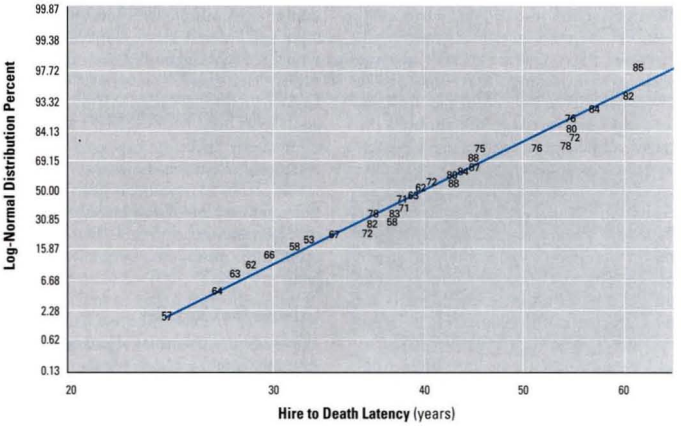


Figure 3. Cumulative log-normal plot for latency.

The study of the distribution of incubation periods may therefore be useful in determining the importance of a particular factor in causing a disease. The absence of a fit (as for termination of employment in this study; Fig. 2) may suggest the factor is not important. The presence of a fit (Fig. 3) is suggestive of an association.

The reasoning by analogy is somewhat like plausibility. If several different carcinogenic agents produce a log-normal latency distribution, then it is easier to accept the idea that the appearance of a latency log-normal distribution is associated with a common etiologic agent or exposure.

Is there a positive association that cannot be explained by bias, confounding, or chance? Misclassification bias was discussed and considered unlikely to obscure the presence of a trend. BMI, MAP, and smoking are three potentially confounding factors. These factors were included in the calculation of adjusted odds ratios and coefficients. Further, the controls were drawn from the same cohort as the cases, so neither bias nor confounding is considered to have any significant impact on the study results.

However, it is possible that no statistically significant association was observed because of the lack of power to detect an effect. For example, the adjusted odds ratio for a cumulative exposure of 100 score-years to stream 3 is 3.3 with the possibility that the "true" odds ratio is as low as 0.57 or as high as 19.4. The wide confidence intervals on the estimates of risk are a reason for concluding that this study has low power and therefore does not provide clear

Table 8. Summary of association of kidney cancer and body mass index among males in population-based case–control studies compared to this study

Reference	ORs (95% CI) by quartiles				Total number	
	Low	2nd Quartile	3rd Quartile	High	Cases	Controls
(22)[a]	1.0	1.01	1.76	1.16	129	256
(13)	1.0	0.9 (0.5–1.1)	0.8 (0.5–1.3)	1.3 (0.8–1.8)	310	426
(48)	1.0			2.0 (1.2–3.2)		
(14)	1.0	1.0 (0.4–2.4)	1.0 (0.5–2.4)	2.2 (1.0–4.9)	104	104
(16)	1.0	1.93 (1.12–3.77)	2.67 (1.49–5.94)	—	189	189
(18)	1.0	1.2 (0.7–2.1)	1.6 (0.9–2.9)	2.5 (1.4–4.6)	206	195
(20)[b]						
No hydrocarbon exposure	1.0	1.0	3.7	3.8	21	35
Low hydrocarbon exposure	1.0	1.12	4.24	0.71	29	35
Moderate hydrocarbon exposure	1.0	1.71	0.83	1.02	53	36
High hydrocarbon exposure	1.0	0.86	4.14	8.14	39	36
This study	1.0	0.93 (0.22–3.84)	2.47 (0.68–8.98)	3.29 (0.93–11.6)	34	141

ORs, odds ratios.
[a]Odds ratios calculated from the data.
[b]Odds ratios of quartiles 2–4 adjusted relative to quartile 1. The odds ratio of first quartile was 1.7, 4.2, and 1.4 for low, moderate, and high hydrocarbon exposure compared to 1st quartile of no hydrocarbon exposure.

evidence for or against an exposure–response relationship or causal association. The study included all the extant cases of kidney cancer in the study population. Hence it is not possible to increase the power of the study until we observe more cases in the study population.

Table 8 summarizes studies that have evaluated the association of kidney cancer with BMI among men, including the results of this study. Practically all of the studies show a trend for the risk to increase as the body weight increases. Wynder et al. (*23*) and McLaughlin et al. (*14*) showed an effect for overweight women but not for men. Goodman et al. (*17*) found a significant trend among both men and women that was somewhat more consistent among men.

The data from this study and from two earlier studies (*15,20*) suggest that a young male 120–130% above normal weight is at two- to threefold increased risk of kidney cancer. Asal et al. (*20*) performed a multivariate analysis and ranked the most important variables as assessed by stepwise regression as 1) recent weight ($p = 0.0001$), 2) petroleum work ($p = 0.0006$), and 3) hypertension ($p = 0.04$) as among the most important risk factors for kidney cancer. Smoking ($p = 0.08$) was ranked 11th. Thus, the study reported here is consistent with other studies suggesting that being overweight increases the risk of kidney cancer among men.

In this study, blood pressure (measured as mean arterial pressure) showed a trend of increasing risk with increasing MAP measured at initial exam. Grove et al. (*46*) found a significant association of blood pressure (10 mm Hg increase) with the incidence of kidney cancer (controlled for age and smoking) among men. After adjustment for blood pressure medication, however, the association was no longer significant. Age at which blood pressure readings were taken ranged from 46 to 68 years. Other case–control studies have not measured blood pressure directly, but categorized exposure as the presence or absence of hypertension, and sometimes adjusted for use of diuretics and hypertension drugs (*22,15,23,29*). Yu et al. (*15*) found that men with high blood pressure and taking diuretics had a slightly increased odds ratio of 1.2 (95% CI, 0.4–4.0) compared to those not taking a diuretic. Both Yu et al. (*15*) and McLaughlin et al. (*47*) found no association with diuretic use alone among men.

The findings of this study are consistent with those in the literature. However, in this study the blood pressure readings are for young men in good health and without the presence of potentially confounding health factors and medications present in older men. The observed trend is suggestive of a causal association but needs to be confirmed.

The association of cigarette smoking and risk of kidney cancer among males has been investigated in a number of case–control studies (*14,15,17,19,22,23,26,28,29,48*). In general, there was a trend for the odds ratios to increase as the level of smoking increased. However, the odds ratios even among the heaviest smokers were only moderately elevated (range of 1.27–2.2). Heavy smokers in this study fall in the lower part of this range. Three studies reported only ever-smokers, and the range was 1.0–2.24. The odds ratio was 1.36 for the study reported here.

These results suggest there is a weak association of kidney cancer among moderate to heavy smokers. The study reported here is consistent with that conclusion based on classification by smoking category. The lack of an association with pack-years may in part be due to the inclusion of former smokers. Most of the studies in the literature do not evaluate pack-years as a risk factor.

Conclusions

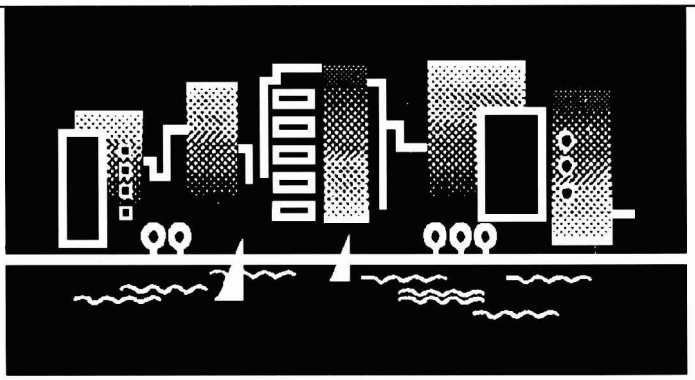
The primary objective of this study was to investigate the association of kidney cancer and hydrocarbon exposure using exposure–response as the measure of association. After controlling for weight, blood pressure, and pack-years smoked, there was no clear-cut exposure–response relationship with tenure or qualitative estimates of cumulative exposure. However, the number of kidney cancer cases is relatively small, and the confidence intervals around both the slope of the exposure–response regression line and the odds ratios by exposure group are so wide that the possibility of a trend cannot be ruled out.

The associations of kidney cancer with BMI and MAP are characteristics of a causal relationship, as the odds ratios increase in a linear fashion. Weight and blood pressure information was collected at the time of first employment or about the start of exposure, so the time sequence is appropriate. For BMI the risk is not observed until the person is overweight, which for men is a BMI >26.5 (*35*). Blood pressure and BMI in this study were measured on physical exam at a young age. Other studies have less specific measures of blood pressure and were obtained at times closer to the disease state. Increased blood pressure does appear to be a risk factor for kidney cancer, but there is a need for more precision in the estimated risk at specific blood pressure (and BMI) levels. Smoking, evaluated both as smoking category and by pack-years, does not show a strong association with kidney cancer.

References

1. Hanis NM, Shallenberger LG, Donaleski D, Sales EA. A retrospective mortality study of workers in three major U.S. refineries and chemical plants, part I: comparisons with U.S. population. J Occup Med 27:283–292 (1985).
2. Shallenberger LG, Acquavella JF, Donaleski D. An updated mortality study of workers in three major U.S. refineries and chemical plants. Br J Ind Med 49:345–354 (1992).
3. Gamble JF. Are petroleum hydrocarbons a kidney carcinogen in humans? A review of the epidemiologic evidence. Int Arch Occup Environ Health (in press).
4. Wong O, Raabe GK. Critical review of cancer epidemiology in petroleum industry employees, with a quantitative meta-analysis by cancer site. Am J Ind Med 15:283–310 (1989).
5. Poole C, Dreyer NA, Satterfield MH, Levin L, Rothman KJ. Kidney cancer and hydrocarbon

