Human Biomonitoring of Environmental Chemicals

Measuring chemicals in human tissues is the "gold standard"
for assessing people's exposure to pollution

Ken Sexton, Larry L. Needham and James L. Pirkle

What chemicals in your daily routine should you be most concerned about? The volatile organic compounds from your carpet? The exhaust fumes on the road to work? The pesticide residues in the apple in your lunch? Most of us are exposed to low levels of thousands of toxic chemicals every day. How can a person—or a nation—decide which substances should be controlled most rigorously?

One strategy is to go after the largest sources of pollution. This approach certainly makes sense when those pollutants have obvious and widespread consequences, such as warming the globe, causing algal blooms, eroding the ozone layer or killing off wildlife. But for protecting human health, this strategy does not serve so well, because the link between a given compound and its biological effects can be difficult to gauge. For epidemiologists to correlate environmental pollutants with health problems, they need to know who has been exposed and at what level.

This knowledge is exceptionally difficult to gain when there is a lag between exposure and the manifestation of illness. In such cases, the data are seldom—if ever—sufficient to deter-

mine the precise agent, the details of contact and the full extent of the affected population. Complicating matters, the scientific understanding of the mechanisms of exposure, such as how various compounds are carried through the air and changed along the way, is often incomplete. As a result, epidemiologists often find it difficult to establish cause-and-effect relationships for environmentally induced sicknesses. Without reliable information some pollutants may be unfairly blamed, whereas others exert their dire effects without challenge. Fortunately, there is hope: a method of accurately measuring not only contact with, but also absorption of toxic chemicals from, the environment—human biomonitoring.

Is It in Me?

Each person's risk of developing an environmentally related disease, such as cancer, results from a unique combination of exposure, genes, age, sex, nutrition and lifestyle. Science doesn't fully understand how these variables interact, but exposure is clearly a key factor. Thus, a fundamental goal of environmental health policy is to prevent (or at least reduce) people taking in chemicals that lead to any of the five Ds—discomfort, dysfunction, disability, disease or death.

Exposure to an environmental chemical is minimally defined as contact with the skin, mouth or nostrils—a meaning that includes breathing, eating and drinking. For the purposes of assessing risk, the most important attributes of exposure are magnitude (what is the concentration?), duration (how long does contact last?), frequency (how often do exposures occur?) and timing (at what age do exposures occur?). The calculation of actual expo-

sure also requires complex detective work to discover all kinds of details, including the chemical identity (for example, the pesticide chlorpyrifos), source (nearby agricultural use), medium of transport (groundwater) and route (drinking contaminated well water). Scientists must consider this information on exposure against the background of people's activity patterns, eating and drinking habits, and lifestyle, and they must also evaluate the influence of other chemicals in the air, water, beverages, food, dust and soil. Overall, this is a daunting challenge.

Historically, those scientists who undertook such a complex task have relied on indirect methods: questionnaires, diaries, interviews, centralized monitoring of community air or water, and a record of broad activity patterns among the population. But the results were often disappointing. Although these circumstantial approaches have the advantages of practicality and frugality, they can also introduce substantial uncertainty into resulting exposure estimates. This shortcoming multiplies the potential for a fundamental error—classifying a person as "not exposed" when he or she has been or vice versa.

A second approach, the direct measurement of an individual's environment, is sometimes a possibility—for example, a person might carry a portable monitor to record contact with airborne chemicals. Although this technique offers an unequivocal record of chemical contact, it is technologically infeasible or prohibitively expensive to measure most pollutants this way. Also, although such monitors document exposure, they tell nothing about the person's uptake of these airborne chemicals—how much truly gets into his or her body, which is, of course, the most relevant

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Figure 1. In July 1945, DDT was widely (and mistakenly) hailed as a progressive measure to eradicate disease-bearing mosquitoes without posing a risk to human health. In this photo from a beach on Long Island, New York, a new insecticide-spraying machine is tested as beachgoers play in the mist. Although this chemical contact is obvious, many other sources of environmental chemical exposure are more difficult to identify. Human biomonitoring examines people's blood and urine to evaluate actual levels of more than a hundred substances.

