Annu. Rev. Pharmacol. Toxicol. 1995. 35:341–68

RISK ASSESSMENT OF ENVIRONMENTAL CHEMICALS

A. Fan and R. Howd
Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Berkeley, California 94704

B. Davis
Department of Toxic Substances Control, California Environmental Protection Agency, Sacramento, California 95812-0806

KEY WORDS: toxicology, mechanism, carcinogenesis, animal-to-human extrapolation

ABSTRACT

Risk assessment is an evolving process, based not only upon toxicology but also upon a broad background of knowledge in fields ranging from chemistry to physiology and molecular biology and from environmental transport processes to applied statistics. Risk assessment procedures must be continually updated to reflect advances in these basic sciences. This review addresses several areas of risk assessment that are receiving heightened attention, including neurotoxicity, immunotoxicity, reproductive and developmental toxicity, genotoxicity, carcinogenicity, and toxicokinetics and modeling. Risk assessors must work with the scientific community at large to incorporate advances in the basic sciences into their extrapolations. A concerted attempt to better define the variability and decrease the uncertainty of hazard estimates will result in more efficient protection of the public and the environment against toxic hazards.

INTRODUCTION

Risk assessment of chemicals is defined by the National Academy of Sciences (1) as a four-step process that includes hazard identification, dose-response

0362-1642/95/0415-0341$05.00

assessment, exposure assessment, and risk characterization. A major objective in performing risk assessment of environmental chemicals is to provide a reliable basis for making decisions on risk management options. Millions of dollars and many lives may depend on such decisions, so they must be made carefully.

Because risk assessment is an evolving art built on sound scientific principles and judgments, its techniques and methodologies are continually undergoing refinement. The present review provides an update of the status of risk assessment, a field sometimes referred to as applied toxicology. Areas undergoing rapid change are emphasized, addressing the biological basis for evaluations, current standing and developments in testing and risk assessment methodology, and future directions.

The increasing concern of scientists, regulators, legislators, and the general public regarding human exposure to environmental chemicals has made regulation of these chemicals and risk management a priority. Inherent in the risk assessment process is the need to know at what level a chemical presents an unacceptable risk; once this level is determined risk may be managed to reduce human exposure. Risk management involves making decisions regarding relative risks, risk-benefit ratios, and the need for emergency actions. To prevent potential contamination, laws and regulations are developed as control mechanisms. Many of these laws mandate risk assessment for the purpose of regulating chemicals, requiring establishment of numerical standards for chemicals in exposure media (e.g. air, water, soil, food). Laws may specify elements such as toxicological endpoints, the assessment approach to be used, the acceptable risk levels, or special considerations such as concern for sensitive populations. Based on these requirements, risk assessment helps manage occupational and residential exposures to a variety of chemicals, such as toxic gases, solvents, and pesticides. It considers industrial, agricultural, commercial, and home uses; accidental chemical spills and leaking waste dumps; safety of chemicals legally added to food or introduced into air, drinking water, and soil; and many other risks such as exposure to lead in pottery.

Not all considerations in risk assessment are specified in any one set of laws or procedural manuals; many are built upon the training and experience of the toxicologists who assess the risk. Thus there may be significant variations in the conduct and final results of risk assessments. The complete process requires a good understanding of chemical effects and toxicological endpoints (e.g. dose response, mechanism of action, pharmacokinetics, threshold vs non-threshold phenomena), data extrapolation (e.g. from animal experimental data to the human situation, intra- and interspecies differences and similarities), exposure scenarios (e.g. route, duration, and frequency), availability of an adequate toxicity data base (e.g. animal experimental or human epidemiological data), and appropriate risk assessment methods and techniques (e.g. math-

ematical or biological models and default assumptions, chemical disposition in the environment, and population data).

In order to eliminate confusion and best utilize existing resources, various agencies have begun to harmonize their toxicity and risk assessment approaches. Ideally, properly conducted assessments generated by one recognized scientific body should be acceptable to all others. Although efforts have been initiated toward achieving this goal, considerable discussion and coordination are anticipated before it can be fully realized.

The four components of the risk assessment process defined previously are carried out roughly in the order given, leading toward a quantitative estimate of the relative risk of a chemical exposure under specified conditions. Hazard identification involves estimating chemical risks for acute (single dose), subchronic (a few doses), or chronic exposures for each possible toxic endpoint, such as liver damage. Risk is generally calculated only for the lowest-dose toxic effect for each exposure duration, assuming that if the lowest-dose toxic effects are protected against, then all other effects will also be prevented.

Dose-response assessment with extrapolation to low doses is required because toxicity tests are generally carried out with much higher doses of chemicals than would result from probable environmental exposures. Many different techniques and models are used for estimating effects from exposure to various levels and across species. Exposure assessment attempts to determine how much of a chemical (or of all similar chemicals) is absorbed from all sources. If the chemical is a pesticide, exposures might be mediated by food, groundwater, air (to those in agricultural areas), and through home and occupational uses. Risk characterization utilizes these estimates to make critical decisions regarding individual and population risks, integrating the previous steps with population demographics to describe the potential adverse outcomes and the strength of both the evidence and the extrapolations. The resulting characterizations are used in risk management, to choose appropriate mitigation strategies.

The many uncertainties in the risk assessment process may result in the restriction of chemical usage to unnecessarily low levels—an inefficient use of resources. With better knowledge about toxic effects and mechanisms, attention and resources can be directed to the most important risks. The need for such knowledge, and progress toward its attainment, are discussed in the following sections.

General Toxicity Considerations

Protecting the public from toxic effects of environmental chemicals primarily involves considering the mechanisms of low-level toxicity and likely biological effects. Thus such high-concentration effects as sedation from hydrocarbon vapors are not usually of concern. Potential cumulative, irreversible effects

such as carcinogenesis and neurotoxicity receive the most attention. Developmental effects are also intensively investigated, because a single exposure during a sensitive period in gestation can have a lifelong detrimental effect. Preventing exposure to mutagens is important for the same reason. Immunotoxicity has been receiving increased attention, particularly in response to concerns about hypersensitivity. The emphasis in risk assessment on specific causes and low-level effects naturally leads to a consideration of more sensitive toxicity tests and a desire for better information on toxicity mechanisms, physiological variables, and exposures.

In the study of problems other than cancer, the goal is to protect an exposed human population against any adverse effects by estimating a safe dose level, called a reference dose. The highest dose that doesn't cause toxicity (the no observed effect level, or NOEL) or, if that is unavailable, the lowest dose observed to have a toxic effect (the lowest observed effect level, or LOEL) in an animal study is divided by safety or uncertainty factors. In calculating the reference dose, cumulative factors of 10 have commonly been used to account for uncertainty caused by extrapolation between species, for variations in sensitivity among humans, for extrapolation from a LOEL to a NOEL (when necessary), and for estimating a safe chronic dose when only acute or subchronic toxicity levels are known (2). The total uncertainty factor used may thus vary from 10 to 10,000, depending on the available data; the most common value is 100. Uncertainty factors are used to account for both the inherent variability in subject responses (e.g. individual differences in size, exposure, metabolic rate, disease states, genetic susceptibility) and uncertainty resulting from undefined toxic effects. The latter include extrapolating from effects in rats to effects in humans when the mechanism of toxicity has not been identified. Also, a different toxic effect might occur in humans than in the tested species. Efforts are in progress to decrease the uncertainty both by estimating the variables more accurately and by providing better information on the unknown elements. Some uncertainty may remain nonquantifiable (3–5).

For cancer risk assessment, dose-response extrapolation is based on the assumption that there may be no threshold for the carcinogenic effect. (Dose-response assessment for noncancer endpoints, on the other hand, generally assumes a threshold below which no toxic effects are expected.) The no-threshold assumption for carcinogens generally means that negligible risk levels are much lower than if a threshold were assumed. Therefore, soil, air, or water contamination is usually regulated at much lower levels for carcinogens than for noncarcinogens.