exposures among petroleum refinery workers. Environ Health Perspect 101(suppl 6):53–62 (1993).
6. Wen CP. Epidemiological studies of the role of gasoline (hydrocarbon) exposure in kidney cancer risk. Adv Mod Environ Toxicol 7:245–258 (1984).
7. Siemiatycki J, ed. Risk factors for cancer in the workplace. Boston, MA:CRC Press, 1991.
8. Borghoff SJ, Short BG, Swenberg JA. Biochemical mechanisms and pathobiology of α2μ-globulin nephropathy. Annu Rev Pharmacol Toxicol 30:349–367 (1990).
9. Hard GC, Rodgers JS, Baetcke KP, Richards WL, McGaughy RE, Valcovic LR. Hazard evaluation of chemicals that cause accumulation of α2u-globulin, hyalin droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environ Health Perspect 99:313–349 (1993).
10. Swenberg JA, Short B, Borghoff SJ, Strasser J, Charbonneau M. The comparative pathobiology of α2μ-globulin nephropathy. Toxicol Appl Pharmacol 97:35–46 (1989).
11. Melnick RL. Mechanistic data in scientific public health decisions. Regul Toxicol Pharmacol 16:109–110 (1992).
12. Melnick RL. An alternative hypothesis on the role of chemically induced protein droplet (α-2μ-globulin) Nephropathy in renal carcinogenesis. Regul Toxicol Pharmacol 16:111–125 (1992).
13. U.S. EPA. $Alpha_{2\mu}$-globulin: association with chemically induced renal toxicity and neoplasm in the male rat. EPA/625/3-91/019F. Washington, DC:Environmental Protection Agency, 1991.
14. McLaughlin JD, Mandel JS, Blot WJ, Schuman LM, Mehl ES, Fraumeni JF. A population-based case-control study of renal cell carcinoma. J Natl Cancer Inst 72:275–284 (1984).
15. Yu MC, Mack TM, Hanisch R, Cicioni C, Henderson BE. Cigarette smoking, obesity, diuretic use, and coffee consumption as risk factors for renal cell carcinoma. J Natl Cancer Inst 77:351–356 (1986).
16. Maclure KM, Willett W. A case-control study of diet and risk of renal adenocarcinoma. Epidemiology 1:430–440 (1990).
17. Goodman MT, Morgenstein H, Wynder EL. A case-control study of factors affecting the development of renal cell cancer. Am J Epidemiol 124:926–941 (1986).
18. Partanen T, Heikkila P, Hernberg S et al. Renal cell carcinoma and occupational exposure to chemical agents. Scand J Work Environ Health 17:231–239 (1991).
19. Asal NR, Risser DR, Kadamani S, Geyer JR, Lee ET, Cherng N. Risk factors in renal cell carcinoma: I. Methodology, demographics, tobacco, beverage, use and obesity. Cancer Detect Prevent 11:359–377 (1988).
20. Asal NR, Lee ET, Geyer JR, Kadamani S, Risser DR, Cherng N. Risk factors in renal cell carcinoma: II. Medical history, occupation, multivariate analysis, and conclusions. Cancer Detect Prevent 13:263–279 (1988).
21. Kadamani S, Asal NR, Nelson RY. Occupational hydrocarbon exposure and risk of renal cell carcinoma. Am J Ind Med 15:131–141 (1989).
22. Fraser GE, Phillips RL, Beeson WL. Hypertension, antihypertension medication and risk of renal carcinoma in California Seventh Day Adventists. Int J Epidemiol 19:832–838 (1990).
23. Wynder EL, Mabuchi K, Whitmore WF. Epidemiology of adenocarcinoma of the kidney. J Natl Cancer Inst 53:1619–1634 (1974).
24. Raynor WJ, Shekelle RB, Rossof AH, Maliza C, Paul O. High blood pressure and 17-year cancer mortality in the Western Electric health study. Am J Epidemiol 113:317–377 (1981).
25. Bennington JL, Laubscher FA. Epidemiologic studies on carcinoma of the kidney, I. Association of renal adenocarcinoma with smoking. Cancer 21:1069–1071 (1968).
26. Domiano SF, Vena JF, Swanson MK. Gasoline exposure, smoking and kidney cancer. J Occup Med 27:398–399 (1985).
27. Brownson RC. A case-control study of renal cell carcinoma in relation to occupation, smoking and alcohol consumption. Arch Environ Health 43:238–241 (1988).
28. McCredie M, Ford JM, Stewart JH. Risk factors for cancer of the renal parenchyma. Int J Cancer 42:13–16 (1988).
29. Armstrong B, Garrod A, Doll R. A retrospective study of renal cancer with special references to coffee and animal protein consumption. Br J Cancer 33:127–136 (1976).
30. Weir JM, Dunn JE. Smoking and mortality: a prospective study. Cancer 25:105–112 (1970).
31. Doll R, Peto R. Mortality in relation to smoking: 20 years observation on male British doctors. Br Med J 2:1525–1536 (1976).
32. Williams RR, Horm JW. Association of cancer sites with tobacco and alcohol consumption and socioeconomic status of patients: interview study from the Third National Cancer Survey. J Natl Cancer Inst 58:525–547 (1977).
33. Hirayama T. Life-style and mortality: a large-scale census-based cohort study in Japan. New York:Karger, 1990.
34. Checkoway H. Methods of treatment of exposure data in occupational epidemiology. Med Lav 77:48–73 (1986).
35. West KM. Computing and expressing degree of fatness. J Am Med Assoc 243:1421–1422 (1980).
36. Halder CA, Holdsworth CE, Cockrell BV, Piccirillo VJ. Hydrocarbon nephropathy in male rats; identification of the nephrotoxic components of unleaded gasoline. Toxicol Ind Health 1:67–87 (1985).
37. Percy C, Stanek E, Gloeckler L. Accuracy of cancer death certificates and its effect on cancer mortality statistics. Am J Public Health 79:242–250 (1981).
38. Copeland KT, Checkoway H, Holbrook RH, McMichael AJ. Bias due to misclassification in the estimate of relative risk. Am J Epidemiol 105:488–495 (1977).
39. Marshall JR, Priore R, Graham S, Brasure J. On the distortion of risk estimate in multiple exposure level case-control studies. Am J Epidemiol 113:464–473 (1981).
40. Maclure KM. Asbestos and renal adenocarcinoma: a case-control study. Environ Res 42: 353–361 (1987).
41. Smith AH, Shearn VI, Wood R. Asbestos and kidney cancer: the evidence supports a causal association. Am J Ind Med 16:159–666 (1989).
42. Edelman DA. Does asbestos exposure increase the risk of urogenital cancer? Int Arch Occup Environ Health 63:469–475 (1992).
43. Armenian HK, Lilienfeld AM. The distribution of incubation periods of neoplastic disease. Am J Epidemiol 99:92–100 (1974).
44. Armenian HK, Lilienfeld AM. Incubation period of disease. Epidemiol Rev 5:1–15 (1983).
45. Armenian HK, Khoury MJ. Age at onset of genetic disease. Am J Epidemiol 113:596–605 (1981).
46. Grove JS, Nomura A, Severson RK, Stemmermann GN. The association of blood pressure with cancer incidence in a prospective study. Am J Epidemiol 134:942–947 (1991).
47. McLaughlin JD, Blot WJ, Fraumeni JF. Diuretics and renal cell cancer (letter). J Natl Cancer Inst 80:378 (1988).
48. Kolonel LN. Association of cadmium with renal cancer. Cancer 37:1782–1787 (1976).
49. Maclure KM, MacMahon B. A case-control study of renal adenocarcinoma [abstract]. Am J Epidemiol 122:520 (1985).



NEW ENGLAND EPIDEMIOLOGY SUMMER PROGRAM

5 DAY AND 10 DAY COURSES

JUNE 10 – JULY 5, 1996

16th annual summer program in Boston offers 19 courses reviewing established concepts and new developments in epidemiology. Registrants may enroll for CME, CEU, Nursing CEU, Industrial Hygiene CM or graduate credit. The following courses will be offered:

Introduction to Epidemiology	Philip Cole
Conducting Epidemiologic Research	Harris Pastides
Theory & Practice of Epidemiology	Kenneth Rothman
Epidemiologic Basis for Causal Inference	Stephan Lanes
Biostatistics for Epidemiologists	Harland Austin
Regression & Categorical Data Methods	Stanley Lemeshow
Survival Analysis	David Hosmer
Meta-Analysis	Anders Ahlbom
Clinical Research	Albert Hofman
Pharmacoepidemiology	Wayne Ray
Health Care Utilization Research	Malcolm Maclure
Epidemiology in Developing Countries	Nicolaus Lorenz
Cancer Epidemiology	Dimitrios Trichopoulos
Perinatal Epidemiology	Lowell Sever
Genetic Epidemiology	Melissa Austin
Occupational & Environmental Epidemiology	Kyle Steenland
Use of Biomarkers in Epidemiology	Paul Schulte
Scientific Writing	Cheryl Iverson & Susan Eastwood
Ethics & Epidemiology	Kenneth Goodman

NEW ENGLAND EPIDEMIOLOGY INSTITUTE

One Newton Executive Park, Dept. PA-EHP, Newton Lower Falls, MA 02162-1450

617-244-1200 Fax 617-244-9669 E-Mail: epidemiol@aol.com

Meeting Report

The Role of the Environment in Parkinson's Disease

Jay M. Gorrell,[1] Donato DiMonte,[2] and Doyle Graham[3]

[1]Department of Neurology, Henry Ford Hospital, Detroit, MI 48202 USA; [2]The Parkinson's Institute, Sunnyvale, CA 94089-1605 USA; [3]Department of Pathology, Vanderbilt University, Nashville, TN 37215 USA

Thirty leading scientists in the field of Parkinson's disease research attended a conference, "The Role of the Environment in Parkinson's Disease," 17-19 September 1995, sponsored and hosted by the National Institute of Environmental Health Sciences. Parkinson's disease investigators working in various basic and clinical disciplines presented and evaluated current scientific findings concerning the etiology of the disease and charted the most fruitful courses for future research. The role of the environment was highlighted, but considerable attention was given to pathological neurochemistry and genetic issues in the etiopathogenesis of Parkinson's disease.

The conference was opened by Annette Kirshner, program administrator at NIEHS and chair of the conference, who framed the context of the discussions to take place. Terri Damstra, acting deputy director of NIEHS, welcomed attendees to the institute and reaffirmed the importance of the work to NIEHS. She emphasized that in Parkinson's disease the potential roles of environmental exposure to one or more agents, genetic susceptibility to such exposures, and the factor of time or aging are likely to play roles in the etiology of the neurodegenerative process.

Formal presentations were divided into three sessions: environmental and genetic risk factors (Doyle Graham, chair), neurotoxins and mechanisms of neuronal injury (Jay M. Gorell, chair), and biological markers of Parkinson's disease (Donato DiMonte, chair). Summaries of each of these sessions follow.

Environmental and Genetic Risk Factors

The relative roles of environmental, endogenous neurochemical and genetic factors in the etiology of Parkinson's disease is currently unclear. For example, the prevalence of Parkinson's disease in a community could be due to a differential distribution of a hypothetical environmental toxicant or be more frequent where a heritable defect is common. Twin studies in the 1980s seemed to discount a significant genetic role in Parkinson's disease when the degree of concordance between monozygotic and dizygotic pairs was found to be similar. An environmental cause of the disease was favored when it was shown that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could enter the brain and be metabolized to 1-methyl-4-phenylpyridinium ion (MPP^+), which could specifically destroy nigral neurons to produce a human condition that closely mimicked Parkinson's disease. Moreover, the fact that the amyotrophic lateral sclerosis/Parkinson's disease/dementia complex could develop in Guamanians decades after their migration from the island strengthened the idea that a long latent period after exposure to an environmental toxicant could be present before a degenerative disorder was fully manifest.

The potential role of genetics in Parkinson's disease was raised again when a number of families with parkinsonism in several generations was reported. Few affected individuals have been autopsied thus far, but typical Lewy body histopathology has been found. Some investigators believe that many more familial Parkinson's disease cases will be found, but, clearly, a biomarker of genetic risk would be optimal for case identification. Allelic association studies and DNA linkage analysis have not tied the occurrence of Parkinson's disease to various candidate genes so far, though a systematic search of the human genome is underway.

As work on genetics proceeds, much productive research continues to identify endogenous neurochemical abnormalities, to study the mechanisms of action of neurotoxins, and to characterize environmental risk factors that may either initiate or perpetuate Parkinson's disease. In the epidemiologic literature, Parkinson's disease has been consistently shown to increase with either advancing age or the passage of time and to be slightly more prevalent in males. Moreover, nearly all studies report an inverse (i.e., protective) association of Parkinson's disease with smoking, though the mechanism by which this influences risk is unclear. Some studies have suggested a decreased risk of Parkinson's disease with a higher intake of antioxidant vitamins, and many others have found an increased risk of Parkinson's disease among those who have lived in rural environments, farmed, or been exposed to pesticides or to well water.