information for assessing health risk. Fortunately, technological advances in biomedicine and analytical chemistry now make it possible to get exactly this information. Biomonitoring measures the actual levels of suspected environmental chemicals in human tissues and fluids. This third approach has come to be the "gold standard" for assessing exposure to chemicals.
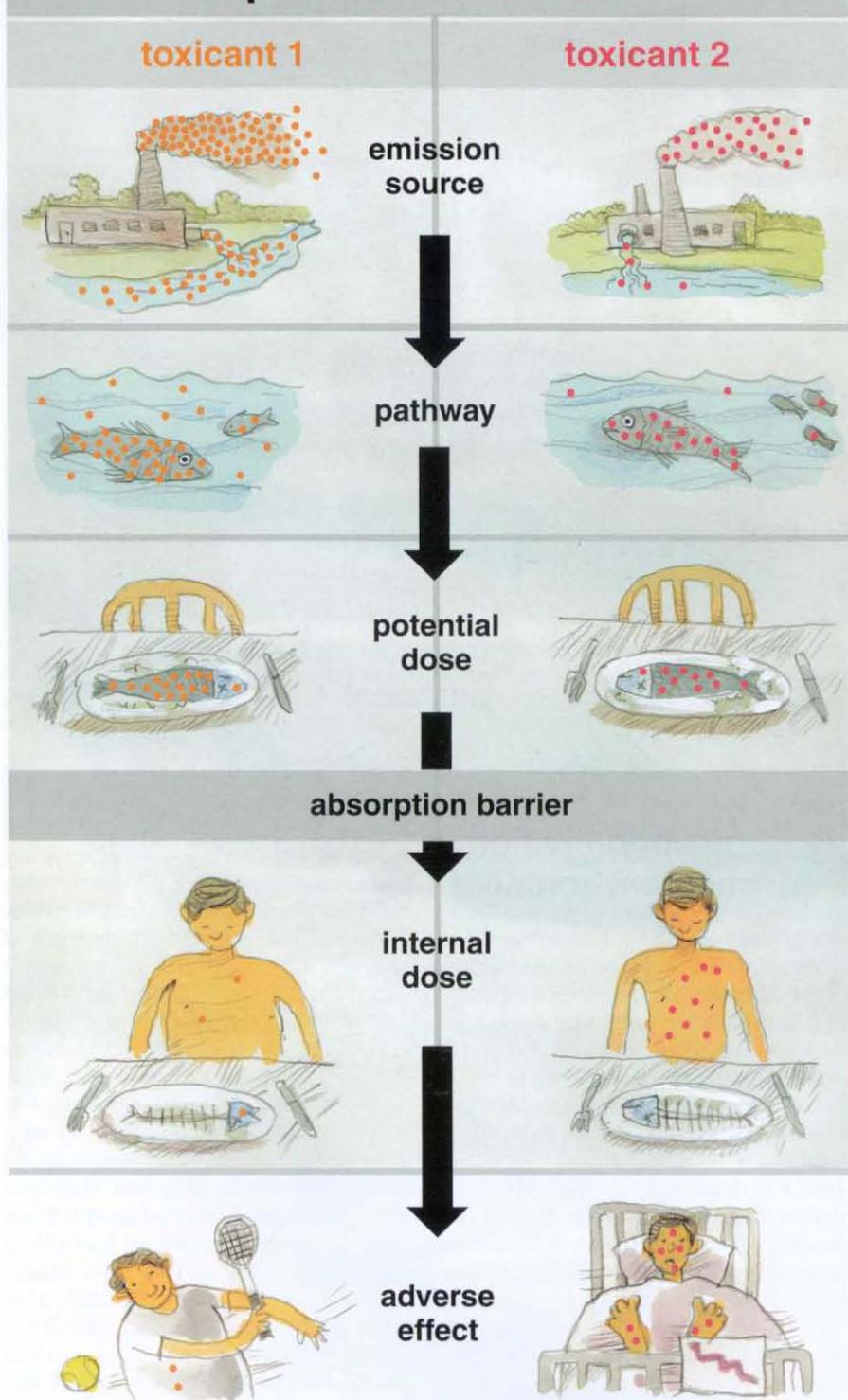
Blood (and Urine) Will Tell

Biomonitoring is not new. It has its roots in the analysis of biological samples for markers for various pharmaceutical compounds and occupational chemicals, efforts that sought to prevent the harmful accumulation of dangerous substances. Although it had a different name at the time, the general idea was

first applied about 130 years ago when doctors monitored the amount of salicyluric acid in the urine of rheumatics who were being treated with large doses of salicylic acid (the precursor of aspirin). And as early as the 1890s, factory workers who were exposed to lead had their blood and urine screened to forestall the elevated levels that produced acute lead poisoning.