Because most risk estimates involve application of toxicological data derived in animals to toxicity estimates for humans, the similarities and differences among species must be considered. Kidney tumors mediated through a

chemical agent's effect on alpha-2-microglobulin in the male rat, for example, should not be used to estimate the carcinogenic potential of that agent in humans because humans do not have this protein (6). Forestomach tumors caused by gavage administration of an irritating chemical such as ethyl acrylate (7) also provide dubious data for direct extrapolation to humans, since humans do not have the same stomach anatomy and would not be exposed in the same way to irritating high concentrations.

The similarities between the genetics, biochemistry, and physiology of rats, mice, and other experimental animals compared to humans are much more significant than the differences. Nevertheless, the focus of a considerable portion of modern research is on the differences. This is seen, for example, in the attempt to document the extent to which simple cross-species extrapolations (based, for instance, on body weight or surface area) are appropriate for a specific case. By default, the US Environmental Protection Agency (EPA) (8) and the California EPA Office of Environmental Health Hazard Assessment have estimated effective doses across species on the basis of the ratio of the two species' body weights to the 2/3 power, i.e.

$$\text{Dose}_{\text{human}} = \text{Dose}_{\text{rat}} \times (\text{BW}_{\text{human}}/\text{BW}_{\text{rat}})^{2/3}.$$

The US Food and Drug Adminstration (FDA) has extrapolated across species directly by body weight, a process equivalent to using a body-weight ratio exponent of 1 rather than 2/3 in the above equation. Using the higher exponent yields a higher (less "conservative" or less health-protective) estimated toxic dose. The true ratio of comparable doses across species varies fairly widely for different chemicals and effects (9). Recent proposals to use an exponent of 3/4 by all government agencies are based more on a desire for consensus than on biology, although a strong biological argument can be made for this value as a default (10). More accurate cross-species extrapolations can often be produced using physiologically based pharmacokinetics (PBPK) when a specific case is important enough to justify the time and expense required by this procedure.

Merging estimates of relative chemical concentrations across species with data on tissue sensitivities to the toxic action would allow more precise estimates of toxicity in different species (11). In vitro tests on both experimental animal and human tissue slices or cells are being developed to provide the needed data on tissue effects. However, estimating in vivo toxicity also requires estimates of biological variability and uncertainty. The variability estimates can be applied to risk assessment using a computer method called Monte Carlo or stochastic analysis, which involves repeatedly calculating effects using different, statistically chosen sets of values for all the variables (12). This can reveal the distribution of potential exposures and effects, although lack of relevant data often limits the utility of the technique. For

example, one suburban community might be agriculture oriented, while another might be dominated by commuters. The risk assessor may not have up-to-date information on these populations, including their diets and patterns of physical activity.

A prominent aspect of risk assessment for environmental chemicals is exposure to multiple chemicals. Because of the near-infinite number of potential mixtures, it is impossible to experimentally test for all interactions, and relevant studies of an effect or interaction are not usually available. For many common mixtures [such as polychlorinated biphenyls (PCBs), dioxins, or polyaromatic hydrocarbons (PAHs)] good data are available for only a few of the component chemicals. Toxicity of the others may be estimated from structure-activity relationships (SARs) and more limited studies, such as in vitro screening methods. Substantial effort is being devoted to the development of Toxicity Equivalent Factors (TEFs) for the chemicals in several common mixtures (13, 14). For this purpose, the chemicals are usually assumed to have additive toxicities based on their relative potencies in one or more simple tests. Dioxin TEFs, for example, could be based on potency of the chemical binding to liver Ah receptor in vitro or its induction in vivo, induction of liver ethoxyresorufin O-deethylase, total liver binding of the dioxin in vivo, its effects on liver binding of epidermal growth factor, induction of cytochrome P450s, or any combination of effects (13, 15–18). Accuracy of TEFs is limited because of multiple effects (which can vary across species) and because effects may not in fact be additive; antagonism, synergism, and nonparallel dose-response curves complicate the issue (19). Although TEFs are essentially provisional ranking schemes, the vast array of potential multiple chemical exposures ensures a role for TEFs in risk assessment for the indefinite future.

NEUROTOXICITY ASSESSMENT

Neurotoxic effects are prominent after high doses of several persistent environmental contaminants. Such effects were observed around Minamata Bay in Japan, when people consumed excessive levels of methyl mercury in fish, and in Morocco among people who consumed cooking oil contaminated with tri-O-cresyl phosphate. However, in most cases, potential human risks must be estimated from much lower levels of contaminants, for which the effects are much more difficult to estimate (20–22). To address this question in detail, it is convenient to divide neurotoxic effects into: (*a*) acute effects related to disruption of neural homeostasis and (*b*) chronic effects caused by nerve damage. Generally, effects of type *a* are reversible, and effects of type *b* are irreversible, though neither generalization is always true. Effects of both types can be mediated either centrally (in the brain and spinal cord) or peripherally (in the autonomic and voluntary nerves).

Comprehensive evaluation of neurological competence is difficult and expensive. Two-tier testing schemes have therefore been developed in which simple preliminary observational tests are followed, when appropriate, by more detailed functional tests. Dozens of procedures to analyze various nervous system functions have been developed over the last few decades to evaluate both acute and chronic effects of chemicals, but no comprehensive test battery has emerged as a standard. Information on neurotoxicity for most environmentally significant chemicals is therefore fragmented and difficult to interpret, and there are no simple guidelines for use of the data in risk assessment. The US EPA has been in the process of formulating neurotoxicity guidelines for several years (23–26). Common tests can be classified as observational (rating of spontaneous motor activity, rearing, social contact, etc); behavioral (disruption of trained activities); neurophysiological (measurement of nerve conduction velocity, evoked potentials, EEG); physiological (measurement of muscle strength, temperature regulation, postural control, reflexes); and biochemical (measurement of alterations in levels of hormones, enzymes, or other biological markers). The fact that some of these tests can be carried out identically in experimental animals and humans makes animal data much easier to interpret for risk assessment.

Behavioral and functional tests are often quite sensitive, capable of detecting effects of chemicals that would be overlooked using standard pathology measures. An excellent example of this is the demonstration of persistent cognitive defects after chronic low-level lead exposure (27). However, in contrast to most structural changes observed in pathology examinations, not every change in a measured parameter is toxicologically relevant. As neurotoxicity tests become more accepted as standard components of chemical evaluations, the controversies regarding interpretation of these data will become more important. Consider an instance in which animals exhibit a behavioral effect such as decreased motor activity during low-level exposure to an organic solvent (perhaps at one tenth the level resulting in observable organ damage). Should risk assessment consider this an adverse effect? Observation of neurochemical changes (i.e. cholinesterase inhibition) in the absence of functional measurements represents a similarly problematic endpoint. In addition, should standard uncertainty factors be applied to such effects?

The risk assessor must determine at what point on the spectrum a transient change should be considered an important toxic effect. When a chemical is administered in drinking water, for example, should a subsequent decrease in an animal's water consumption be attributed to sickness or to bad-tasting water? It must be decided whether the LOEL is the threshold for decreased water consumption, for decreased weight gain at a higher chemical concentration, or for "failure to thrive." (Failure to thrive is a severe inhibition of weight gain that may be observed at high concentration—an inhibition that is often

irreversible, persisting even after the chemical has been removed from the water.) At present, decreased weight gain when chemicals are administered in water is usually not considered a toxic effect unless it is accompanied by corroborating signs or confirmed by a similar effect when the chemical is administered by another route. The range of effects, from (potentially) inconsequential to frank toxicity, will be observed for virtually every other behavioral and functional measure. Risk assessors do not yet interpret such data in a consistent way or agree on the uncertainty factor to be used for various gradations of effects.

Neurochemical and biochemical changes that are not clearly secondary to pathology are equally problematic for risk assessment. Severe inhibition of nerve acetylcholinesterase (AChE) by organophosphate or carbamate pesticides is rapidly fatal; less severe inhibition can chronically impair neurologic function (28). Lower pesticide doses cause reversible AChE inhibition that can often be detected in blood at doses far lower than those associated with any clinical signs or symptoms—although pesticide toxicity studies generally do not include functional or behavioral tests. The question for risk assessors is whether the AChE inhibition should be deemed to represent a potential for harm or whether only frank symptoms should be considered as adverse effects (29, 30). In the absence of accurate behavioral, functional tests, regulators may insist on preventing any significant AChE inhibition in blood or tissues (31).