Two population-based case–control studies have been conducted. Semchuk and colleagues, at the Universities of Calgary and Saskatchewan, found that a family history of Parkinson's disease was the strongest predictor of Parkinson's disease risk, followed by a history of head injury severe enough to require medical attention, and then by a history of occupational exposure to herbicides. Gorell and colleagues, at Henry Ford Hospital in Detroit, Michigan found an increased risk of Parkinson's disease with more than 10 years of occupational exposure to copper, manganese, or lead, but no increased risk with iron, zinc, or mercury exposure. Gorell et al. also found an increased risk of Parkinson's disease with occupational exposure to either herbicides or insecticides.

There is active investigation of potential risk of Parkinson's disease in the systemic metabolism of xenobiotics, operating either via cytochrome P450 enzymes (e.g., CYP2D6 in the detoxication of MPTP and isoquinolines) or through impaired sulfation and sulfoxidation. Finally, there is an ongoing, large study of identical twins, examining their concordance for Parkinson's disease, which could help define the relative roles of genetic and nongenetic factors.

Ultimately, there may be a varying mixture of factors that produce Parkinson's disease in different human populations. For example, in families showing an autosomal dominant pattern of Parkinson's disease transmission, genetic factors may dominate. Among other populations (currently believed to account for the majority of cases) a variety of environmentally acquired neurotoxic exposure(s) may interact under the oxidatively stressed conditions in the Parkinson's disease substantia nigra (SN) to produce the disease. More than one agent (e.g., xenobiotics, metals) may produce Parkinson's disease in sequential stages. Finally, various nongenetic and genetic mechanisms may be linked if the genome is damaged by environmental or endogenous toxins. However, any model proposed to explain the occurrence of Parkinson's disease must account for the selective vulnerability of nigral neurons, the susceptibility of particular individuals, and disease progression.

Neurotoxins and Mechanisms of Neuronal Injury

The chemical pathology of neuromelanin and oxidative stress in Parkinson's disease

Address correpondence to A.G. Kirshner, MD 3-03, National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park, NC USA.

may be pivotal in nigral neuronal death. Neuromelanin, made from orthoquinones derived from catecholamine metabolism, forms during the gradual depopulation of pigmented catecholaminergic neurons in the central nervous system in Parkinson's disease. The chemical milieu surrounding neuromelanin is characterized by transition metal-catalyzed redox cycling between semiquinone and catecholamine compounds, as well as by the formation of hydrogen peroxide from the catabolism of catecholamines by either the action of monoamine oxidase (MAO) or by their autoxidation. Cellular factors that increase SN oxidative reactions, such as potential activation of xenobiotics by MAO (as with MPTP), interference with mitochondrial oxidative phosphorylation (e.g., by inhibition of complex I), or action by excitotoxins, can exacerbate local oxidative stress.

There is an apparently Parkinson's disease-specific 35–40% decrease in SN mitochondrial complex I function, the same defect seen with MPP^+ action. This deficiency could generate superoxide and other free radicals that may contribute to neuronal damage in the SN. However, it is unclear whether the SN complex I deficiency in Parkinson's disease is due to genetic damage, exogenous or endogenous factors, or both. A possible genetic defect may be due to alterations in nuclear and/or mitochondrial DNA, as several complex I subunits are encoded by mitochondrial DNA.

Pharmacological study of model systems such as P19 neuroglial cultures can shed further light on neurochemical changes in oxidatively stressed brain. For example, the cytotoxicity of lipophilic dopamine congeners in this system is enhanced by manganese coincubation and is associated with cross linking of neurofilament protein into high molecular weight aggregates. Moreover, redox-cycling catechols induce lipid peroxidation that generates E-4-hydroxy-2-nonenal, a protein crosslinking agent. These results suggest that lipid peroxidation, and perhaps Lewy body formation with subsequent neurodegeneration, may be related to catechol oxidation.

Iron levels are increased approximately 35% in the Parkinson's disease SN zona compacta, and laser microprobe and X-ray microanalysis studies show increased iron (and aluminum) in SN neuromelanin in such patients. Moreover, iron infusion into SN in animals can cause both acute and progressive neuronal loss. The importance of these findings may lie in the fact that iron, if liberated from neuromelanin (or ferritin) stores, can greatly potentiate the formation of highly cytotoxic hydroxyl radicals from hydrogen peroxide, which is produced from dopamine metabolism. This process may be more likely in the Parkinson's disease brain, in which a deficiency of reduced glutathione (GSH), the major defense against hydroxyl radical formation, has been found.

In a monkey model of manganism produced by intravenous infusion of $MnCl_2$, the metal accumulated in putamen, with a subsequent massive periventricular accumulation of iron and an increase in aluminum in globus pallidus. These findings raise the possibility that environmental exposure to manganese may also reflect exposure to iron and aluminum. Because the liver is the major organ involved in the clearance of manganese, liver failure could predispose an individual to manganese-induced neurotoxicity. Finally, though aluminum does not promote lipid peroxidation, it greatly potentiates the ability of iron to do so, raising the possibility that all three metals may participate in the pathogenesis of Parkinson's disease.

Several classes of neurotoxins have been studied as potential nigral toxins, with varying results. For example, some β-carboline compounds are structurally similar to MPP^+ and have been shown to inhibit mitochondrial complex I. Though substances studied so far do not show dopaminergic cytotoxic specificity in rat primary mesencephalic cultures, 2,9-di-*N*-methyl β-carbolinium derivatives are detectable in the cerebrospinal fluid of some Parkinson's disease patients, and such compounds are eight-fold more concentrated in Parkinson's disease SN than in cerebral cortex. The organochlorine pesticide dieldrin was present in 6 of 20 Parkinson's disease brains in one study, though it was not more concentrated in Parkinson's disease samples, suggesting that it was not sufficient to cause Parkinson's disease.

Four models were considered in the identification of agents with potential SN toxicity: 1) utilization of the dopamine uptake system in nigrostriatal neurons to confer specificity (e.g., MPP^+); 2) interaction of toxins with dopamine (e.g., methamphetamine-induced striatal terminal damage); 3) utilization of iron in free radical generation and/or in the bioactivation of an environmental toxin (e.g., MPP^+ generation by iron-activated, MAO-independent means); and 4) potentiating effects of two agents at one or more sites (e.g., diethyldithiocarbamate plus L-dopa may act by inhibition of superoxide dismutase, increased bioactivation by MAO, decreased dopamine re-uptake, decreased mitochondrial oxidative function, and by glutamatergic excitotoxicity).

Biological Markers

Brainstem Lewy body formation is a neuropathological hallmark in the diagnosis of Parkinson's disease postmortem. However, little is known about its role in the pathogenesis of Parkinson's disease, nor is much known about the role of neuronal inclusions in MPTP-parkinsonism. The reasons for differences in the inclusions in these disorders is needed to clarify the pathogenesis of both Parkinson's disease and MPTP-parkinsonism.

Thus far, it has not been possible to find a specific biomarker of Parkinson's disease in accessible tissues of living patients. For example, there has been an extensive search for a mitochondrial complex I defect in peripheral tissues. However, the existence such a deficiency in skeletal muscle is in doubt. Moreover, the consistent decrease in complex I activity in Parkinson's disease platelets shows overlap with control values, and the extent of the deficiency is variable.

No significant associations have been found between superoxide dismutase-1 (SOD-1), Huntington (IT-15), ApoE4 and ApoE3, CYP2D6 alleles, or MAO-A haplotypes and Parkinson's disease. Analysis has failed to show genetic linkage in three Parkinson's disease families with glutathione peroxidase, tyrosine hydroxylase, amyloid precursor protein, SOD-1, CYP2D6, or chromosomal regions expressing choline acetyltransferase or brain-derived neurotrophic factor. Further studies are in progress to define a role for a mutant allele of CYP2D6 and to test the hypothesis that alterations in detoxification enzymes may be markers of risk factors for Parkinson's disease.

The value of cerebrospinal fluid data as a marker of the dopaminergic system in Parkinson's disease has been limited. A decrease in cerebrospinal fluid homovanillic acid (HVA) has been shown in Parkinson's disease in many, but not all, studies, and there is no correlation between the HVA level and either the duration or severity of the disease. There has been no follow-up of an earlier report suggesting that the 5-*S*-cysteinyl forms of dopamine, DOPAC and L-dopa, in cerebrospinal fluid may be indices of oxidative stress in basal ganglia.

Imaging studies offer promise in providing markers of Parkinson's disease. For example, positron emission tomography (PET) studies have shown a linear correlation between the extent of reduced 6-fluorodopa uptake in striatum and the numbers of SN neurons in subsequent autopsies in some Parkinson's disease patients. Higher resolution PET scanners can differentiate

caudate and putamen, and Parkinson's disease patients can be completely separated from control subjects. The two populations can be mostly resolved by imaging striatal binding of the dopamine antagonist raclopride. The single-photon emission tomography radioligand, [^{123}I]β-carbomethoxy-3-β(4-iodophenyl)-tropane, a cocaine analogue that binds to the dopamine transporter in striatum, has been used to show a direct relationship between diminished uptake and motor disability in Parkinson's disease patients. Finally, a new MRI method has been developed to assess iron accumulation at 3 Tesla in SN. The method relies on the calculation of $1/T_2'$ (R_2'), the relaxation rate due to local paramagnetic material. R_2' in the dark area of the SN on T_2 or T_2^*-weighted images is significantly higher in Parkinson's disease patients, and does not overlap with control values, reflecting the higher iron content in Parkinson's disease SN postmortem. A direct correlation has been shown between the right/left asymmetry of SN R_2' versus the left/right asymmetry of simple reaction time in Parkinson's disease subjects, suggesting that SN iron accumulation is related to disease severity.

Conclusions

Areas of focus for future research were identified, which should be pursued by several NIH institutes. Epidemiological studies need to 1) identify exposures to particular toxins that can specifically damage SN or other basal ganglionic structures, 2) clarify the age-specific rates of disease to understand trends over time, 3) more specifically define risk factors being assessed, 4) examine whether a family history of other neurodegenerative diseases increases the risk for Parkinson's disease, 5) study Parkinson's disease subgroups (e.g., young, old, familial, and nonfamilial cases) to determine differential risk for acquiring the disease, and 6) bank specimens of blood and other tissues to link environmental exposures to markers of Parkinson's disease; i.e., of the disease itself, of mechanisms of biochemical damage, and of potential genetic risk. Finding biomarkers for Parkinson's disease would enhance the reliability of clinical diagnosis, helping to ensure the homogeneity of cases selected for clinical and epidemiologic studies; permit preclinical diagnosis, enabling full ascertainment of familial cases, identification of those at risk for selected environmental toxicants, and allow selection of individuals for trials of possibly neuroprotective drugs; potentially permit the screening of large populations; and possibly provide clues about the etiopathogenesis of the disease.

Understanding the mechanism(s) underlying brainstem Lewy body formation and its role in the neurochemical pathology of Parkinson's disease could elucidate the etiopathogenesis of the condition. Identifying early or initiating neurochemical events is critical, including factors conferring selective vulnerability or protection to nigral neurons (e.g., chemical characteristics of toxins; the role of glial cells in the bioactivation of toxins or in providing neuroprotection; neurotrophic factors). The role of bioaccumulation of toxicants (e.g., metals, glutamate, pesticides, infectious agents, others), with slow release over time, should be investigated. The development of models of chronic *in vivo* neurotoxicity that more closely resemble the progressive nature of Parkinson's disease should be a priority.