These investigators soon learned that the degree of contact with a substance doesn't necessarily determine the biologically relevant exposure to that chemical. As a result, this measure didn't help much in predicting the risks of lead poisoning. However, they did find that the amount of a compound that crosses the body's boundaries (called the internal or absorbed

dose, or sometimes the body burden) has considerable value for estimating the risk to health. Today, it is relatively affordable to measure the absorbed doses for hundreds of chemicals by looking for biomarkers of exposure in accessible human tissues and fluids, including saliva, semen, urine, sputum, hair, feces, breast milk and fingernails (all of which can be collected readily), and blood, lung tissue, bone marrow, follicular fluid, adipose tissue and blood vessels (which require incursion into the body). Although procedures to collect any of the first set would, technically, be considered "noninvasive," in fact, that categorization rests on cultural, psychological and social factors. So obtaining the right material can sometimes be awkward. Fortunately

exposure assessment

toxicant 1	toxicant 2

emission source

pathway

potential dose

absorption barrier

internal dose

adverse effect

Figure 2. Which toxicant is more dangerous? Because of the multiple steps through which an environmental chemical must pass before it becomes a potential health threat, the answer is not always clear. Here, toxicant 1 is more abundant in the environment, but the specific properties of the chemical may mean that it poses less medical risk than another compound. Different methods of exposure assessment can evaluate each of these steps, but biomarker analysis, which measures internal doses of specific substances, provides the most relevant information for human health.

natural compounds in the body, such as proteins.

Choosing the appropriate tissue or fluid for biological monitoring is based primarily on the chemical and physical properties of the chemical of interest and, in some cases, the time interval since the last exposure. For example, some chemicals including dioxins, polychlorinated biphenyls and organochlorine pesticides have long biological residence times in the body (months or years) because they are sequestered in fatty tissues. They are thus said to be fat-loving or, to use the proper term, lipophilic. By contrast, other chemicals such as organophosphate pesticides and volatile organic compounds, which don't accumulate in fats (being lipophobic), have relatively short biological residence times (hours or days) and tend to be metabolized rapidly and excreted in the urine.

The time since the last exposure can also play a key role in determining the best biological specimen for analysis. For example, a persistent chemical, such as a dioxin, remains present in blood for a much longer period (years) than does a nonpersistent compound such as benzene (hours), but dioxin does not form significant urinary metabolites, whereas benzene does. For these reasons, persistent chemicals are typically measured in blood, and nonpersistent chemicals are measured in urine (as soon after exposure as possible), although they can also be detected in blood soon after exposure if the analytical methods are sufficiently sensitive—and they usually are. Specialists can now detect extremely low levels—parts-per-billion, parts-per-trillion, even parts-per-quadrillion—of multiple markers using a relatively small sample, say, 10 milliliters or less.

Clearly, the sensitivity of the analysis is important in choosing what to measure—but it's not everything. Other issues must be considered before the results can be considered meaningful. Well before attempting to discern trace amounts of target chemicals, an investigator should be able to answer three broad questions: How is the measurement related to the magnitude, duration, frequency and timing of exposure? How do subsequent processes within the body—such as absorption, distribution, metabolism and excretion—influence the targeted biomarker? And is this particular marker specific for a certain chemical or does it indicate an entire class of substances?

for those of us in the biomonitoring field, it's never necessary to collect all of those samples—blood and urine are typically sufficient. These are analyzed for the presence of biological markers of exposure—generally the targeted chemical, its primary metabolites or the products of its reaction with certain

Because the science underpinning human biomonitoring has improved significantly in recent years, these questions are now easier to answer. The rapid advancement in knowledge of what the body does to chemicals that are inhaled, ingested or absorbed through the skin has led to better interpretation of the range of concentrations for various biomarkers. And the number of testable compounds has increased dramatically: Sensitive and specific biomarkers are available for many environmental chemicals, including metals, dioxins, furans, polychlorinated biphenyls, pesticides, volatile organic compounds, phthalates, phytoestrogens and environmental tobacco smoke. As research continues, the list will surely continue to grow.

Exposure *and* Uptake

Biomonitoring has many advantages over traditional methods. For example, biological samples reveal the integrated effects of repeated contact. Also, this approach documents all routes of exposure—inhalation, absorption through the skin and ingestion, including hand-to-mouth transfer by children. Such specimens also reflect the modifying influences of physiology, bioavailability and bioaccumulation, which can magnify the concentrations of some environmental chemicals enough to raise them above the detection threshold. Perhaps most importantly, these tests can help establish correlations between exposure and subsequent illness in individuals—which is often the key observation in proving whether or not a link exists.