Another controversy in applying animal neurotoxicity test results to risk assessment involves interpretation of chronic or persistent effects. In a recent case, a herbicide was reported to cause irreversible neuropathy at very low levels in rats after lifetime dietary administration. No such effect was observed in smaller dog studies after one-year exposures at higher doses. Humans are exposed to significant levels of the herbicide for only a few weeks annually, during the planting season. Should the intermittent human exposures be regulated based on the rat or the dog data? Use of the rat data assumes that effects on humans would accumulate over a lifetime; application of the dog data, on the other hand, assumes that the shorter-term dog study is more relevant because these exposures were much longer than any annual human exposure. In addition, no neuropathy has been observed in humans, although past exposures were much higher than presently allowed. A public health protective approach might be to use the rat data in the risk assessment until or unless it can be shown that intermittent exposures will not produce neuropathy. Formal guidelines on how to interpret various exposure patterns, considering duration and reversibility of effects, have not yet been developed.

Recently a National Academy of Sciences committee, in evaluating the safety of the nation's food supply to infants and children, recommended a better evaluation of the neurotoxicity of pesticides (32). Finalization of US EPA guidelines for neurotoxicity testing and use of the data in risk assessment

will help protect against potential neurotoxic effects of environmental chemicals.

IMMUNOTOXICITY ASSESSMENT

Risk assessment of immunotoxic chemicals is a new and challenging area. Reasons for its significance include: recent emphasis on the human health implications of the immune system; public awareness that chemicals or biological agents can alter immune responses, as seen in acquired immune deficiency syndrome (AIDS); the ability of immune function tests to detect changes at very low doses; the fact that pesticides, a major class of chemicals, are known to modulate immune system response; special vulnerability to immune system distress among young, old, pregnant and malnourished individuals; the potential for development of hypersensitivity, including multiple chemical sensitivity (MCS); and the current lack of regulatory data requirements and risk assessment guidance for immunotoxic compounds and effects. Owing to such considerations, testing methods and risk assessment guidelines are being developed by various organizations.

Impaired immune response can result in increased susceptibility or decreased resistance to bacterial, fungal, and viral infections, and to some forms of cancer. The latter phenomenon is well documented in transplant patients, in whom prolonged use of immunosuppressive drugs is associated with a high incidence of tumors. In immunosuppressed patients with AIDS, too, susceptibility to infections and certain forms of cancer increases as the capacity for immune response diminishes. Impaired immune function has also been associated with exposure to environmental contaminants, spurring efforts to characterize the nature, magnitude, and health implications of any effects of environmental chemicals on the immune system (33).

That environmental chemicals might affect the human immune system was discussed initially in a symposium organized by the US FDA in 1978 (34) and was pursued in 1981 by Sharma (35). Considerations of immunotoxicity testing and assessment and public health were included in both. A summary of testing methodologies and of federal research and regulatory activities in immunotoxicity has been provided recently by the US Congress (36). In spite of the well-documented immunomodulative effects in animals of chemicals such as halogenated aromatic hydrocarbons, metals, solvents, polycyclic aromatics, and pesticides, it has been difficult to relate these changes to a definitive health risk or disease process (37). Data on occupational exposure to more than 40 pesticides has suggested a danger of contact dermatitis, although supporting evidence is sporadic (38).

Attention was drawn to the effect of low-level pesticide exposure on the immune system when suppression of antibody response was reported in mice

administered drinking water containing only 1 ppb of aldicarb (39). Later epidemiological studies suggested that low-level chronic ingestion of aldicarb in groundwater was associated with altered T cell numbers, including a decreased $CD4^+$:$CD8^+$ ratio in otherwise healthy women (40). However, none of these changes has been directly associated with a change in health status. Other more comprehensive studies in mice did not show immunologic effects from aldicarb (41, 42). The current uncertainties in scientific knowledge and lack of guidance for interpretation of immunologic data have stimulated research on animal test batteries that can predict immunotoxic effects of chemicals in humans.

The immune system is a complex network of lymphoid organs and cells in circulating blood and interstitial tissue spaces that interact to generate the immune responses. No single test is adequate to measure the immunologic effects of chemicals on these multiple system components. A variety of immunological techniques are available to test various parameters of immunological responsiveness, but few have been used in routine toxicological studies and still fewer have been validated for use in risk assessment. A tiered approach to assessing the immunotoxicity of chemicals has been used by the National Toxicological Program (43), with the mouse as the species of choice.

The simple tests in Tier 1 include various endpoints involving several cell types and serum factors called lymphokines. If these analyses suggest immunotoxicity, more comprehensive Tier II tests are conducted to determine the extent and possible mechanism of the effects. Tier I includes endpoints such as immunopathology (involving analyses of hematology, organ weight, cellularity, histopathology), humoral-mediated immunity (analyses of IgM antibody production in response to T cell–dependent antigen, mitogen response to lipopolysaccharide), cell-mediated immunity [analyses of mitogen response to concanavalin A and mixed lymphocyte response (MLR)], and nonspecific immunity [analysis of natural killer (NK) cell activity]. Tier II includes immunopathology (quantitation of T and B cells), humoral-mediated immunity (analysis of IgG antibody response to T-cell dependent and T-cell independent antigens), cell-mediated immunity [analyses of cytotoxic T lymphocytes (CTL) and delayed hypersensitivity response (DHR)], nonspecific immunity (tests of macrophage function), and host resistance to challenge (tests using bacteria, virus, parasites and syngeneic tumor cells).

For environmental chemicals, the respiratory system and skin are the two primary routes of exposure and targets of immunotoxicity. The major concerns are related to respiratory and skin (contact) sensitization. Contact sensitivity, asthma, rhinitis, and urticaria are likely clinical manifestations of allergic immunotoxicity. Concern over these effects arose during investigation of the health effects of malathion applied aerially against the Mediterranean fruit fly in southern California (44). Data from animal sensitization tests (e.g. guinea

pig sensitization) as well as human predictive and diagnostic studies (e.g. the patch test) exist for many pesticides. The guinea pig sensitization test appears to be an appropriate method for predicting contact sensitivity. However, animal data may suggest higher incidences of sensitization than are supported by human data (38).

The dose-response relationships for immunologic responses are different from those that occur for other toxic endpoints. This is because a high dose may induce tolerance whereas a low dose may sensitize (45). Socioeconomic factors, nutrition, and age may play roles in the development of immunotoxicity of environmental chemicals. Human data concerning chemically induced modulations of immune-response parameters are scarce. Where modulations have been observed, the biological significance has often been unclear. Given the debate over the functional reserve of the various components of the human immune system, current testing has not been able to predict whether suppression of an immune parameter is likely to damage health. It is not unreasonable, however, to suppose that significant immunologic changes in exposed individuals will produce adverse outcomes. This concept is supported by human data from individuals with genetic or virus-induced (e.g. AIDS) immunodeficiencies and from those being therapeutically immunosuppressed (e.g. organ transplant recipients). In addition, a good correlation between immune test results and altered host resistance has been demonstrated in animal studies (46).

To date little immunotoxicity information is available for environmental chemicals. In the US EPA Integrated Risk Information System (IRIS), which provides summary toxicity data on over 200 chemicals, only one chemical has a reference dose based on immunotoxicity. The National Academy of Sciences (NAS), in its recent study of pesticides in the diets of infants and children, recommended immunotoxicity assessment as part of a complete pesticide safety evaluation. Regulatory agencies such as the US EPA and the US FDA are developing testing guidelines and data requirements for immunotoxicity testing. Presently the US FDA is attempting to revise its 1982 guidelines, which set forth the criteria used by the agency to judge the safety of food additives, to include recommendations for immunotoxicity studies and a strategy for assessing immunotoxic potential (47). The US EPA is preparing a requirement for an immunotoxicity screen in its pesticide assessment guidelines that would provide basic data for risk evaluation and might suggest additional appropriate studies (48). The revised guidelines would also require such data to be considered in human risk characterizations.