Continued search for one or more genes in Parkinson's disease with linkage to brainstem Lewy body pathology is an important area of investigation. More autopsies are needed in large kindreds to establish the pathological basis for their clinical condition. Results of the ongoing study of a large number of identical twins with one Parkinson's disease twin could clarify a potential genetic role in Parkinson's disease.

The potential interaction between the environment, pathological neurochemistry, anatomy, and genetic factors was seen as the most fruitful overall research direction to unravel the etiology and pathogenesis of Parkinson's disease. Sharing insights in the basic and clinical neurosciences is the best vehicle for maximally using diverse scientific talent. Interdisciplinary research should be encouraged by NIH institutes.

Co-sponsors of the Workshop on the Role of the Environment in Parkinson's Disease

NIEHS Division of Extramural Research and Training

NIEHS Superfund Basic Research Program

Organizing Committee

Annette G. Kirshner
NIEHS
Research Triangle Park, North Carolina

Jay M. Gorell
Department of Neurology
Henry Ford Hospital
Detroit, Michigan

Donato DiMonte
The Parkinson's Institute
Sunnyvale, California

Doyle Graham
Department of Pathology
Vanderbilt University
Nashville, Tennessee

Thomas Montine
Department of Pathology/Toxicology
Vanderbilt University
Nashville, Tennessee

The Catholic University of America

The Center for Advanced Training in Cell and Molecular Biology Washington, DC

For information:
Office Manager, CATCMB/103 McCort-Ward Bldg.
The Catholic University of America
620 Michigan Ave., NE, Washington, DC 20064
202-319-6161 Fax: 202-319-4467

JUNE

June 3–7
Plant Biotechnology, Gene Transfer and Expression

June 10–14
Expression of Recombinant DNA in Mammalian Cells

June 17–21
DNA-binding Proteins & Transcriptional Regulators

June 24–28
In situ Hybridization

JULY

July 8–12
Recombinant DNA Methodology

July 15–19
Non-radioactive Labeling Technology

July 19–20
Capillary Electrophoresis: Methods and Applications for Molecular Biology

July 22–26
Molecular Immunology and Immunochemistry

July 22–26
Mitochondrial Molecular Biology

New Books

Basic Toxicology; Fundamentals, Target Organs, and Risk Assessment
Frank C. Lu
Washington DC: Taylor and Francis, 1996, 376 pp. ISBN: 1560323795 (cloth: alk. paper), $99.95. 1560323809 (paper: alk. paper), $44.95.

Carcinogens and Anticarcinogens in the Human Diet; A Comparison of Naturally Occurring and Synthetic Substances
National Research Council
Washington, DC: National Academy Press, 1996, 417 pp. ISBN: 0309053919, $37.95.

Developing Environmental Awareness through Children's Literature; A Guide for Teachers and Librarians K-8
Nancy Lee Cecil
Jefferson, NC: McFarland and Company, 1996, 224 pp. ISBN: 0786402210, $28.50.

Don't Hazard a Guess: Addressing Community Health Concerns at Hazardous Waste Sites
National Association of County and City Health Officials
Washington, DC: NACCHO, 1996, 70 pp. $15 for members, $20 nonmembers.

Food, Climate, and Carbon Dioxide
Sylvan H. Wittwer
Boca Raton, FL: CRC Press, 1995, 256 pp. ISBN: 0873717961, $69.95

Ecology and Democracy
Freya Mathews, ed.
Portland, OR: Frank Cass, 1995. ISBN: 0714642525, $18.50.

Ecology of Marine Bivalves; An Ecosystem Approach
Richard F. Dame
Boca Raton, FL: CRC Press, 1996. ISBN: 0849380456, $85.

The Economic Appraisal of Environmental Projects and Policies: A Practical Guide
Organization for Economic Cooperation and Development
Washington, DC: OECD Publications, 1995, 155pp. ISBN: 9264145834, $34.

The Economics of Pollution Control in the Asia Pacific
Robert Mendelsohn, Daigee Shaw
Brookfield, VT: Edward Elgar Publishing, 1996. ISBN: 185898307X, $79.95.

The Environment Industry: The Washington Meeting
Organization for Economic Cooperation and Development
Washington, DC: OECD Publications, 1996, 285 pp. ISBN:9264147683, $51.

Environmental Life-Cycle Assessment
Mary Ann Curran
New York: McGraw-Hill, 1996. ISBN: 007015063X (acid-free paper), $65.

Environmental Policy Law; Problems, Cases, and Readings
Thomas J. Schoenbaum, Ronald H. Rosenberg
Westbury, NY: Foundation Press, 1996. ISBN: 1566623448 (alk. paper), $127.

Global Change and Arctic Terrestrial Ecosystems
Walter C. Oechel
New York: Springer, 1996. ISBN: 0387943560 (alk. paper), no price available.

The Global Environmental Goods and Services Industry
Organization for Economic Cooperation and Development
Washington, DC: OECD Publications, 1996, 55 pp. ISBN: 9264146938, $16.

Global Forests and International Environmental Law
Canadian Council of International Law
Boston: Kluwer Law International, 1996. ISBN: 9041108971 (alk. paper), $127.

Handbook of Comparative Veterinary Pharmacokinetics and Residues of Pesticides and Environmental Contaminants
Stephen F. Sundlof, Jim Edmond Riviere, Arthur L. Craigmill
Boca Raton, FL: CRC Press 1996, 592 pp. ISBN: 0849332133, $166.95.

Indoor Air Quality One Stop Reference
National Association of County and City Health Officials
Washington, DC: NACCHO, 1996, 70 pp. $15 for members, $20 nonmembers.

Integrating Environment and Economy: Progress in the 1990s
Organization for Economic Cooperation and Development
Washington, DC: OECD Publications, 1996, 63 pp. ISBN: 9264147748, $19.

Mineral and Metal Neurotoxicology
Masayuki Yasui et al., eds.
Boca Raton, FL: CRC Press, 1996. ISBN: 0849376645, $129.95.

Your Natural Home; A Complete Sourcebook and Design Manual for Creating a Healthy Beautiful Environmentally Sensitive House
Janet Marinelli, Paul Bierman-Lytle
Boston: Little Brown and Company, 1995, 256 pp. ISBN: 0316093025 (cloth), $45. 0316093033 (paper), $21.95.

Principles of Toxicology
Karen Stine, Thomas M. Brown
Boca Raton, FL: CRC Press, 1996. ISBN: 0873716841 (alk. paper), $49.95.

Promising the Earth
Robert Lamb, in Collaboration with Friends of the Earth
New York; Routledge, 1996, 208 pp. ISBN: 0415144434, $55.

Redefining Nature; Ecology, Culture, and Domestication
Roy Ellen, Katsuyoshi Fukui
Washington, DC: Berg Publishers, 1996, 608 pp. ISBN: 1859731309 (cloth, alk. paper), $45.95. 185973135X (paper, alk. paper), $19.95.

Statistical Methods for Health Sciences
M.M. Shoukri and V.L. Edge
Boca Raton, FL: CRC Press, 1996, 320 pp. ISBN: 0849376440, $59.95.

Statistics in Epidemiology
Hardeo Sahai, Anwer Khurshid
Boca Raton, FL: CRC Press, 1996, 352 pp. ISBN: 0849394449, $59.95.

The Terror of the Machine; Technology, Work, Gender and Ecology on the US-Mexico Border
Devon G. Pena
Austin, TX: University of Texas at Austin, 1996, 412 pp. ISBN: 0292765614 (cloth, alk. paper), $45. 0292765622 (paper, alk. paper), $19.95.

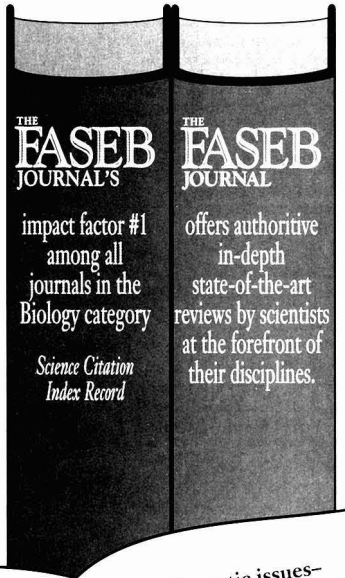
Understanding Environmental Administration and Law
Susan J. Buck
Washington, DC: Island Press, 1996. ISBN: 1559630213 (cloth), $34.95. 155963474X (paper), $21.95.

YOUR LIBRARY IS NOT COMPLETE...

without a subscription to

THE
FASEB JOURNAL

A library in itself and the #1 review and communication biology journal.



key biological areas...

- ANATOMY
- BIOCHEMISTRY
- BIOPHYSICS
- CELL BIOLOGY
- DEVELOPMENTAL BIOLOGY
- GENETICS
- IMMUNOBIOLOGY
- MOLECULAR BIOLOGY
- NEUROBIOLOGY
- NUTRITION
- PATHOLOGY
- PHARMACOLOGY
- PHYSIOLOGY

FEATURES

- Invited reviews
- Innovations in methodology
- Original communications
- Articles covering public affairs
- News items about research funding people, and institutions
- Calendar of Scientific Events
- New Products
- Employment Opportunities
- Hypotheses Section

PLUS 2 thematic issues–
Structural Immunology
Vascular Biology

PLUS 3 abstract issues from–
- Experimental Biology 95 APS, ASPET, ASIP, AAI, AIN, AAA
- ASBMB/DBC-ACS 1995 Annual Meeting

PLUS the Journal is 100% guaranteed!
We are so certain of your satisfaction, that should you become dissatisfied, you may cancel your subscription at any time and receive a refund for the unused portion.

SUBSCRIPTION RATES:		ADD FOREIGN POSTAGE:	
FASEB Society Members	$ 47	*Expedited delivery except*	
Students	$ 47	*U.S., Canada & Mexico.*	
Nonmembers	$110	Canada & Mexico	$ 20
Institutions	$295	Elsewhere	$ 48

Maryland residents add 5% sales tax
D.C. residents add 6% sales tax
Canadian subscribers add GST number + 7%

For Rates in Japan, contact USACO. Corp., 13-12 Shimbashi 1-Chome, Minato-Ku. Tokyo, 105 Japan; Tel: 011-81-33-502-6473 Fax: 011-81-33-593-2709

☐ VISA ☐ Mastercard

Card Number: _____

Expiration Date _____

Cardholder's Name _____

Cardholder's Signature _____

Cardholder's Phone Number _____

THE FASEB JOURNAL

SUBSCRIBE

TO EXPEDITE YOUR ORDER: Charge by FAX: 301-530-7001
Questions? Call 800-43-FASEB ext. 7027

☐ *YES!* Please enter my 1995 subscription to *The FASEB Journal*

(Please print clearly)

NAME _____ TITLE _____

ORGANIZATION _____ DEPARTMENT _____

ADDRESS _____ BLDG/ROOM _____

CITY _____ STATE _____ ZIP _____

POSTAL CODE _____ COUNTRY _____

☐ Check enclosed. *U.S. currency drawn on U.S. bank only.*

Make checks payable to: *The FASEB Journal.*
Charge & fax your order OR mail your check to: *The FASEB Journal*
9650 Rockville Pike, Bethesda, MD 20814-3998 U.S.A.