A great strength of biomonitoring is that it provides unequivocal evidence that both exposure and uptake have taken place. In some cases these data can confirm the findings of traditional exposure estimates. For example, in 1979, residents of Triana, Alabama, were notified that fish from a nearby creek had forty times more DDT than the allowable limit, even though the local DDT manufacturing plant had been inactive since 1971. The announcement was especially concerning because many people in that area caught and ate the fish regularly. In response to this discovery, the Centers for Disease Control and Prevention (CDC) constructed an evaluation based on DDT concentrations in fish and the amount of fish eaten per week. This estimate indeed correlated with levels of DDT and its metabolites,
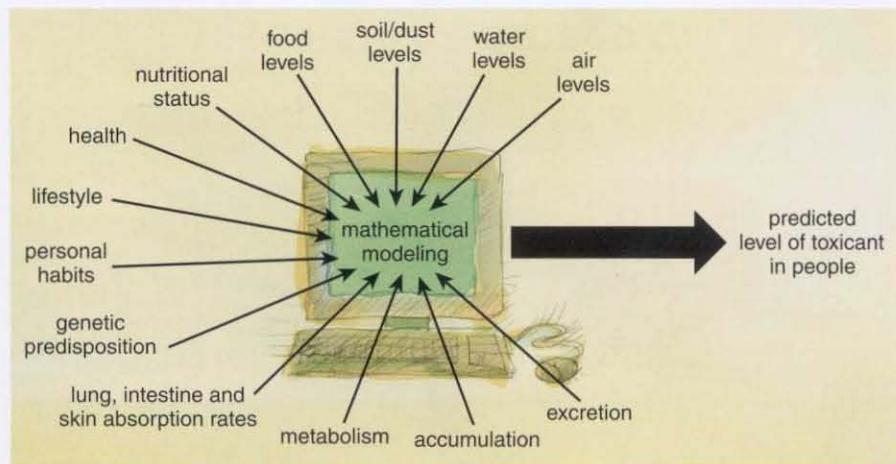

Figure 3. Traditional estimates of human exposure have to account for many variables, including some that demand assumptions about factors that are poorly understood. The result is often uncertain.
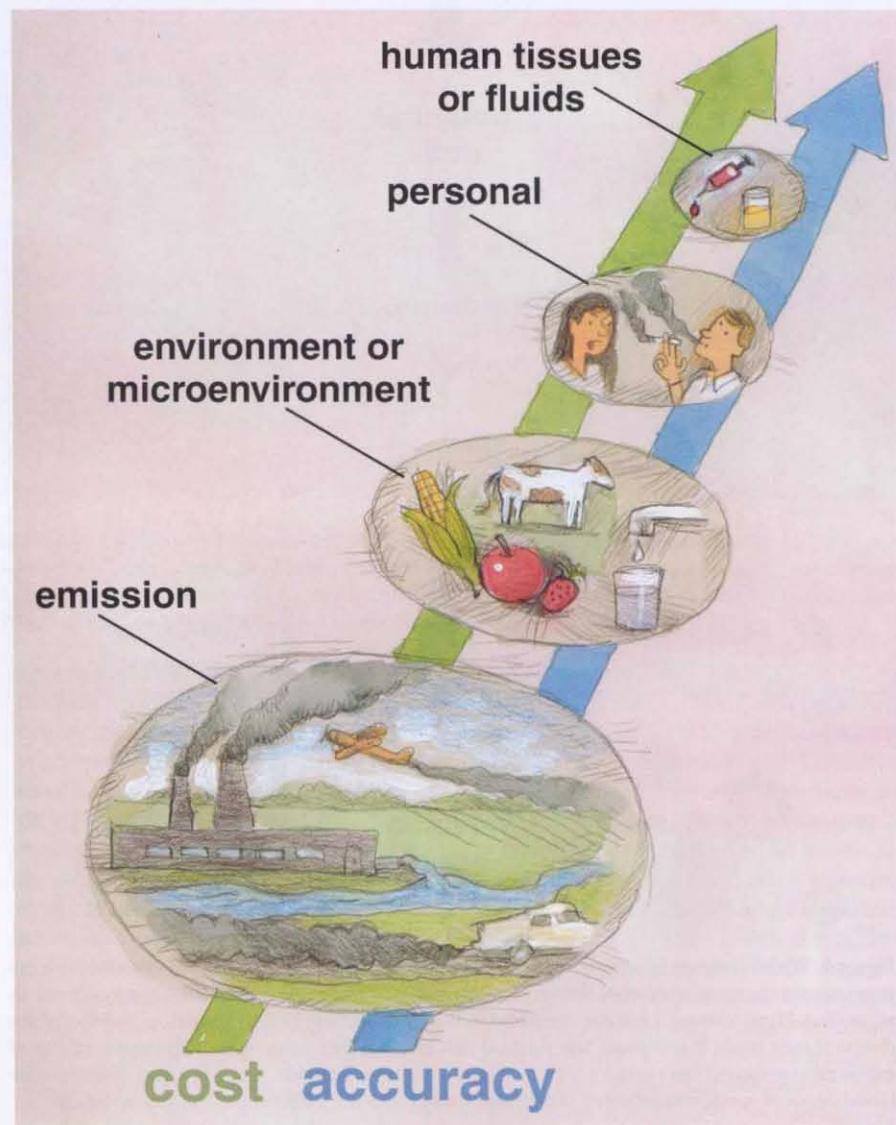

Figure 4. Exposure to environmental chemicals can be assessed in several ways. Generally, the accuracy and cost vary together. Monitoring emission sources is the least expensive and least accurate means of determining human exposure, whereas biomarker measurement is more costly but also highly informative for that person.