Immunotoxicity tests and evaluation will make an important contribution to the overall risk assessment of environmental chemicals. Integration of an understanding of the mechanism by which a chemical can alter immune function at the cellular and molecular levels with a quantitative description of tissue dosimetry and epidemiological data would help in the development of biol-

ogy-based mechanistic models for immunotoxicity; such a model would aid human health risk assessment (49). Although formal guidelines for the use of immunotoxicity data in risk assessment do not exist, use of immunotoxicity testing data has been addressed by Luster et al (46, 50). These authors reported that as few as two or three immune system tests are sufficient to predict the immunotoxicity of various compounds in rodents. They also discussed the significance of changes in immunological test parameters in the context of human biological and toxicological dose-response relationships, and the relationship between changes in selected immune function tests and host resistance tests. Thus progress is being made toward gathering the data and developing the interpretation methods necessary to assess the immunotoxicity of environmental chemicals.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY ASSESSMENT

Although closely related, the toxicologies of reproduction and development are distinct disciplines. (The two topics are combined in this section for convenience and brevity.) Developmental toxicity is toxicity that adversely affects offspring through maternal exposure to toxic agents prior to conception, through exposure in utero, or through exposure during the period from birth to sexual maturity. Adverse developmental effects include structural abnormalities, growth alteration, functional defects, and death (51). The most common test for developmental toxicity involves exposing females, typically rodents and rabbits, to a toxic chemical in the air, water, or diet at various stages of pregnancy. By limiting exposure to specific periods of gestation, it is possible to define the period of development that is most vulnerable.

Reproductive toxicity is toxicity that adversely affects any aspect of male or female reproductive function. Effects may be observed as changes in reproductive cells or organs, in endocrine functions, or in behavior. Because they affect reproductive cells (germ line, meiotic cells, and gametes), genotoxic effects may be included in reproductive toxicity. A mutational event in such a cell could theoretically be caused by a single molecule. This possibility is the basis for assertions that some developmental and reproductive toxins are nonthreshold agents.

Reproductive toxicity can be assayed in a two-generation rodent study. Treatment begins with the parental generation and continues throughout the development and breeding of the offspring, followed by a second generation of offspring. Both generations of offspring are evaluated for the abnormalities listed above. For a discussion and comparison of US FDA guidelines with those in Europe and Japan, see Ref. 52. Several detailed reviews of the per-

formance and outcome of reproductive and developmental toxicity assays for pesticides and other chemicals are available (53–56).

It is difficult to overstate the impact on the general public of this area of risk assessment. The fear of poisons insidiously released into the environment combines with deep feelings about reproduction and love of one's children to produce a powerful emotional mix. These feelings, and a mistrust of government, can lead to legislation imposing restrictions that may not be scientifically valid. California's Proposition 65, "The Safe Drinking Water and Toxic Enforcement Act of 1986" (California Health and Safety Code, Section 25249.5 et seq.), was overwhelmingly approved by the voters through the initiative process. It has been evaluated at length (57–59). One of its interesting and debatable features is the mandatory 1000-fold margin of safety for all developmental and/or reproductive toxicants.

Neubert & Kavlock (60) have recently discussed prenatally induced adverse health effects. An issue unique to this area of risk assessment is the relevance of maternal toxicity to fetal effects. Adverse fetal effects that occur at dose levels below those that cause maternal toxicity are clearly of concern. At doses associated with maternal toxicity, however, fetal effects may be secondary to the maternal toxicity. Disagreement arises over the contribution of maternal toxicity to prenatal toxicity when the thresholds overlap.

Regulators want to assure that sufficiently high doses of the agent in question will be tested—that is, doses high enough to cause some maternal toxicity, even though the relevance of fetal effects at such high doses is difficult to ascertain. Also, regulators want to set protective levels of exposure—they want to know whether the customary safety factor of 100 from the maternal or fetal NOEL is adequate to protect the developing fetus. Proposition 65 and the recent NAS study (32) make the assumption that a 1000-fold factor may be necessary to protect the fetus. Further studies should be directed toward resolving this issue.

Another issue is the use of in vitro techniques for evaluating reproductive and developmental toxicity. A recent review of the potential of these techniques concluded that they are useful adjuncts but cannot now and are unlikely in the future to be able to supplant in vivo tests (61).

For both reproductive and developmental toxicity, risk assessors must extrapolate from animals to humans while recognizing that the same effects do not necessarily occur in different species. One aspect of this extrapolation problem is the interpretation of structural defects in rodent studies. The case has been made that such defects have less relevance to humans than dysfunctions, especially of the immune and endocrine systems (60).

While our understanding of the molecular biology of development has not kept pace with that of carcinogenicity, important new insights have been reported. Of great interest is the evidence for an overlap in genes important in

both development and carcinogenicity. Cyclins and cyclin-dependent kinases (cdks) are key regulators of cell division during development, just as they are in the adult (see the discussion, below, of risk assessment for carcinogenicity). Interactions among the cyclins, kinases, and other critical regulatory molecules, such as maturation-promoting factor, are currently being elucidated (62, 63).

Targeted gene replacement, the knockout technique (64), was used to generate mice lacking the tumor suppressor gene for the p53 protein (65). Extensive testing indicated that the mice had no DNA for the deleted coding portion of the *p53* gene, no messenger RNA, and no p53 protein. That these mice are able to go through normal development is consistent with a negative function of *p53* in regulating cell division. The absence of this *p53* function does manifest itself in the mice, 74% of which developed obvious neoplasms by six months of age.

Modeling techniques that have been applied with great success to other areas of toxicology should be brought to bear on reproduction and development studies. As these methods mature and are refined, they should greatly improve the biological accuracy of developmental and reproductive risk assessment. In addition, exploration of the molecular biology of development should allow progress far beyond the level of cataloguing structural defects in rodent assays, to application of a mechanistic understanding of specific effects of toxicants on critical genes, regulatory molecules, and organ systems.

GENOTOXICITY ASSESSMENT

Risk assessment for genetic effects is both important and difficult. The outcome in some 5–10% of live human births is a significant birth defect (66, 67). It has been estimated that among all congenital effects about 20% have environmental causes, 20% have predominantly genetic causes, and 60% result from a combination of the two. In addition to problems that are severe enough to be recognized as birth defects, genetic changes can lead to more subtle results: A child may lead a less healthy life, be more susceptible to disease, or have a shortened productivity and life span.

The first difficulty in genetic risk assessment comes at the hazard identification step. Any genetic system in any organism can be adapted to screen chemicals for genotoxicity. Earlier, effects were usually divided into chromosomal mutations and point mutations. Ideally, a point mutation would be confined to a single gene, but in practice the definition is an operational one that depends on the limits of cytogenetic resolution. Molecular biology can now directly measure the interactions between DNA and potential genotoxins. Sophisticated techniques, such as transgenic mouse assays (68, 69), can identify specific gene alterations. Molecular biology has also provided insight into the mechanisms of mutation (70–79).

A major problem in hazard identification is to determine the relevance to human health of the hundreds of available genotoxicity assays. Hereditary diseases in humans can result from chromosome alterations, either structural or numerical, a variety of changes within structural or regulatory genes, or polygenic mutations (80). Genotoxicity at any of these levels should therefore be relevant, and different tests have been designed to discern each of these effect types. However, the extrapolation required in the case of genotoxins may be not merely from rodent to human, but also between organisms as phylogenetically distant as bacteria and humans. It is possible to use human cells in tissue culture for some endpoints, but such a recourse merely replaces the uncertainties of interspecies extrapolation with those of extrapolating from an in vitro system to a living organism.

Risk assessors must also distinguish between somatic cell and reproductive cell mutations. The former are of interest with respect to carcinogenesis, while the latter may be more relevant to birth defects and effects on the human gene pool. Animal assays for germ line mutations are costly and difficult (81, 82), while human germ line data are never available (83). Indeed, no agent has yet been demonstrated to induce germ line mutations in humans (66, 84).