Calendar

July

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

21–28 July, Sun–Sun. Fourth International Symposium on the Geochemistry of the Earth's Surface, Ilkley, Yorkshire, England. Information: Conference Secretariat, Department of Continuing Education, Leeds University, Leeds LS2 9JT, UK, 01132-333-241, FAX 01132-333-240

23–27 July, Tue–Sat. Western Pacific Geophysics Meeting, Brisbane, Australia. Information: AGU Meetings Department, 2000 Florida Avenue NW, Washington, DC 20009, (202) 462-6900

August

4–14 August, Sun–Wed. International Geological Congress, Beijing, China. Information: Z. Xun, Deputy Secretary General, 30th International Congress, PO Box 823, Beijing 100037, P.R. China, 86-1-8327772, FAX 86-1-8328928

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

12–15, August, Mon–Thu. International Symposium on Representation of the Cryosphere in Climate and Hydrological Models, Victoria, British Columbia, Canada. Information: Secretary General, International Glaciological Society, Lensfield Road, Cambridge, CB2 1ER, UK, +44-1223-355974, FAX +44-1223-336543

17–22, August, Sat–Thu. Institute on Economics for Journalists, Jackson Lake Lodge, Grand Teton National Park, Wyoming. Information: Doug Ramsey, Senior Vice President, Foundation for American Communications, 3800 Barham Boulevard, Suite 409, Los Angeles, CA 90068

24–29, August, Sat–Thu. Seventeenth International Congress of Biochemistry and Molecular Biology In Conjunction With 1997 Annual Meeting of the American Society for Biochemistry and Molecular Biology, San Francisco, California. Information: Congress Secretariat, 9650 Rockville Pike, Bethesda, MD 20814-3996, e-mail: 171UBMB@asbmb.-faseb.org

29–30, August, Thu–Fri. Dietary Fat and Cancer: Genetic and Molecular Interactions, Loews L'Enfant Plaza Hotel, Washington, DC. Information: Judith Cohn, (202) 328-7744, FAX (202) 328-7226, e-mail: jcohn@capcon.net

September

1–7 September, Sun–Sat. Cellular and Molecular Biology, Second World Congress, Ottawa, Canada. Information: Second World Congress Secretariat, Suite 353, 2660 Southvale Crescent, Ottawa, Ontario, Canada K1B 4W5, (613) 247-1344, FAX (613) 247-2187, e-mail: mhamelin@ottawa.net.

11–13 September, Wed–Fri. Biological Monitoring in Occupational Environmental Health, Espoo, Finland. Information: Biological Monitoring, c/o Finnish Institute of Occupational Health Symposium Secretariat, Topeliuksenkatu 41 a A FIN-00250 Helsinki, Finland, 358-0-47-471, FAX 358-0-47-47548

12–15 September, Thu–Sun. The Extracellular Matrix: Its Synthesis, Function, and Degradation, Holiday Inn, Lake Placid, NY. Information: The Organizing Committee, W. Alton Jones Cell Science Center, Inc. 10 Old Barn Road, Lake Placid, NY 12946 (518) 523-1252, FAX (518) 523-1849

12–21 September, Thu–Sat. XVIII Quadrennial Ozone Symposium-96, Rome, Italy. Information: R.D. Bojkov, c/o World Meteorological Organization, C.P. 2300, Geneva-2, CH-1201 Switzerland, FAX +41 22 7400984

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

22–27 September, Sun–Fri. Third USA/CIS Joint Conference on Environmental Hydrology and Hydrogeology, Tashkent, Uzbekistan. Information: American Institute of Hydrology, 3416 University Avenue SE, Minneapolis, MN 55414-3328, (612) 379-1030, FAX (612) 375-0169

24–29 September, Tue–Sun. 42nd Annual Eastern Pacific Oceanic Conference, Stanford, California. Information: M. Korso, College of Oceanic and Atmospheric Sciences, Oregon State University, Ocean Administration, Building 104, Corvallis, OR 97331-5503, (503) 737-3079, FAX (503) 737-2064, e-mail: kosro@oce.orst.edu

29 September–3 October, Sun–Thu. Trefoil/P-Domain Peptides: From Basic Research to Molecular Medicine, Aix-les-Bains (Savoy), France. Information: INSERM Institut National De La Sante, Conferences Philippe Laudat, 101 rue de Tolbiac, 75654 Paris, Cedex 13 France, 33 (1) 44 23 60 89/87, FAX 33 (1) 44 23 60 89, e-mail: laudat@tolbiac.inserm.fr

October

12–15 October, Sat–Tue. Fourteenth International Neurotoxicology Conference. Arlington Hotel, Hot Springs, Arkansas. Information: Joan Cranmer, Department of Pediatrics, #512, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205, (501) 320-2986, FAX (501) 320-4978.

13–17 October, Sun–Thu. V(D)J Recombination and Other Models of DNA Repair and Mutagenesis, Aix-les-Bains (Savoy), France. Information: INSERM Institut National De La Sante, Conferences Philippe Laudat, 101 rue de Tolbiac, 75654 Paris, Cedex 13 France, 33 (1) 44 23 60 89/87, FAX 33 (1) 44 23 60 89, e-mail: laudat@tolbiac.inserm.fr

16–19 October, Wed–Sat. Conference on the Integrative Biology of Exercise, Vancouver, British Columbia, Canada. Information: Conference Management, 9650 Rockville Pike, Bethesda, MD 20814, (301) 530-7010, FAX (301) 530-7014, e-mail: vancouver96@faseb.org

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

20–24 October, Sun–Thu. Seventh North American ISSX Meeting, San Diego, California. Information: International Society for the Study of Xenobiotics, PO Box 3, Cabin John, MD 20818, FAX (301) 983-5357

25–31 October, Fri–Thu, Molecular Genetic Approaches to the Treatment of Genetic Disease, Hyatt Regency, Lake Tahoe, Nevada. Information: Cambridge Symposia, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1399, FAX (617) 630-1395, e-mail: symposia@xensei.com

29–31 October, Tue–Thu. Water Resources & Environmental Research: Towards the 21st Century, Kyoto, Japan. Information: S. Ikebuchi, Water Resources Research Center, Kyoto University, Gokasho, Uji, Kyoto 611 Japan +81-774-32-3093, e-mail: conf@wrcn2.-dpri.kyotou.ac.jp

31 October–5 November, Thu–Tue. Molecular Genetic Approaches to the Treatment of Genetic Disease, Hyatt Regency, Lake Tahoe, Nevada. Information: Cambridge Symposia, 1037 Chestnut Street, Newton Upper Falls, MA 02164, (617) 630-1399, FAX (617) 630-1395, e-mail: symposia@xensei.com

November

3–7 November, Sun–Thu. CFTR (Cystic Fibrosis Trnasmembrane Conductance Regulator) Protein: Traffic-King, Expression and Cellular Functions, Aix-les-Bains (Savoy), France. Information: INSERM Institut National De La Sante, Conferences Philippe Laudat, 101 rue de Tolbiac, 75654 Paris, Cedex 13 France, 33 (1) 44 23 60 89/87, FAX 33 (1) 44 23 60 89, e-mail: laudat@tolbiac.inserm.fr

How to Reach Us

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

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

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

Public Service Advertising—On a space-available basis, we will accept your public service announcements in a variety of formats. We can accept full, half, and one- or two-column camera-ready ads. If you do not have a formal ad but can provide an electronic version of your text via electronic mail or on a computer disk, we will include it for a limited time in the journal.

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

3–7 November, Sun–Thu. International Conference on Radiation and Health, Ben Gurion University of the Negev, Beer Sheva, Israel. Information: Conference Secretariat, Ortra Ltd., 2 Kaufman Street, PO Box 50432, Tel Aviv 61500, Israel, 972-3 517-7888, FAX 972-3-517-4433

10–14 November, Sun–Thu. Environmental Impact on Male Reproductive Function, Aix-les-Bains (Savoy) France. Information: INSERM Institut National De La Sante, Conferences Philippe Laudat, 101 rue de Tolbiac, 75654 Paris, Cedex 13 France, 33 (1) 44 23 60 89/87, FAX 33 (1) 44 23 60 89, e-mail: laudat@tolbiac.-inserm.fr

December

1–5 December, Sun–Thu. The American Society of Tropical Medicine and Hygiene 45th Annual Meeting, Hyatt Regency, Baltimore, Maryland. Information: The American Society of Tropical Medicine and Hygiene, 60 Revere Drive, Suite 500, Northbrook, IL 60062, (847) 480-9592, FAX (847) 480-9282

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

1997

August

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

Fellowships, Grants & Awards

Postdoctoral Fellowship in Cardiovascular Disease Epidemiology and Prevention:

NIH-sponsored two- to three-year fellowship emphasizes research methods in the epidemiology and prevention of cardiovascular disease. Fellows contribute to and gain competency in designing, administering, and analyzing cardiovascular population studies. Fellows can seek an M.P.H. degree. Candidates must have a doctoral degree and permanent U.S. residency status. Inquiries: Dr. Aaron R. Folsom (folsom@epivax.epi.umn.edu), Division of Epidemiology, School of Public Health, University of Minnesota, Suite 300, 1300 South 2nd St., Minneapolis, MN 55454-1015. The University of Minnesota is an equal opportunity educator and employer.

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 who are studying pulmonary epidemiology. Programs are tailored to individuals. Training can lead to an MPH degree from UNM. Laboratory research or pathogenesis of disease can focus on one of several disciplinary areas, including cell biology, molecular biology, biochemistry, immunology, pathology, physiology, toxicology, radiobiology, aerosol science, or mathematics modeling, depending on interests and qualifications. Participants must be US citizens or permanent resident aliens.

Contact: Dr. David Coultas, MD, The Mexico Tumor Registry, University of New Mexico, School of Medicine, 900 Camino de Salud NE, Albuquerque, NM 87185, (505) 277-5541. We are an Equal Opportunity/Affirmative Action Employer.

European Cancer Centre Two-Year Fellowships for Oncologists

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

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

Eligibility Criteria: Candidates must meet the following conditions:

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

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

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

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

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

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

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

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

Earthwatch Field Grants

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

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

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

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

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

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

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

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

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

Great Lakes Protection Fund Call for Preproposals

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

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

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

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

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

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

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

Forest History Society Offers 1996 Travel Grants

The Forest History Society announces the availability of Alfred D. Bell, Jr. travel grants for 1996. Those wishing to study at the Society's library and archives may receive up to $750 in support of travel and lodging expenses. Five Bell grants were awarded during 1995. For information on the Society's holdings and application procedures, write: Bell Travel Grants, Forest History Society, 701 Vickers Avenue, Durham, NC 27701 or call: 919 682-9319.

Forest Ecosystem

The U.S. Forest Service is soliciting proposals to develop a quality assurance and scientific assessment program within forest ecosystem research, monitoring and socioeconomic projects under its Southern Global Change Program. Applicant evaluation factors include: 1. experience in developing a quality assurance program for biological research , especially for field research; and 2. knowledge, educational background and experience regarding air pollution and potential climate change impacts to forest resources. For solicitation copy, immediately write: USFS, PO Box 2750, Attn: Nancy H. Meadows, Asheville, NC 28802. or Fax (704) 257-4876. Telephone for more information only: (704) 257-4297 or Alan Moore at (704) 257-4291. Reference: RFP SE-96-28.