Figure 5. At its Environmental Health Laboratory, CDC scientists use several types of high-resolution mass spectrometry to analyze human tissue and fluid samples. The equipment shown here is being used to measure dioxin levels in a sample of blood serum. (Photograph courtesy of James L. Pirkle.)

gasoline lead decreased by approximately 55 percent) there was a parallel decline in the amount of lead coursing through the veins of the U.S. population. Overall, average blood concentrations decreased from about 16 to less than 10 micrograms of lead per deciliter of blood. These data demonstrated the effectiveness of removing lead from gasoline, and they were a dominant factor in the decision by the Environmental Protection Agency (EPA) to remove lead from gasoline more rapidly—a task that was effectively complete by 1991. Today, the average blood-lead level in the U.S. population is less than 2 micrograms per deciliter
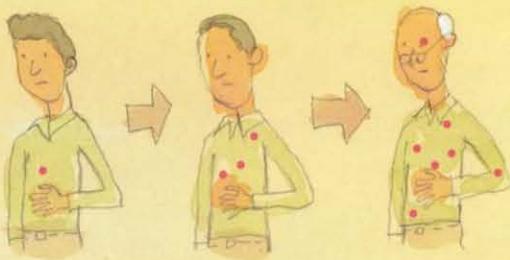
Exposure Disclosure

The study that revealed the tight connection between the lead in people's gas tanks and the lead in their blood was mounted by the CDC, which conducts the National Health and Nutrition Examination Surveys (NHANES for short). Although no environmental chemicals were measured as part of NHANES I (1971–1975), starting with NHANES II (1976–1980), the CDC began measuring blood lead levels in the U.S. population, ironically enough, after the Food and Drug Administration voiced concerns about possible exposures from eating food stored in lead-soldered cans, which turned out to be a very minor risk compared with leaded gasoline. As part of NHANES II, the EPA tested for certain persistent pesticides in people's blood and nonpersistent pesticides or their metabolites in urine. After an eight-year hiatus, NHANES III was conducted in two three-year phases from 1988 to 1994. In that iteration, the CDC measured lead and cadmium and began testing for cotinine, the major metabolite of nicotine, in blood. Additionally, the CDC began a separate pilot program to measure new compounds, testing for trace amounts of 32 volatile organic chemicals in blood and 12 pesticides or their metabolites in urine from approximately 1,000 of the NHANES III participants.

Then came another long gap in coverage. But thankfully, in 1999, NHANES became a continuous survey of the noninstitutionalized U.S. population. (It is thought that excluding members of isolated organizations, such as military personnel, college students and prisoners, provides a better cross-section of America.) In the current design,

DDE and DDD, in the blood of Triana residents. In a similar story that unfolded in the late 1980s, chemical-plant workers in New Jersey and Missouri discovered that they had been exposed to dioxin-contaminated compounds up to the early 1970s. They had come into contact with the dioxin in various ways—breathing it, swallowing it and taking it in through the skin. Despite the complexities of their interaction with this dangerous substance—and the time interval since exposure—a scheme that used occupational records to calculate the duration of potential exposure was able to accurately estimate internal doses. This finding was confirmed by the correlation of these results with the concentration of dioxins in their blood.

Having information about exposure *and* uptake is more than a *pro forma* detail: There are many cases in which traditional estimates of exposure (questionnaires, proximity to sources, environmental concentrations, constructed scenarios) are not correlated with measured biomarkers. For example, from 1962 to 1971, the U.S. Air Force sprayed the defoliant known as "Agent Orange" in Vietnam. Many service members who participated in that operation touched or breathed the herbicide, potentially exposing themselves

to high levels of dioxin. The Air Force first estimated the risk to soldiers using a scenario approach, which included the average dioxin concentration in Agent Orange, the number of gallons used during a soldier's tour of duty, and the frequency and duration of potential contact based on job description. Despite a considerable scientific effort that went into these predictions, CDC studies in the late 1980s proved that none of the exposure estimates were correlated with the measured blood levels of dioxin in at-risk troops. A subsequent investigation of personnel with the highest dioxin levels did identify some patterns that explained their increased contact—for example, small-statured enlisted men often climbed into the chemical tanks to clean out residual Agent Orange.