In spite of the complexities of hazard identification, regulatory agencies in various countries have defined guidelines for genotoxicity testing (85–89). Because direct chemical interactions with genes represent nonthreshold phenomena, quantifying the risks in this domain shares pitfalls with carcinogenicity dose-response assessment. The US EPA has developed guidelines for mutagenicity risk assessment (85) and in a series of papers has attempted to demonstrate appropriate risk assessment procedures, using ethylene oxide as an example (83, 90–93). These results were offered in order to stimulate discussion and progress in this area, but a consensus on genetic risk assessment is not yet in view.

CARCINOGENICITY ASSESSMENT

Risk assessments evaluate carcinogenicity differently from other toxic effects, which are grouped together as noncarcinogenicity. The primary reason for this dichotomy is the treatment of carcinogenicity as having no threshold and all other toxicity as having a threshold. The basis for this hypothesis is that mutations in a few critical genes can lead to a loss of the regulation of cell division. Thus a few molecules of a carcinogen can produce an unregulated cell that divides to form a clone and ultimately a tumor composed of unregulated cells. The consequence of this assumption is that any dose of a carcinogen, no matter how small, should produce a calculable risk.

Noncarcinogens may damage or kill cells, but at low enough doses, the cells can be repaired or replaced. At doses above a threshold level, enough cells die

to cause an observable adverse effect. Risk assessment attempts to identify a safe exposure level for noncarcinogenicity below which no significant adverse toxicity is anticipated.

Although the mechanism of carcinogenicity probably does have a nongenetic, threshold basis for some chemicals, regulatory agencies currently treat all carcinogens as nonthreshold agents (8, 94). Carcinogens dominate the health considerations of most risk assessments because of the assumption that they have some risk at any level. Genotoxic chemicals should also lack a threshold, but because of the difficultes in risk assessment described in the previous section, they are not evaluated in the same manner nor with the same concern as carcinogens.

Several schemes are used to classify carcinogens during the hazard identification stage of risk assessment. These take into account evidence from epidemiology, animal studies, structure-activity relationships, and genotoxicity. Animal bioassays for carcinogenicity have been reviewed recently (95), including a discussion of the US National Toxicology Program classification scheme for evaluating those bioassays. The US EPA carcinogen ranking system has been used most often in US risk assessments. Chemicals are divided into six groups, beginning with group A, a "known human carcinogen" based on sufficient evidence of carcinogenicity in epidemiological studies. Group B chemicals are "probably carcinogenic to humans." They are subdivided into B1, indicating limited evidence of carcinogenicity in humans and perhaps some evidence from animal studies, and B2, indicating sufficient evidence of carcinogenicity in animal studies and inadequate or no evidence in humans. A group C chemical is considered a "possible human carcinogen" based on limited evidence of carcinogenicity in animals. A group D chemical is one that has not been adequately tested, while a group E chemical has been tested and appears to be free of carcinogenic effects (8). A similar six-category system is used by the International Agency for Research on Cancer (IARC). Other classification systems are those of the US Department of Health and Human Services, the European Community, and the American National Standards Institute. The US EPA is currently proposing to replace its rating system with a narrative statement describing the evidence concerning carcinogenicity for each chemical.

Low-dose extrapolation for a quantitative risk assessment is performed following the qualitative hazard identification described above. In most cases extrapolation is done over several orders of magnitude from dose levels used in rodent carcinogenicity assays to dose levels of potential human exposures. Mathematical models for low-dose extrapolation include the probit, Weibull, and time-to-tumor models. The model adopted by the US EPA and most frequently used in the United States is the linearized multistage model (96). The equation for this model is

$$P(d) = 1 - \exp(-q_0 - q_1 d - q_2 d^2 - \ldots - q_k d^k),$$

where $P(d)$ is the probability or risk of cancer, exp is the natural base e raised to the exponent in parentheses, d is the dose, and k, q_0, q_1, ... q_k are parameters. For the low-dose range of interest the equation reduces to approximately the linear term of the exponent, which is represented by q_1 (the slope of the low-dose, linear portion of the curve) times the dose. Regulatory agencies in the United States use the 95th percent upper confidence limit on q_1, which is called the cancer slope factor or q_1* (pronounced "q one star"). Cancer risk at low doses equals Dose $\times$ q_1*, and the dose associated with a specific risk (i.e. one in one million) equals Risk/q_1*. Because the slope factor is an upper-bound estimate, calculated risks are also upper-bound estimates and calculated doses are lower-bound estimates.

Several uncertainties plague carcinogen risk assessments that seek to apply results from rodent assays to humans. The chemistry of the genetic material is the same in rodents and humans, and compounds that interact with rodent DNA are surely also able to interact with human DNA. However, absorption, metabolism, and excretion of mutagens may be quite different in the two intact organisms. Concordance as to carcinogenicity between well-conducted mouse and rat carcinogenicity assays using consistent protocols is no greater than 75% (95, 97, 98), a percentage that presumably reflects a combination of the statistical limitations of the bioassays and the biological species differences. Prediction of human carcinogenicity from rodent bioassays is unlikely to be better. Risk assessment methods currently deal with this problem by asserting that in the absence of compelling epidemiologic studies there is no alternative to the assumption that a rodent carcinogen is a probable human carcinogen as well.

The low-dose extrapolation method introduces more uncertainty. Carcinogens are regulated at risk levels in the range of 1×10^{-4} to 1×10^{-6}. Such levels are based on rodent studies with about 50 males and 50 females in each dose group. Because male and female outcomes are treated separately, the limits of resolution for these assays are on the order of a few percent for a response. Extrapolation for risk assessment extends two to four orders of magnitude beyond this level for effect and corresponding doses, introducing tremendous statistical (as well as mechanistic) uncertainty. The issue of dose extrapolation across orders of magnitude may apply to risk assessment for other toxic endpoints, but it is most cogent for carcinogenicity because of the nonthreshold assumption discussed above. The low-dose extrapolation problem is complicated by the fact that the linearized multistage model appears to respond in a less than ideal manner to changes in the numbers of animals and in dose levels.

The working assumption among regulators that all carcinogens act through

nonthreshold mechanisms has been called into question because only a 60% concordance was found between rodent carcinogenicity assays and each of four different genetic toxicity tests (98). No combination of the four tests had a substantially better concordance than the *Salmonella* (Ames) assay alone. In some cases cancer was found in animal bioassays without positive results for genetic toxicity; in others, no cancer was found when genetic toxicity tests were positive. Cancer in the absence of positive mutation assays might be explained by nongenetic mechanisms. The failure of mutagens to act as rodent carcinogens is more troublesome. It may be explained by inadequacies in the rodent assays and problems with the genotoxicity assays, but it could also call into question the mutational basis of carcinogenicity.

The agreement between genotoxicity and carcinogenicity assays can be improved by considering such other factors as structure-activity relationships and formation of reactive metabolites or covalent adducts. The following section describes current research on oncogenes, tumor suppressor genes, and repair genes that demonstrates without question the importance of mutation in carcinogenesis. However, it is clear that a variety of nongenetic mechanisms can also lead to cancer (for a review, see 99). In short, it is valid to treat some carcinogens as nonthreshold but others as threshold agents.

Cancer Molecular Biology: Oncogenes, Suppressor Genes, and Repair Genes

Recent advances in the molecular biology of carcinogenesis have so far influenced risk assessment very little. The discovery of oncogene-carrying tumor viruses was soon followed by the demonstration that such viral, cancer-inducing genes were aberrant forms of normal cellular genes that carried out a variety of cell-regulatory functions. Subsequently, tumor cells were found to contain regulatory-gene alterations associated either with abnormal stimulation of cell division (oncogenes) or with loss of inhibition of division (tumor suppressor genes). In most cases these alterations do not appear to be virus derived. Many tumor cells exhibit multiple genetic changes. More than 100 oncogenes have been described (100). The most common of these, the *ras* mutation, is found in 10–15% of solid tumors. Mutant oncogenes are characteristically dominant over the wild-type allele, indicating that the mutant gene's functions overcome potential interference from the normal gene or its products.

Altered tumor suppressor genes are also common. Mutant forms of the p53 protein gene are best known (101), found in about 50% of human cancers. On the basis of the number of familial cancer genes known, Knudson (102) has estimated that there may be about 50 such tumor suppressor genes in humans.