Environmental Restoration

Applied research and development of technologies for environmental restoration and waste management are sought by the U.S. Department of Energy. Areas of interest include: 1.) contaminant plume containment and remediation; 2.) mixed waste characterization, treatment and disposal; 3.) landfill stabilization; 4.)sensor technology; and 5.) robotic technology. Proposals should address new concepts, long-term technology needs, and barriers and gaps in current technology. For solicitation copy, write: DOE, Morgantown Energy Technology Center, PO Box 880, Attn: Crystal A. Sharp, Morgantown, WV 26507-0880. Phone for more information only: (304) 285-4442. e-mail: csharp@metc.doe.gov

NTP to Examine Toxic Equivalence Factors (TEF) and Cancer Potency for Dioxin and Dioxin-Like Chemicals

The National Toxicology Program (NTP) intends to commission a series of two-year rodent cancer studies to determine the extent to which toxic equivalence factors (TEFs) for dioxin and dioxin-like chemicals predict a chemical's cancer-causing potential, target organ specificity, or relative potency.

Several hundred polyhalogenated aromatic compounds share biological response characteristics with dioxins, specifically with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Such compounds include certain polychlorinated and polybrominated biphenyls, polychlorodibenzofurans, and other polychlorinated dibenzo-*p*-dioxins. TEFs have been generated for many of these chemicals, primarily through short-term studies of noncancer endpoints that compared the chemical's potency in producing pleiotropic responses to that of dioxin. Although studies to date have indicated a good correspondence of TEFs to cancer promotion in initiation–promotion studies, this correspondence has never been adequately tested using the traditional rodent bioassay.

The NTP plans to examine single, or combinations of several, representatives of the major classes of dioxin like chemicals found in human adipose tissue using the female Sprague Dawley rat, which is the strain and sex of rodent most sensitive to the carcinogenic effects of dioxin and whose response is used as the basis for human risk assessments. The NTP tentatively plans to study 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, and 3,3',4,4',5-pentachlorobiphenyl (PCB 126), as well as TCDD. A noncoplaner, nondioxin-like PCB, 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), would also be studied alone and in a binary combination with TCDD to examine possible synergism or antagonism for the carcinogenic effect of TCDD. Finally, a combination of all chemicals other than PCB 153 will be tested as a representative mixture of dioxin-like chemicals. Doses will be selected using published information from existing prechronic and chronic rodent studies, and the dose ranges will be constructed to provide a rigorous test of the relationship between TEFs and carcinogenic potency. Selected tissue concentrations of the chemicals will be determined along with a subset of measures typically used to determine TEFs at various points during the studies such as CYP 1A1, 1A2, thyroid hormone levels, and others.

Because of the potential importance of these studies in determining future regulatory approaches to dioxin-like chemicals, the NTP plans to make available a limited number of small research grants (RO3) through the NIEHS extramural grants program. This RO3 program, via a competitive review process, will allow researchers at eligible organizations to utilize animals, sera, or tissues from these studies to perform experiments to enrich the dataset. Of particular interest would be studies that would provide a firmer mechanistic foundation for the TEF concept and its relationship to the carcinogenic activity of TCDD. Possible areas of exploration include changes in tissue receptor levels for estrogen, EGF or glucocorticoids, changes in CDC2, src activity, or measures related to oxidative stress. To allow completion of the study design of the two-year cancer studies, the NIEHS invites interested investigators to contact Dr. John Bucher, Deputy Director, Environmental Toxicology Program (Ph 919 541 4532, Fax 919 541 4255) to discuss ideas on how you would like to participate in these studies. Discussions would be for information only and would not preclude or replace submission of an RO3 application at the appropriate time.

Position Announcements

Retinal Anatomy/Physiology
Postdoctoral Position availbable now to work on NIH-funded study of interactions between rod and cone signals in macaque monkey retina. Training is offered in physiology and anatomy of primate retina in the lab of Dr. Dennis Dacey (Department of Biological Structure). Background in electrophysiology, visual psychophysics, and/or color science would be helpful. Annual salary: $25,000 for one to three years. Please send curriculum vitae and names of three references to : Dr. Steven Buck, Department of Psychology, University of Washington, Box 351525, Seattle, WA 98195. FAX: 206-685-3157, e-mail: sbuck@u. washington.edu

Public Health Scientist
The Natural Resources Defense Council, a national nonprofit public interest organization, seeks a Senior Scientist to bring scientific analysis and knowledge to advocacy in various forums for the prevention of adverse health and ecological effects of toxic chemical pollution. A PhD or MD/MD with 5 or more years highly relevant experience is required. Candidates should be knowledgeable about cutting-edge toxics issues such as disproportionately impacted subpopulations, noncancer endpoints, and emerging issues regarding carcinogenesis. The salary is $50,000--$65,000, commensurate with experience. Send resume to: Public Health Program, NRDC, 1350 New York Avenue, NW, Suite 300, Washington, DC 20005. Equal Opportunity Employer.

Postdoctoral Research Opportunities at the National Institute of Environmental Health Sciences
Listed below are outstanding opportunities to conduct research with leading scientists in Research Triangle Park, North Carolina.
To apply, please send a cover letter, curriculum vitae, bibliography, and names of three references to the hiring scientist at the maildrop and laboratory listed using the following address: NIEHS, PO Box 12233, Research Triangle Park, North Carolina 27709. In your cover letter, list the position title and the HNV number.
Minorities, women and handicapped individuals are encouraged to apply. All applicants receive consideration without regard to race, religion, color, national origin, sex, physical or mental handicap, political affiliation, age (with statutory exceptions) or any other nonmerit factor. Positions are open until filled.

Molecular Neurobiology (HNV94)
The signal transduction pathways regulating the expression of neuropeptide and cytokine genes in neural and glial systems are being investigated. Studies on the effects of neuropeptides on the biosynthesis and release of cytokines in microglial cells and potential roles of cytokines in neurodegeneration will be conducted. Applicants should have experience in neuropharmacology, neurochemistry or molecular biology.
Contact: J.S. Hong, (919) 541-2358, Laboratory of Environmental Neurosciences, Maildrop E1-01, e-mail: Hong3@niehs.nih.gov

Characterization of Receptor–Ligand Interactions (HNV96-1)
Mass spectrometry combined with protection assays is being used to probe structural motifs involved in molecular interactions, such as the interaction of HIV rgp120 and immunoglobin, relevant to an understanding of the basic processes occurring during HIV viral infection. Candidates should have expertise in protein and immunochemistry.
Contact: Kenneth Tomer, (919) 541-1966, Laboratory of Molecular Biophysics, Maildrop 6-01, e-mail Tomer@niehs.nih.gov

Eicosanoids, Airway Inflammation and Asthma (HNV96-2)
The expression and/or activation of the enzymes that metabolize arachidonic acid to inflammatory lipids; prostaglandin H synthase, lipoxygenases and phospholipases as related to the inflammatory response of trachea-bronchial epithelium is being investigated. A rat or human culture system is used for these studies in which differentiation to mucociliary or squamous phenotypes is regulated by retinoids. Differentiation of the epithelium has profound effects on the arachideonic acid metabolism. Experience with molecular biology techniques required and an interest in pulmonary biology desirable.
Contact: Thomas Eling, (919) 541-3911, Laboratory of Molecular Biophysics, Maildrop B3-01, e-mail: Eling@niehs.nih.gov

ATM Function in Cell Cycle Checkpoints and Senescence (HNV96-3)
Signal transduction mechanisms regulating cell cycle checkpoints and cellular senescence are being investigated, focusing particulary on the role of the ataxia telangiectasis mutated (ATM) gene product. Studies focus on the regulation of certain cyclin/cyclin-dependent kinase complexes in response to DNA damage following exposure to selected environmental carcinogens and in response to the normal aging process. Candidates should have experience in molecular biology, cell biology, or biochemistry.
Contact: Richard S. Paules, (919) 541-3710 or Cynthia Afshari (919) 541-1310, Laboratory of Environmental Carcinogenesis and Mutagenesis, Maildrop C1-09, e-mail: Paules@niehs.nih.gov or Afshari@niehs.nih.gov

Signal Transduction/Protein Purification (HNV96-6)
Inhibition of calcium-dependent chloride secretion by $Ins(3,4,5,6)P_4$ represents a new field of signal transduction regulating salt and fluid secretion, osmoregulation and neurotransmission; pharmacological intervention is relevant to cystic fibrosis and cardiac hypertrophy. The successful applicant will contribute to purification and characterization of the $Ins(3,4,5,6)P_4$ receptor and the enzymes of $Ins(3,4,5,6)P_4$ receptor synthesis and metabolism.
Contact: Stephen Shears, (919) 541-0793, Laboratory of Cellular and Molecular Pharmacology, Maildrop 7-10, e-mail: Shears@niehs.nih.gov

X-ray Crystallography (HNV 96-8)
The structural basis for the broad substrate specificity in the drug-metabolizing enzymes including P450s and sulfotransferases is studied. Contact: Masahiko Negishi, (919) 541-2404, Laboratory of Reproductive and Developmental Toxicology, Maildrop E4-07, e-mail: Negishi@niehs.nih.gov

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

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

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

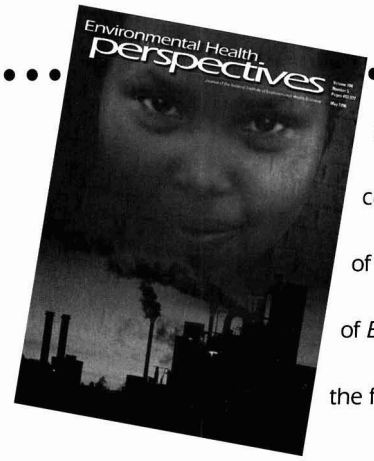
Molecular Biology and Fatty Acid Biochemistry (HNV112)
Novel human cytochrome P450 enzymes that metabolize fatty acids are cloned and expressed, and the catalytic properties of the recombinant, purified proteins are evaluated by HPLC. Regulation of the gene expression is studied using Northern analysis, RT-PCR, and protein immunoblotting, immunohistochemistry and *in situ* hybridization. Applicants should have a strong background in cell and molecular biology.
Contact: Darryl Zeldin, (919) 541-1169, Laboratory of Pulmonary Pathobiology, Maildrop D2-01, e-mail: Zeldin@niehs.nih.gov

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

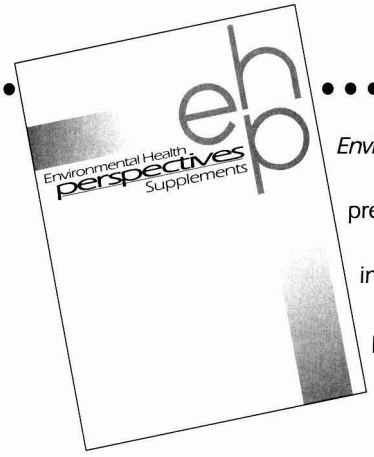
of Cellular and Molecular Pharmacology, Maildrop 19-04, e-mail: Yakel@niehs.nih.gov

Toxicokinetic Modeling (HNV121)
Toxicokinetic models are being studied as a means of relating the response of laboratory animals and humans to exposure to environmental toxins. Research relating to the utility of these models for the design and analysis of laboratory and epidemiology studies and for risk estimation is planned. Applicants should have a strong background in computer modeling and some experience in modeling biological systems.
Contact: Christopher J. Portier, 919-541-3519, Laboratory of Quantitative and Computational Biology, Maildrop A3-06, e-mail: Portier@niehs. nih.gov

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.