A more striking example of the value of biomonitoring came in the mid-1970s when the United States elected to start phasing out leaded gasoline. Prior to this decision, traditional models had suggested that eliminating lead in gasoline would have only a slight effect on people's uptake of that metal. However, biomonitoring data from the CDC's Second National Health and Nutrition Examination Survey revealed that from 1976 to 1980 (as unleaded fuel was first introduced and

Identifying priority exposures. Out of thousands of chemicals, which are the most dangerous? Biomarkers can help set priorities for public health and regulatory follow-up.

Recognizing time trends in exposure. Periodic measurement of biomarkers in the population shows how body burdens of chemicals vary from season to season, year to year and decade to decade.

**Identifying at-risk populations.** Large biomarker studies can distinguish exposure differences among racial, geographic or socioeconomic groups.

Establishing reference ranges for comparison. A blood test shows that you've been exposed to some chemical. Should you be worried? Your doctor can't tell without data from people with little or no exposure.

Providing integrated dose measurements. Biomarker analysis provides a direct assay of body burden that integrates exposure from all sources, even ones that are hard to measure.
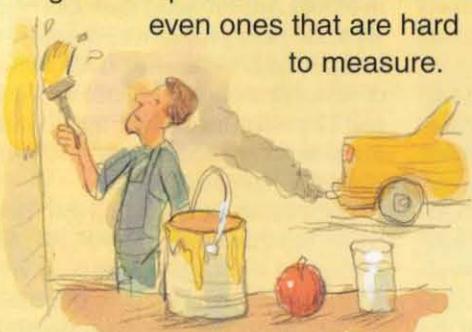
Evaluating exposure prevention efforts. Our government is entrusted with reducing people's exposure to environmental chemicals. Do they succeed? Before-and-after biomarker tests can tell.
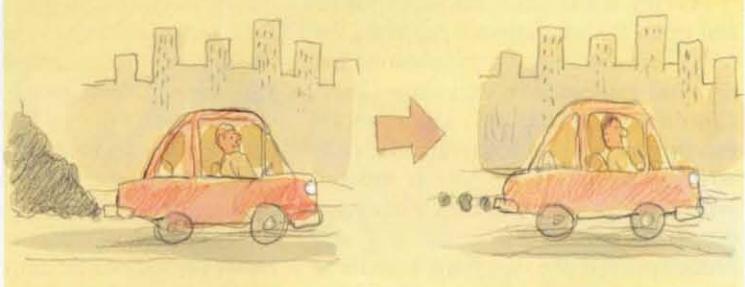
Figure 6. When used to establish levels of human chemical exposure, biomonitoring has six major uses that can help to protect public health.
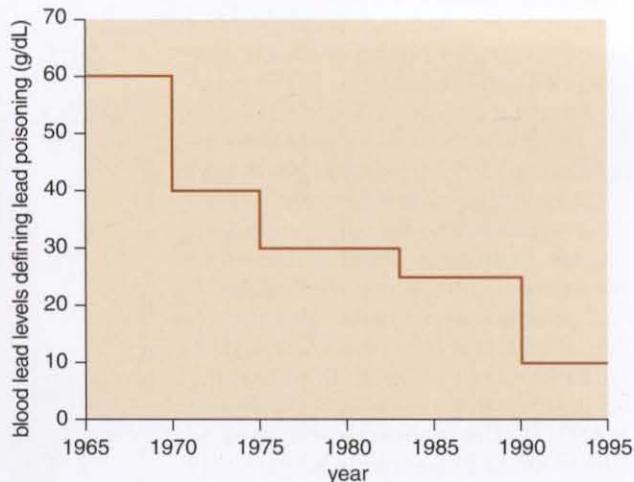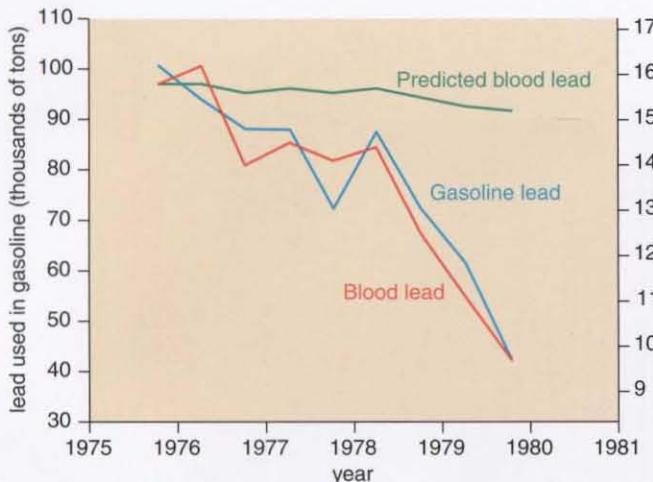
Figure 7. Leaded gasoline began to be phased out in the 1970s. Although the predicted effect on blood lead was minimal, actual lead exposure in the U.S. population (measured in micrograms of lead per deciliter of blood) sharply declined between 1976 and 1980, paralleling the changes in gasoline *(left)*. Blood lead and gas lead continued to follow nearly identical decreases up to 1990. At the same time, a series of studies on lead toxicity showed that lower doses could still cause adverse effects, prompting a steady decline in the level defining lead poisoning *(right)*.