Tumor suppressor mutant alleles are recessive to their wild-type alleles, indicating that cancer results from the absence of a normal (suppressive) product. Thus, in the human hereditary propensity for retinal cancers known

as retinoblastoma, a defective tumor suppressor allele is inherited from either parent. A mutation in the remaining wild-type, functional allele in a single cell (sometimes referred to as loss of heterozygosity or LOH) can result in the disease. Nonfamilial or sporadic cases result from the unlikely mutation of the gene on both chromosomes in the same cell.

Research on oncogenes and tumor suppressor genes is now coming together with knowledge about the cell cycle and its regulatory genes. Cell division is carefully regulated, involving both genes that bring about the next stage and genes able to block progression (63, 103–106). Cyclins are important proteins in this regulation, acting through a group of kinases called cyclin-dependent kinases (cdks) (107, 108). The *p53* tumor suppressor gene appears to block cell division by activating a gene whose protein product binds to a cyclin and cyclin-dependent kinase complex and prevents the kinase activity (109, 110).

A useful system has been developed in the teleost *Xiphophorus* to allow genetic and molecular manipulation of an oncogene and a tumor suppressor gene that interact in melanoma formation (111). Cyclin D1 has been shown to be an oncogene (112). Kamb et al recently reported a gene called *Multiple Tumor Suppressor 1* (*MTS1*) that may be even more common in tumor cells than the *p53* mutant (100). *MTS1* encodes an inhibitor (p16) of cyclin-dependent kinase 4. Thus *MTS1* is a tumor suppressor gene directly involved in the cell-cycle machinery.

The emerging picture is that oncogenes normally function to promote cell division whereas mutant oncogenes stimulate inappropriate cell division. Tumor suppressor genes normally block cell division, either because it is inappropriate or because there is a defect in the genetic material. The study of cell-cycle mutants has demonstrated two important checkpoints (106). Cells are blocked prior to DNA synthesis if abnormalities in the genetic material are detected, and they are blocked prior to mitosis until synthesis has been completed. The *p53* gene appears to be critical in this process; a mutation allows aberrant cells to proceed through the cell cycle.

A third class of genes involved in human carcinogenesis are the DNA repair genes (113–117). Mutations of either of two genes involved in DNA base mismatch repair are found in 30% of sporadic colon cancer cases and 90% of cases of hereditary nonpolyposis colon cancer, a common (1/200 people) genetic disease.

Elucidation of the molecular biology of cancer offers several implications for risk assessment. First, although it does not preclude nongenetic means for some carcinogens (99), it provides irrefutable grounds for the mutational basis of chemical carcinogenesis. Second, it provides strong evidence for a multistep process of carcinogenesis. No single mutation in an oncogene, tumor suppressor gene, or DNA repair gene is found in a majority of tumors. Several different combinations of mutations may lead to carcinogenesis, perhaps with different

pathways for different tumor types (118). Third, it provides a better understanding of sensitive subpopulations and of interspecies differences. Hereditary retinoblastoma and other familial cancer propensities, associated with heterozygosity for a tumor suppressor gene, provide extreme examples of sensitive subpopulations. Other susceptibilities may be much subtler. Fourth, information is emerging on carcinogenesis pathways in assorted tissues and organs. Tumor types show quite different mutation frequencies for various genes. For example, about 75% of melanomas (but no colon tumors and no neuroblastomas) have some form of *MTS1* mutation (100). The *p53* mutations also differ in human cancers of various tissues (119). Fifth, specific toxins can be associated with particular mutations in critical cancer genes. For example, a strong geographical correlation has been shown between exposure to aflatoxin B and a nucleotide substitution in codon 249 of the *p53* gene in hepatocellular carcinomas (120). The challenge for risk assessment is to use such new information to improve regulatory guidelines and actions.

TOXICOKINETICS AND MODELING

Predicting toxicity in humans by extrapolating to much lower human doses from animal studies using high-level acute, subacute, and chronic exposures, often with different routes of administration, yields very uncertain results. Large uncertainty factors have consequently been employed. However, if the mechanisms of action of a chemical and its disposition in the body are well understood, the animal data may be incorporated into a comprehensive model extrapolable to humans. Such a model could correct for species differences in pattern of toxic effects, total daily dose, dose rate, concentration × time parameters necessary to produce toxicity, variation in biological repair mechanisms, and physiological differences.

A first consideration is that the various dosing methods are not equivalent. Administration of chemicals in a bolus (such as oral gavage) may produce results different from those of slow dosing (exposure in feed, drinking water, or by inhalation over several hours). If the effect is acutely concentration dependent (either at the site of administration or in a sensitive tissue), the dosing method will greatly influence toxicity. Rat forestomach tumors developed following gavage exposure to ethyl acrylate (7) appear to be a good example. However, if the effect in question is related to accumulation of a chemical (such as neurotoxicity from methyl mercury exposure), then acute dose rate is irrelevant.

Risk assessors must often decide whether to use the data from bolus-dose studies for estimating human risks from long-term, low-level environmental exposures. Any decision to discount toxic effects observed only after gavage dosing requires a high degree of confidence in the quality of the various studies

and an understanding of mechanisms and pharmacodynamics. Partly because it is difficult to relate effects seen after gavage dosing to the patterns of human exposures, gavage dosing is no longer recommended for most cancer studies. Exposures to chemicals in feed or drinking water or by inhalation are more relevant to the usual patterns of environmental exposure.

In the case of long-term toxic effects, toxicity may be associated either with accumulation of a chemical or its metabolite, or with accumulation of damage from repeated acute toxic effects (such as alcohol-induced cirrhosis of the liver). In order to distinguish between these alternative etiologies investigators attempt to determine whether the daily chemical dose causes acute adverse effects on the organ system, whether the chronic symptoms are related to total dose irrespective of daily dose rate, and of course whether any chemical products accumulate in the body. Chronic toxicity models are of little value unless it is known whether the rate-limiting factor is the rate of tissue repair or the rate of chemical elimination.

Information on reactivity and disposition pathways of a chemical is also essential in evaluating its potential toxic effects. If it is known, for example, that a highly reactive intermediate is formed (as for methylene chloride; 121), then there is a greater potential for carcinogenic effects of the chemical. In vitro studies to identify reactive intermediates and other metabolites, and to determine the potential for the formation of protein and DNA adducts, are extremely useful (though it is never easy to apply these data directly to risk assessment). If it is discovered that the chemical is metabolized in the liver by cytochrome P450s, many further factors are worth considering: Induction of the cytochrome P450 may occur, and liver weight may increase after subchronic administration of the chemical. Turnover of thyroid hormones (metabolized by cytochrome P450s) may increase, and in chronic studies such an increase might be accompanied by thyroid hyperplasia and a higher incidence of thyroid tumors (99).

Induction of metabolic enzymes may result in either decreased toxicity of a chemical or increased toxicity, depending on whether the toxic effects are caused by a precursor or a product of the reaction. Manipulation of the metabolism of chemicals by administration of liver enzyme inhibitors and inducers has commonly been used in determining the toxic species.

When basic questions about chemical properties, effects, and biological mechanisms are answered, computer modeling of the in vivo uptake and disposition may be appropriate. Physicochemically based exposure models, biologically based dose-response (BBDR) models, and physiologically based pharmacokinetic (PBPK) models are increasingly being applied to risk assessment of environmental chemicals. The exposure and BBDR models use information on chemical properties [vapor pressure, octanol-water partition coefficient (K_{ow}), etc] and specific effects (such as enzyme or receptor affinities

or reaction rates) to help predict in vivo responses (122–125). PBPK models utilize mathematical modeling of organ systems and chemical reaction rates to describe the disposition of chemicals in the body (16, 126–129). The major premise of these techniques is that a good understanding of chemical properties, toxic mechanisms, and body compartments (as demonstrated by matching predictions with experimental observations) allows extrapolation to other chemicals and conditions for which there are few or no data.