Environmental Health Perspectives Supplements presents state-of-the-art information in the form of monographs, conference proceedings, and an annual review of environmental science.

To receive *EHP Supplements*, fill out the lower form.

United States Government
INFORMATION
PUBLICATIONS ★ PERIODICALS ★ ELECTRONIC PRODUCTS

Order Processing Code:
*5665

Charge your order. It's easy! MasterCard VISA

To fax your orders (202) 512-2250

To phone your orders (202) 512-1800

☐ **YES,** please send me _____ subscription(s) to **Environmental Health Perspectives** (EHPM) at $39 ($48.75 foreign) per year so I can get cutting-edge research articles and news of the environment.

The total cost of my order is $_____. Price includes regular shipping and handling and is subject to change.

For privacy, check box below:
☐ Do not make my name available to other mailers.

Check method of payment:
☐ Check payable to the Superintendent of Documents
☐ GPO Deposit Account ☐☐☐☐☐☐–☐
☐ VISA ☐ MasterCard
☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
☐☐☐☐ (expiration date) *Thank you for your order!*

_____ Company or personal name (Please type or print)

_____ Additional address/attention line

_____ Street address

_____ City, state, ZIP code

_____ Daytime phone including area code

_____ Purchase order number (optional)

_____ (Authorizing signature) (5/96)

Mail To: Superintendent of Documents
P.O. Box 371954, Pittsburgh, PA 15250-7954

Important: Please include this completed order form with your remittance.

United States Government
INFORMATION
PUBLICATIONS ★ PERIODICALS ★ ELECTRONIC PRODUCTS

Order Processing Code:
*5720

Charge your order. It's easy! MasterCard VISA

To fax your orders (202) 512-2250

To phone your orders (202) 512-1800

☐ **YES,** please send me _____ subscription(s) to **Environmental Health Perspectives Supplements** (EHP) at $46.00 ($57.50 foreign) per year so I get state-of-the-art information in the form of monographs, conference proceedings, and an annual review of environmental health.

The total cost of my order is $_____. Price includes regular shipping and handling and is subject to change.

For privacy, check box below:
☐ Do not make my name available to other mailers.

Check method of payment:
☐ Check payable to the Superintendent of Documents
☐ GPO Deposit Account ☐☐☐☐☐☐–☐
☐ VISA ☐ MasterCard
☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
☐☐☐☐ (expiration date) *Thank you for your order!*

_____ Company or personal name (Please type or print)

_____ Additional address/attention line

_____ Street address

_____ City, state, ZIP code

_____ Daytime phone including area code

_____ Purchase order number (optional)

_____ (Authorizing signature) (5/96)

Mail To: Superintendent of Documents
P.O. Box 371954, Pittsburgh, PA 15250-7954

Important: Please include this completed order form with your remittance.

Editorial Policy

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

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

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

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

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

Perspectives

The journal is a forum for the expression of ideas and opinions. Opinions and ideas should be carefully considered and based on scientific principles. Three formats are offered:

Editorial statements are published by our editors, members of our editorial boards, and occasional guest editors. These statements are intended to focus attention on important or neglected areas of environmental health, offer opinions and ideas, and stimulate discussion.

Commentaries are up-to-date articles that may present commentaries offering perspective and insight on a particular topic. Commentaries are subject to peer review.

Correspondence is encouraged. Opinions, perspectives, and insight are welcome. Comments on articles published in *Environmental Health Perspectives* are also welcome, but criticism will always be balanced by the opportunity for defense and clarification.

Research

To ensure fairness in the review process, we routinely seek opinions from three reviewers. Suggestions for reviewers of manuscripts will be considered. The research portion of the journal consists of four formats:

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

Research Advances are concise articles intended to address only the most recent developments in a scientific field. Clarity of presentation is of primary importance because these articles are intended to be educational though targeted to the expert audience.

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

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

Environews

The news section provides up-to-date information on important issues in environmental health covering a variety of areas including policy, legislative, and regulatory actions; innovative technological and conceptual research advances; conference and meeting summaries; and emerging environmental problems. The news section consists of several components:

Forum articles are brief reports on matters of potential environmental health significance such as chemical spills and contamination episodes. Brief reviews of recent scientific advances are also included.

NIEHS News summarizes significant activities or accomplishments at NIEHS and the National Toxicology Program.

Focus articles are substantive news items about important issues in environmental health. Examples include reports on risk assessment, risk management dilemmas, women's health initiatives, environmental equity, relevance of animal models to toxicity testing, and structure–activity approaches to toxicity evaluation.

Spheres of Influence is a legal/regulatory column that presents reports on significant events and decisions involving the executive branch, Congress, and regulatory agencies. Examples include new directions of White House policies, impact of Clean Air Act legislation, and coverage of congressional hearings on environmental health issues.

Innovations presents emerging opportunities in environmental health based on new discoveries or approaches in biology, chemistry, engineering, or information sciences. Examples include the use of transgenic animals in toxicity testing, new advances in molecular biology, development of more rapid and efficient methods for clean-up of hazardous wastes, and methods for early detection of environmental damage and environmentally mediated diseases.

Announcements includes a calendar of upcoming events such as conferences, workshops, and public hearings. Appropriate listings are made for industrial, academic, regulatory, and legal activities. This section also includes listings of fellowship and grant announcements and positions available.

Environmental Health Perspectives Supplements

During the last 20 years, we focused on the development of a series of monographs that have generally arisen from symposium or conference proceedings. Monographs are now published as supplements to the main journal. Six to eight supplements are published per year: four to seven of these consist of conference, workshop, or symposium proceedings, and one issue is dedicated to solicited and unsolicited comprehensive reviews on environmental health. Conference manuscripts must be of the highest scientific quality and are subject to rigorous peer review. Manuscripts that do not meet *EHP* standards are not published.

Each supplement resulting from a conference should address a specific area of concern, a research problem, or a particular scientific issue. Supplements are, in general, dedicated to scientific issues and not to programmatic themes. Each supplement should form a landmark statement for a particular subject and must be an up-to-date, balanced source of reference material for researchers, teachers, legislators, and the informed public. Publication of conference proceedings in *EHP Supplements* requires the submission of a proposal as described in Instructions to Authors.

Supplement Articles from conferences are generally the result of research investigations, reviews, or a combination of both; however, brief reports and commentaries are also appropriate.

Perspective Reviews are targeted to the one or two specific issues of *EHP Supplements* set aside for the publication of reviews in environmental health sciences. Perspective reviews are in-depth, comprehensive articles that address developments in specific areas. Perspective reviews must not be simply a compilation of the literature but should be scholarly, landmark statements offering a complete and balanced perspective as well as insight into the environmental significance of the research.

Instructions to Authors

Environmental Health Perspectives covers all disciplines engaged in the broad field of environmental health. Authors should therefore write in a clear and simple manner, avoiding unnecessary technical jargon, so that the article is understandable to readers in other disciplines.

All submitted manuscripts are acknowledged upon receipt and subjected to three independent peer reviews. Submit four copies of the manuscript, along with three sets of publication-quality figures. Authors may suggest reviewers when submitting a manuscript, although suggested reviewers may not be chosen. Peer review is generally completed within four weeks and authors are notified of necessary revisions or rejection of the manuscript. Revisions are requested within three weeks of notification. Authors must submit two copies of the revised manuscript, a letter responding to reviewer's comments, and a diskette containing the revised manuscript. Articles are generally published three months after receipt of revisions. Corresponding authors are sent 200 free reprints of their article upon publication.

Manuscript Preparation

All manuscripts must be typed, double-spaced, in English, on only one side of the paper. Type the article on white paper, 216 × 279 mm (8.5 × 11 in) or ISO A4 (212 × 297 mm), with margins of at least 25 mm (1 in). Number pages consecutively, beginning with the title page. Reference lists, tables, and figure legends should be on separate pages, and should also be double-spaced. If the manuscript is accepted for publication, a computer disk copy must be submitted along with two hard copies of the revised manuscript.

Titles should not exceed 20 words and should generally not contain abbreviations or numerical values. The title page should also list authors (first or second names spelled out in full), full address of the institution where the work was done, and affiliation of each author. Indicate author to whom galley proofs and reprints should be sent (include complete address for express mail service, telephone and FAX numbers).

Place a **running title,** not to exceed 50 characters and spaces, on the second page of the manuscript. Also on this page, list 5–10 **key words** for indexing purposes, list and define all abbreviations, and include **acknowledgments** and grant information, not to exceed 50 words. Nomenclature and symbols should conform to the recommendations of the American Chemical Society or the International Union of Pure and Applied Chemistry (IUPAC).

All articles except meeting reports and commentaries must include an **abstract,** not to exceed 250 words, which should be placed on the third page of the manuscript. Do not include details of materials and methods or references in the abstract.

Text should begin on the fourth page. For research involving human subjects, include a statment that informed consent was obtained. For animal subjects, include a statement that care and treatment was conducted in accordance with established guidelines. Concise headings (not to exceed 8 words) may be used to designate major sections. Recommended headings, where appropriate, are "Materials and Methods," "Results," and "Discussion" or "Conclusion." **References** must be listed by number, in order of citation. Reference numbers should be italicized, if possible, and placed in parentheses in the text.

The **reference list** should begin on a separate page. Personal communications, unpublished observations, manuscripts in preparation, and submitted manuscripts should not be included in the reference list, nor should explanatory text (footnotes). Such references should be inserted at appropriate places in the text, in parentheses, without a reference number. "In press" articles should be included in the reference list. Abbreviate journal names according to *Index Medicus* or *Serial Sources for the BIOSIS Previews Database.* List all authors and editors; do not use et al. in the bibliography. Include the title of the journal article or book chapter and inclusive pagination. For reports, include the authoring organization, report number, "publisher" and location, and year of publication. Some examples are shown below:

Journal Article:

1. Canfield RE, O'Connor JF, Birken S, Kirchevsky A, Wilcox AJ. Development of an assay for a biomarker of pregnancy in early fetal loss. Environ Health Perspect 74:57–66 (1987).

Book Chapter:

2. Lohman AHM, Lammers AC. On the structure and fiber connections to olfactory centers in mammals. In: Progress in brain research: sensory mechanisms, vol 23 (Zotterman Y, ed). New York:Elsevier, 1967;65–82.

Report:

3. U.S. EPA. Status of pesticides in reregistration and special review. EPA 738-R-94-008. Washington, DC:Environmental Protection Agency, 1994.

Each **table** must be on a separate page. Tables should be numbered with Arabic numerals, followed by a brief title (not to exceed 25 words). General footnotes to tables should be indicated by lowercase superscript letters beginning with *a* for each table. Footnotes indicating statistical significance should be identified by asterisks (*, **) and daggers (†, ‡). Type footnotes directly after the table. Tables should contain no more than three layers of column headings, and the entire table should fit on one journal page.

Figure legends should be typed, double-spaced, on a separate page. Legends should be as brief as possible without compromising explanation of the figure. Use Arabic numerals to number figure legends. Define any abbreviations in the legend on first mention.