a new national sample is collected every two years. Although some other studies have focused on specific populations or on more restricted data, NHANES is the only national survey that includes both a medical examination and collection of biological samples from participants. Individuals selected for NHANES are representative of the U.S. population, meaning that they do not necessarily have high or unusual exposures. About 5,000 participants are examined annually from 15 locations throughout the country.

Reporting For Duty

In March 2001, the CDC released the National Report on Human Exposure to Environmental Chemicals, which included data from 1999 on 27 chemicals. A second report was published in January 2003 that examined 116 chemicals in samples from 1999–2000. Both studies used biomonitoring to provide an ongoing assessment of exposure to a variety of substances. Although various studies of workplace exposure, for example, had raised concerns about the health effects of such chemicals, most of them had never before been measured in a representative slice of the U.S. population.

The inventory of tested substances in the second CDC report includes lead, mercury, cadmium and other metals; persistent (organochlorine-based) and nonpersistent (organophosphate- and carbamate-based) insecticides, herbicides and other pesticides; pest repellents and disinfectants; cotinine; phthalates; polycyclic aromatic hydrocarbons;

dioxins, furans and polychlorinated biphenyls; and phytoestrogens. Results from the general population are subdivided by age, gender and ethnicity.

An important feature of the CDC report is that it provides reference ranges for exposure among the general U.S. population. If people are concerned that they may have been excessively exposed to an environmental chemical, they can compare their biomarker levels to those standards. These reference ranges are immensely beneficial to public-health scientists who must decide if certain high-exposure groups need follow-up action. If average levels among the cohort are similar to those of the general public, then the group's exposure is unlikely to cause unique problems. On the other hand, if levels are substantially higher than national
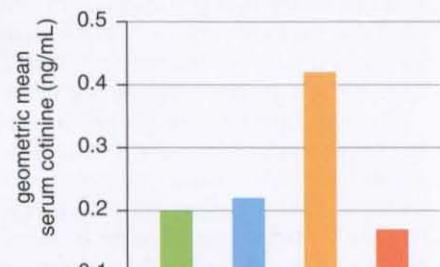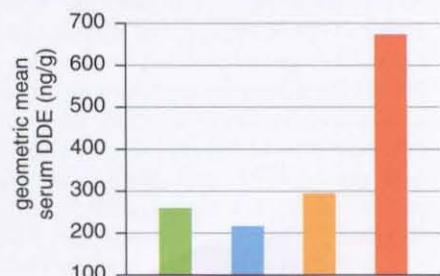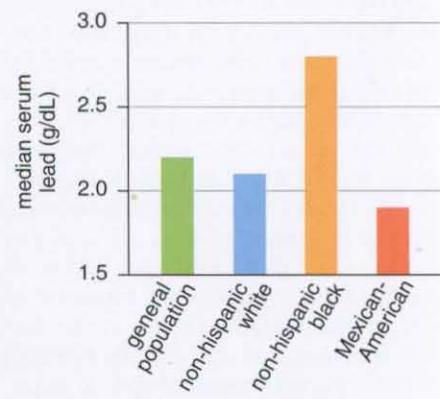
Figure 8. One important function of biomonitoring is that it can identify specific subpopulations that may be more vulnerable to exposure from a particular chemical. For example, *p,p'*-DDE, a long-lasting metabolite of DDT, is more than twice as high in Mexican-Americans compared with the general population. By contrast, cotinine levels are the lowest among this group, indicating that they have the least exposure to environmental tobacco smoke. For both cotinine and lead, non-hispanic blacks showed the highest levels. DDE (in nanograms per gram of lipid) and lead (in micrograms per deciliter of blood serum) data are from the CDC's Second National Report on Human Exposure to Environmental Chemicals, published in 2003. Information on cotinine (in nanograms per milliliter of blood) is from the third National Health and Nutrition Examination Survey (NHANES III), 1988-1991.

norms, epidemiologists can confirm the unusual exposure, identify the sources and provide continuing health care as appropriate. The reference ranges provide indirect financial ad-

vantages too, because distinguishing common from unusual chemical contact helps direct resources to the most-pertinent exposure situations.

The overarching purpose of these reports is to help scientists, physicians and health officials to prevent, reduce and treat environmentally induced illnesses. However, some caution must be exercised in interpreting the findings: It is important to remember that detecting a chemical in a person's blood or urine does not by itself mean that the exposure causes disease. Separate scientific studies in animals and humans are required to determine which levels are likely to do harm. For most chemicals, toxicologists simply don't have this information.