These techniques are just beginning to be accepted for use in risk assessment. Recent evaluations of the use of benchmark dose models to estimate cholinesterase inhibitor toxicity represent one example of how BBDR modeling may help improve the accuracy of risk assessments (31). PBPK has so far been applied mostly to low-molecular-weight organic solvents, including several chemicals that are significant environmental contaminants. However, because of the many additional uncertainties, use of PBPK models has often had little effect on the potency estimates (126, 127). Monte Carlo methods to incorporate explicit measurements of the uncertainties and the distribution of predicted exposures and effects into risk assessments can provide useful perspectives (12). As risk assessors become more acquainted with the concepts and gain more experience with their application to environmentally relevant chemicals, these mathematical modeling techniques should contribute significantly to regulatory decision-making.

SUMMARY AND DISCUSSION

To ensure appropriate use of resources for public health and environmental protection, risk assessment must continue to pursue vital data and improve its methods. Coordination of research is needed both to fill data gaps on important environmental contaminants and to improve exposure assessment. Finalization of guidelines for neurotoxicity and immunological testing, and application of these methods to suspect chemicals, are essential. Risk assessors must then determine how best to utilize the data obtained from these newer procedures. When tests are more sensitive, less severe effects can be documented at lower exposure levels, which will help to clarify the low-dose extrapolation issue discussed above.

Improved in vitro testing procedures continue to provide more detailed information on toxicity mechanisms. This information must be incorporated into risk assessment through increased use of mechanistic, biologically based toxicodynamic models. Computerized modeling methods will be invaluable to integrate the wealth of new information being generated. Within the next few years, risk assessors will be attempting to quantify the risks from combined exposures to chemicals with similar effects. When better information is available on the potential sensitivities of various subpopulations (infants, children,

the elderly), it will be possible to document the extent to which current practices protect the entire public. In order to estimate risks from exposure to pesticides, for instance, we need better information on the dietary patterns of these and other subpopulations (e.g. vegetarians, ethnic and religious groups) (32).

Multimedium exposure assessments need to be improved with better data on concentrations of chemicals in food, water, air, and soil, and on the parameters of combined exposures (i.e. water consumption, soil ingestion, and dermal exposure to soil among children who play outdoors in hot, dry climates). Monte Carlo or other stochastic techniques should be examined further as a means to estimate the distributions of these exposures and of the resulting cancer and noncancer risks. Such methods will greatly improve not only the overall accuracy of risk estimates but also our ability to relate (excess) risks to specific parameters and chemicals. An important goal will be to identify and minimize the components of uncertainty that result from deficiencies in measurement as opposed to actual variability.

Better prediction of the toxic effects of both existing and future chemicals should greatly increase the social benefits of toxicology and risk assessment by enabling regulators to focus ameliorative efforts on the most important risks. The challenge for risk assessors is to develop enough of a global view to be able to apply their greater predictive power wisely, in the service of public needs and goals. Current efforts to involve the public in the ranking of risks and to assess the social consequences of regulatory decisions about toxic substances (130) are a valuable step in that direction.

Literature Cited

1. National Academy of Sciences. 1983. *Risk Assessment in the Federal Government: Managing the Process.* Washington DC: Natl. Acad. Press
2. Dourson ML, Stara JF. 1983. Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol.* 8:288–99
3. NIEHS News. 1993. Strengthening the foundation of risk assessment. *Environ. Health Perspect.* 101(5):392–94
4. Focus. 1993. Toxicity tests in animals: extrapolating to human risks. *Environ. Health Perspect.* 101:396–401
5. Ziegler J. 1993. Health risk assessment research: the OTA report. *Environ. Health Perspect.* 101(5):402–6
6. Borghoff SJ, Lagarde WH. 1993. Assessment of binding of 2,4,4-trimethyl-2-pentanol to low-molecular-weight proteins isolated from kidneys of male rats and humans. *Toxicol. Appl. Pharmacol.* 119:228–35
7. Gillette DM, Frederick CB. 1993. Quantitation of an epithelial S-phase response in the rat forestomach and glandular stomach following gavage dosing with ethyl acrylate. *Toxicol. Appl. Pharmacol.* 122:244–57
8. US Environmental Protection Agency.

1989. *Risk Assessment Guidance for Superfund.* Vol. 1. Human Health Evaluation Manual (Part A). Off. Emerg. Remedial Response, US EPA No. 540/1-89-002
9. Watanabe K, Bois FY, Zeise L. 1992. Interspecies extrapolation: a reexamination of acute toxicity data. *Risk Anal.* 12(2):301–10
10. Sidhu KS. 1992. Basis for body weight exponent (0.75) as a scaling factor in energy metabolism and risk assessment. *J. Appl. Toxicol.* 12(5):309–10
11. Koeman JH. 1991. From comparative physiology to toxicological risk assessment. *Compar. Biochem. Physiol. C* 100 (1–2):7–10
12. Thompson KM, Burmaster DE, Crouch EAC. 1992. Monte Carlo techniques for quantitative uncertainty analysis in public health risk assessments. *Risk Anal.* 12(1):53–63
13. Harris M, Zacharewski T, Safe S. 1993. Comparative potencies of Aroclors 1232, 1242, 1248, 1254, and 1260 in male Wistar rats—assessment of the toxic equivalency factor (TEF) approach for polychlorinated biphenyls (PCBs). *Fund. Appl. Toxicol.* 20:456–63
14. Tysklind M, Tillitt D, Eriksson L, Lundgren K, Rappe C. 1994. A toxic equivalency factor scale for polychlorinated dibenzofurans. *Fund. Appl. Toxicol.* 22:277–85
15. Whitlock JP. 1990. Genetic and molecular aspects of 2,3,7,8,tetrachlorodibenzo-p-dioxin action. *Annu. Rev. Pharmacol. Toxicol.* 30:251–77
16. Kohn MC, Lucier GW, Clark GC, Sewall C, Tritscher AM, Portier CJ. 1993. A mechanistic model of effects of dioxin on gene expression in the rat liver. *Toxicol. Appl. Pharmacol.* 120: 138–54
17. De Vito MJ, Maier WE, Diliberto JJ, Birnbaum LS. 1993. Comparative ability of various PCBs, PCDFs and TCDD to induce cytochrome P450 1A1 and 1A2 activity following 4 weeks of treatment. *Fund. Appl. Toxicol.* 20:125–30
18. De Vito MJ, Ma X, Babish JG, Menache M, Birnbaum LS. 1994. Dose-response relationships in mice following subchronic exposure to 2,3,7,8,-tetrachlorodibenzo-p-dioxin: CYP1A1, CYP1A2, estrogen receptor, and protein tyrosine phosphorylation. *Toxicol. Appl. Pharmacol.* 124:82–90
19. Warshawsky D, Barkley W, Bingham E. 1993. Factors affecting carcinogenic potential of mixtures. *Fund. Appl. Toxicol.* 20:376–82
20. Annau Z, ed. 1986. *Neurobehavioral Toxicology.* Baltimore, MD: Johns Hopkins Univ. Press
21. Hartman DE. 1988. *Neuropsychological Toxicology. Identification and Assessment of Human Neurotoxic Syndromes.* New York: Pergamon
22. Committee on Neurotoxicology and Models for Assessing Risk, National Research Council. 1992. *Environmental Neurotoxicology.* Washington DC: Natl. Acad. Press
23. US Environmental Protection Agency. 1991. *Pesticide Assessment Guidelines—Subdivision F Hazard Evaluation: Human and Domestic Animals.* Addendum 10—Neurotoxicity series 81, 82, 83. Off. Pestic. Programs, US EPA No. 540/09-91-123
24. Risk Assessment Forum, US Environmental Protection Agency. 1992. *Report of the neurotoxicity risk assessment guidelines peer review workshop,* June 2–3, 1992. US EPA No. 630/R-92-003
25. Office of Pesticide Programs, US Environmental Protection Agency. 1992. *Proc. US EPA Workshop Cholinesterase Methodologies, December 4–5, 1991* Arlington, VA: Eastern Res. Group
26. US Environmental Protection Agency. 1993. Draft report: principles of neurotoxicity risk assessment: request for comments. *Federal Register* 58(148): 41556–99
27. Angle CR. 1993. Childhood lead poisoning and its treatment. *Annu. Rev. Pharmacol. Toxicol.* 33:409–34
28. Steenland K, Jenkins B, Ames RG, O'Malley M, Chrislip D, Russo J. 1994. Chronic neurological sequelae to organophosphate poisoning. *Am. J. Publ. Health* 84(5):731–36
29. US Environmental Protection Agency. 1990. *Review of cholinesterase inhibition and its effects.* Report of the SAB/SAP joint study group on cholinesterase. US EPA No. SAB-EC-90-014
30. Padilla S. 1993. *Regulatory and research issues related to cholinesterase inhibition.* Presented at US EPA Health Effects Res. Lab. Symp., Research Triangle Park, NC
31. Jowa L, Howd R, DiBartolomeis M, Fan A, Jackson R. 1993. An approach for using cholinesterase inhibition in risk assessment. *Toxicologist* 13:415
32. National Academy of Sciences. 1993. *Pesticides in the Diets of Infants and Children.* Washington DC: Natl. Acad. Sci.
33. Rest KM, ed. 1992. Advancing the understanding of multiple chemical sensitivity. *Proc. Assoc. Occup. Environ. Clinics Workshop on Multiple Chemical*