Three sets of publication-quality **figures** must be submitted. Electronic versions of figures are encouraged, but should be submitted in addition to, not in lieu of, hard copies of the figures. Dot matrix computer drawings are not acceptable as original art. The style of figures should be uniform throughout the paper. Identify all figures on the back with the authors' names and figure number and indicate orientation. Label axes of graphs clearly and define all symbols used.

Material suitable for inclusion as **on-line documentation,** such as kinetic studies, is welcome. Contact the *EHP* office for instructions regarding submission.

Electronic copies of accepted manuscripts are required. We prefer 3.5-inch diskettes, Macintosh platform, Microsoft Word, but IBM PC-compatible files are acceptable. The file should contain ***all*** parts of the manuscript in ***one*** file. Label the diskette with title, author, manuscript number, and software used. Diskettes are not returned to authors. Electronic files created by word processors or similar equipment are not acceptable.

Environmental Health Perspectives Supplements

Supplement Manuscripts result from conferences, symposia, or workshops and may take several forms. 1) Manuscripts reporting original research should be formatted as described for Research Articles, 2) opinions and discussion about a particular topic should be formatted as described for Commentaries, 3) manuscripts reviewing a topic or reporting a combination of review and original research should be formatted as described below for Perspective Reviews.

Perspective Reviews are in-depth, comprehensive reviews of a specific area. They should begin with a title and second page as described for research articles. Introduction and presentation of information should be continuous with specific items and discussion indentified by using subheadings. Abstracts, references, abbreviations, figures, and tables should also be handled as described for research articles.

Proposals for the publication of conference, symposium, and workshop proceedings will be considered; however, space is limited. We turn away many excellent proposals simply because we do not have space to publish them.

All proposals are reviewed and examined with a number of specific questions in mind. In developing a proposal, consider the following: Proposals are assessed according to their originality and scientific merit. Is the supplement needed? Is the subject matter timely and potentially useful to workers in the field? What is the environmental significance of the topic being addressed? Is the proposed supplement a complete representation of the field? Are there other aspects that should be included? Does the proposal contain sufficient information for evaluation? Is the presentation clear? Can the organizers integrate the participants into a cohesive unit? Are the contributors appropriate for the topic listed and do they have scientific credibility?

The source of funding is also considered. Scientific objectivity is extremely important, and it must be clear that organizers are not being used to present a bias favored by the funding body. Contributions from an interested party to a conference need not disqualify a proposal, but

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

All proposals must be submitted at least six months in advance of the conference. In the publication of conference proceedings, timeliness is essential. Because it takes at least six months to publication, no proposal will be considered after the conference has been held.

Submission of Manuscripts and Proposals

Submit all manuscripts and proposals in quadruplicate to:

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 news editor at (919) 541-5377.

Published Volumes of EHP Supplements

Year Vol/No	Subject
1972	
1	Polychlorinated Biphenyls[1]
2	Review–Perspective Articles[1]
1973	
3	Phthalate Esters[1]
4	Review–Perspective Articles[1]
5	Chlorinated Dibenzodioxins and Dibenzofurans[1]
6	The Evaluation of Chemical Mutagenicity Data in Relation to Population Risk[1]
1974	
7	Low Level Lead Toxicity and the Environmental Impact of Cadmium[1]
8	Review–Perspective Articles[1]
9	Asbestos[1]
1975	
10	Mobile Air Emission; Biometerological Hazards; Abstracts on Heavy Metals in the Environment, Conference II[1]
11	Components of Plastics Manufacture[1]
12	Heavy Metals in the Environment[1]
1976	
13	US–USSR Environmental Health Conference[1]
14	Human Health Effects of New Approaches to Insect Pest Control[1]
15	Target Organ Toxicity: Liver and Kidney[1]
16	Target Organ Toxicity: Lung[1]
17	WHO/NIEHS Symposium on Plastics Manufacture[1]
18	Target Organ Toxicity: Development[1]
19	Arsenic and Lead[1]
1977	
20	Proceedings of the Second NIEHS Task Force; NIEHS Science Seminar[1]
21	Vinyl Chloride Related Compounds[1]
1978	
22	Air Pollution and Human Health: Extrapolation from Animal to Man[1]
23	Polybrominated Biphenyls[1]
24	Target Organ Toxicity: Gonads; PCBs[1]
25	Factors Influencing Metal Toxicity[1]
26	Target Organ Toxicity: Cardiovascular System, Nervous System[1]
27	Higher Plants as Monitors of Environmental Mutagens; Hazardous Solid Wastes and Their Disposal[1]
1979	
28	Cadmium[1]
29	Pollutants and High Risk Groups[1]
30	USA/USSR Cooperative Research[1]
31	Aneuploidy[1]
32	JAPAN/USA Biostatistics; Statistics and the Environment[1]
33	Target Organ Toxicity: Intestines; Effects of Increased Coal Utilization; NIEHS Science Seminar[1]
1980	
34	Aquatic Toxicology; Biological Effects of Mineral Fibers and Particulates[1]
35	Experimental Models for Pulmonary Research[1]
36	Application of Negative Ion Spectrometry[1]
1981	
37	Pollen Systems[1]
38	Target Organ Toxicity: Endocrine Systems[1]
39	Target Organ Toxicity: Blood[1]
40	Role of Metals in Carcinogenesis[1]
41	Polyvinyl Chloride[1]
42	Environmental Epidemiology[1]
1982	
43	Target Organ Toxicity: Immune System[1]
44	Target Organ Toxicity: Eye, Ear, and Other Special Senses; Author and Subject Indexes, Volumes 26–40[1]
45	Phthalate Esters[1]
46	Drinking Water Disinfectants[1]
1983	
47	Mutagenicity and Carcinogenicity of Air Pollutants[1]
48	Health Effects of Toxic Wastes[1]
49	*N*-Substituted Aryl Compounds[1]
50	Tumor Promotion[1]
51	*In Vitro* Effects of Mineral Dusts[1]
52	Environmental Epidemiology; Air Quality Control; NIEHS Science Seminar[1]
53	Asbestos[1]
1984	
54	Metallothionein and Cadmium Nephrotoxicity[1]
55	Monograph on Pulmonary Toxicology[1]
56	Methods in Pulmonary Toxicology; Proliferative Pancreatic Lesions[1]
57	Glycol Ethers; Mechanisms of Cell Injury[1]
58	Carcinogenic Potency Database; Formaldehyde[1]
1985	
59	PCBs: Japan–U.S. Symposium[1]
60	PCBs: U.S. Symposium; Finland–U.S. Symposium[1]
61	Monograph on Structure–Activity and Molecular Mechanisms[1]
62	DNA Adducts; Environmental Epidemiology[1]
63	Health Effects of Acid Precipitation; Environmental Risk Assessment[1]
64	Monograph on Free Radical Metabolites[1]
1986	
65	Metal-Binding Proteins; Phthalic Acid Esters[1]
66	Cotton and Grain Dusts[1]
67	Dietary Mutagens[1]
68	SENCAR Mouse; Author and Subject Indices, Volumes 41–60[1]
69	Drinking Water Disinfectants[1]
70	Airborne Pollutants and Respiratory Cancer; Reproductive and Developmental Toxicology[1]
1987	
71	Aquatic Toxicology[1]
72	Methyl Isocyanate[1]
73	Ovarian Pathology; Nitrogen Oxides[1]
74	Biomarkers in Reproductive and Developmental Toxicology[1]
75	NIEHS 20th Anniversary[1]
76	Basic Research in Risk Assessment[1]
1988	
77	Male Reproductive System; Extrapolation of Carcinogenicity Data[1]
78	Lead–Blood Pressure Relationships, Author and Subject Indices, Volumes 61–73[1]
1989	
79	Acid Aerosols[1]
80	Monograph on Regulation of Differentiation in Eukaryotic Cell Systems[1]
81	Scientific Advances in Environmental Health[1]
82	Benzene Metabolism, Toxicity and Carcinogenesis[1]
83	Monograph on Groundwater Quality[1]
1990	
84	Calcium Messenger Systems[2]
85	Chemicals and Lung Toxicity; Upper Respiratory System[1]
86	Butadiene; Environmental Health in the 21st Century[2]
87	Biostatistics in Human Cancer; Structure–Activity[1]
88	Risk Factors and Mechanisms in Carcinogenesis[1]
89	Advances in Lead Research[1]
1991	
90	Contaminated Aquatic Food Resources; Quantitative Risk Assessment[2]
91	Lead in Bone[2]
92	Chromium[2]
93	Target Genes in Chemical Carcinogenesis[2]
94	Environmental Epidemiology[2]
95	Indoor Air Quality[1]
96	Genotoxicity and Carcinogenicity Databases; Global Warming[2]
1992	
97	Particle Clearance by Alveolar Macrophages[2]
98	Biomarkers in Human Cancer–Part I: Predisposition and Use in Risk Assessment[2]
1993	
99	Biomarkers in Human Cancer–Part II: Exposure Monitoring and Molecular Dosimetry[1]
100	Twenty Years of Environmental Health Research[2]
101/1	NTP Abstracts, 1976–1992[2]
101/2	Impact of the Environment on Reproductive Health[2]
101/3	Environmental Mutagenesis in Human Populations at Risk[2]
101/4	Environmental Epidemiology[2]
101/5	Cell Proliferation and Chemical Carcinogenesis[2]
101/6	Health Effects of Gasoline[1]
1994	
102/1	Biostatistics and the Study of Toxicology[1]
102/2	Reviews in Environmental Health, 1994; Human Developmental Neurotoxicity[2]
102/3	Molecular Mechanisms of Metal Toxicity and Carcinogenicity[2]
102/4	Risk Assessment of Urban Air[2]
102/5	Biopersistence of Respirable Synthetic Fibers and Minerals[2]
102/6	Carcinogenic and Mutagenic *N*-Substituted Aryl Compounds[2]
102/7	Health Effects of Boron[2]
102/8	Biostatistics in the Study of Human Cancer[2]
102/9	Toxicological Evaluation of Chemical Interactions[2]
102/10	Oxygen Radicals and Lung Injury[2]
102/11	Dosimetry for Risk Assessment[1]
102/12	Genetic and Molecular Ecotoxicology[2]
1995	
103/1	Fate, Transport and Interactions of Metals[2]
103/2	Health Effects of Ozone; Environmental Epidemiology; Perinatal Exposure to Dioxins[2]
103/3	Human Tissue Monitoring and Specimen Banking[2]
103/4	Wildlife Development[2]
103/5	Biodegradation[2]
103/6	Child Health; Asthma[2]
103/7	Estrogens in the Environment[3]
103/8	Carcinogenic Potency Database; Avoidable Cancer Causes[3]
103/9	Great Lakes and Human Health[3]
1996	
104/1	Reviews in Environmental Health, 1996; Indices, Vol 103, Suppl 1–9 (1995)[3]
104/2	Neurobehavioral Toxicity[3]
104/3	Environmental Mutagens[3]

[1]Out of print. [2]Available free of charge from NIEHS-EHP, P.O. Box 12233, Research Triangle Park, NC 27709. [3]Available by subscription only from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

U. S. Department of Health & Human Services
Public Health Service
National Institutes of Health
NIH Publication 96-218

Official Business
Penalty for Private Use $300

Bulk Rate
U.S. Postage
PAID
Permit 2058
St. Louis, Mo.