But even if scientists are not sure of the overall level of risk, they can make concrete statements about whether situations are getting better or worse. The latest CDC report, in addition to listing current biomarker levels in the population, also highlights some interesting exposure trends gleaned from earlier NHANES findings. For example, from 1991 to 1994, 4.4 percent of children between the ages of one and five had levels of blood lead greater than or equal to 10 micrograms per deciliter, the Federal action level. By the second collection period (1999 and 2000), only 2.2 percent of this age group exceeded this threshold. This decrease suggests that efforts to reduce lead exposure for children have been successful. It also serves as a reminder that some children, including those living in homes with lead-based paint or lead-contaminated dust, remain at unacceptably high risk.

The last report also indicates a hopeful trend in the exposure to environmental tobacco smoke, as shown by tests for the biomarker cotinine in the blood of nonsmokers. Median levels of cotinine fell more than 70 percent in roughly a decade—that is, between the second (1988 to 1991) and third (1999 and 2000) periods of data collection. This drop provides objective evidence of reduced exposure to environmental tobacco smoke for the general U.S. population. Nevertheless, the fact that more than half of American youth continue to be exposed to environmental tobacco smoke remains a public-health concern.

The CDC plans to release future reports that document their biomonitoring efforts every two years. In the next edition, they will also add the findings from separate studies of special popu-
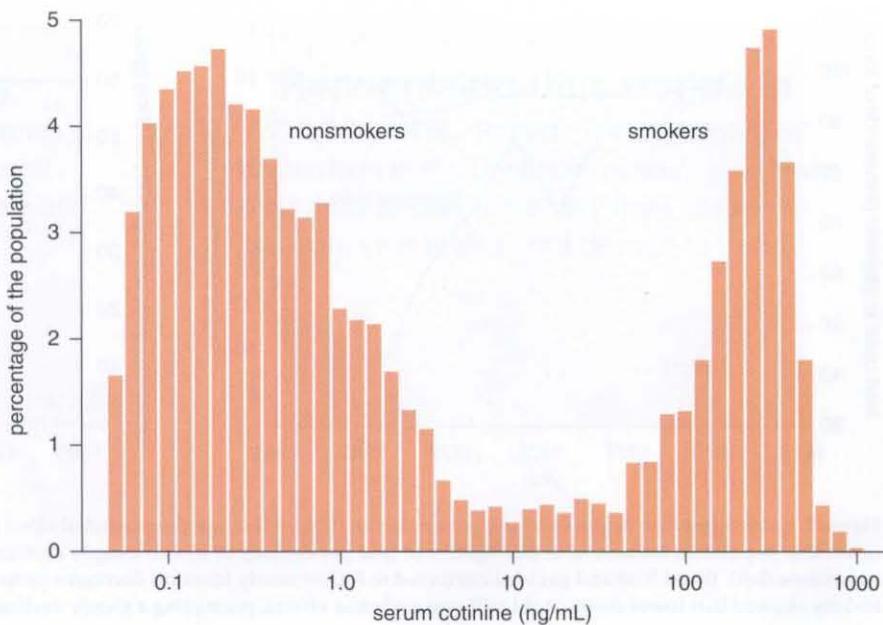

Figure 9. U.S. population clearly segregates into smokers and nonsmokers based on the level of cotinine in blood. The working threshold for distinguishing the two groups is 10 nanograms per milliliter of blood serum. Among nonsmokers, the highest values of cotinine were found in children under 12, and they were strongly reflective of the number of smokers in the home. The data are from NHANES III, 1988-1991.

lations, such as the laborers who apply pesticides to crops, people living near hazardous-waste sites and workers in lead smelters, all of which are likely to have higher-than-average exposures to certain environmental chemicals.

Annual Check-Up With Biomarkers?

As the 21st century unfolds, the CDC surveys and other well-designed biomonitoring studies will continue to build an understanding of people's exposure to toxic environmental chemicals. Nonetheless, these data will not obviate the need to collect other kinds of relevant information—to monitor sources of pollution, to conduct surveys of toxic substances in the environment and to study human activities and behaviors that contribute to exposure. Moreover, further research in toxicology and epidemiology is necessary before specialists can interpret the health significance of exposure biomarkers for most environmental chemicals. Particularly as detection methods improve—enabling investigators to measure lower concentrations of more chemicals from smaller samples at less cost—scientific understanding of what the body does to the chemical (and vice versa) must keep pace. If this effort is successful, a full screen of exposure biomarkers may be a part of every routine physical exam in the not-too-distant future.

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