Sensitivity, 20–21 September, 1991. Washinton, DC, Toxicol. Indus. Health (Spec. Issue) 8(4): July-August 1992

34. US Food and Drug Administration. 1978. Inadvertent modification of the immune response. The effects of foods, drugs and environmental contaminants. *Proc. 4th FDA Sci. Symp., US Food and Drug Admin., US Naval Academy, Annapolis, August 28–30, 1978.* Washington, DC: FDA
35. Sharma RP. 1981. *Immunologic Considerations in Toxicology.* Boca Raton: CRC
36. Office of Technology Assessment. 1991. Identifying and controlling immunotoxic substances—background paper. OTA-BP-BA-75. Washington DC: US GPO

36a. Miller K, Turk JL, Nicklin S, eds. 1992. *Principles and Practice of Immunotoxicology.* Oxford: Blackwell Scientific

37. Murray MJ, Thomas PT. 1992. Toxic consequences of chemical interactions with the immune system. See Ref. 36a, pp. 65–85
38. Kimber I. 1992. Contact sensitivity. See Ref. 36a, pp. 104–24
39. Olsen LJ, Erickson BJ, Hinsdill RD, Wyman JA, Porter WP et al. 1987. Aldicarb immunomodulation in mice: an inverse dose response to parts per billion levels in drinking water. *Arch. Environ. Contam. Toxicol.* 16:433–39
40. Fiore MC, Anderson HA, Hong R, Golubjatnikov R, Seiser JE et al. 1986. Chronic exposure to aldicarb-contaminated drinking groundwater and human immune function. *Environ. Rev.* 41:633–45
41. Thomas PT, Ratajczak HV, Eisenberg WC, Furedi-Marchacek M, Ketels KV et al. 1987. Evaluation of host resistance and immunity in mice exposed to the carbamate pesticide aldicarb. *Fund. Appl. Toxicol.* 9:82–89
42. Thomas PT, Ratajczak HV, Demetral D, Hagen K, Raron R. 1990. Aldicarb immunotoxicology: functional analysis of cell mediated immunity and quantitation of lymphocyte subpopulations. *Fund Appl. Toxicol.* 15:221–30
43. Luster MI, Munson AE, Thomas PT, Holsapple MP, Fensters JD, et al. 1988. Development of a testing battery to assess chemical-induced immunotoxicity. *Fund. Appl. Toxicol.* 10:2–19
44. California Department of Health Services. 1991. *Health Risk Assessment of Aerial Application of Malathion-Bait.* Off. Environ. Health Hazard Assess. (formerly Calif. Depart. Health Services). Berkeley, CA: Calif. Environ. Protect. Agency
45. Critchfield JM, Racke MK, Zuniga-Pflucker JC, Cannella B, Raine CS, et al. 1994. T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. *Science* 263:1139–43
46. Luster MI, Portier C, Pait DG, Rosenthal GJ, Germolec DR, et al. 1993. Risk assessment in immunotoxicology. II. Relationships between immune and host resistance tests. *Fund. Appl. Toxicol.* 21:71–82
47. US Food and Drug Administration. 1993. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. "Redbook II."* Washington DC: US Food Drug Admin.
48. US Environmental Protection Agency. 1991. *Proposed Amendment to the Subdivision F Guideline Requirements to Provide for an Evaluation of the Immunotoxicity of Chemical Pesticides.* Washington DC: US Environ. Protect. Agency
49. Wierda D, Greenlee WF. 1992. Integrating in vitro systems with animal models; mechanistic studies of immunotoxic agents. See Ref. 36a, pp. 324–43
50. Luster M, Portier C, Pait DG, Rosenthal GJ, Germolec DR, et al. 1992. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fund. Appl. Toxicol.* 18:200–10
51. Wilson JG. 1973. Principles of teratology. In *Environment and Birth Defects,* pp. 11–34. New York: Academic
52. Sullivan FM. 1988. Reproductive toxicity test: retrospect and prospect. *Human Toxicol.* 7:423–27
53. Clegg ED, Sakai CS, Voytek PE. 1986. Assessment of reproductive risks. *Biol. Reprod.* 32:5–16
54. Christian MS. 1986. A critical review of multigeneration studies. *J. Am. Coll. Toxicol.* 5:161–80
55. Johnson EM. 1986. The scientific basis for multigeneration safety evaluations. *J. Am. Coll. Toxicol.* 5:197–201
56. National Research Council. 1984. *Toxicity Testing Strategies to Determine Needs and Priorities.* Washington DC: Natl. Acad. Press
57. Nossaman, Gunthner, Knox & Elliott and Carrick, R. L. 1987. *Surviving Proposition 65: A Basic Guidebook to The Safe Drinking Water and Toxic Enforcement Act of 1986.* Los Angeles: Nossaman, Gunthner, Knox & Elliott
58. Kizer KW, Warriner TE, Book SA. 1988. Sound science in the implementation of public policy. A case report on

California's Proposition 65. *J. Am. Med. Assoc.* 260:951–55

58a. Neubert D, Kavlock RJ, Merker HJ, Klein J, eds. 1992. *Risk Assessment of Prenatally-Induced Adverse Health Effects.* Berlin: Springer-Verlag

59. Chernoff GF, Book SA. 1992. Reproductive hazard evaluation and risk assessment under California's Proposition 65. See Ref. 58a, pp. 181–94

60. Neubert D, Kavlock RJ. 1992. Introduction. See Ref. 58a, pp. 1–23

61. Klug W, Neubert D. 1992. Possible contribution of in vitro methods to risk assessment in reproductive and developmental toxicology. See Ref. 58a, pp. 449–473

62. O'Farrell PH, Edgar BA, Lakich D, Lehner CF. 1989. Directing cell division during development. *Science* 246:635–40

63. Murray AW, Kirschner MW. 1989. Dominoes and clocks: the union of two views of the cell cycle. *Science* 246:614–21

64. Capecchi MR. 1989. Altering the genome by homologous recombination. *Science* 244:1288–92

65. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, et al. 1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 356: 215–21

66. Committee on the Biological Effects of Ionizing Radiation (BEIR). 1980. The effects on populations of exposure to low levels of ionizing radiation: 1980. BEIR Report, pp. 71–134. Washington DC: Natl. Res. Counc.

67. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). 1988. *Sources, Effects and Risks of Ionizing Radiation.* Report to the General Assembly, with annexes. New York: United Nations

68. Myhr BC. 1991. Validation studies with Muta™ Mouse: a transgenic mouse model for detecting mutations in vivo. *Environ. Mol. Mutag.* 18:308–15

69. Kohler SW, Provost GS, Fieck A, Kretz PL, Bullock WO, et al. 1991. Analysis of spontaneous and induced mutations in transgenic mice using a lambda ZAP/lacI shuttle vector. *Environ. Mol. Mutag.* 18:316–21

70. Kupchella E, Cebula TA. 1991. Analysis of *Salmonella typhimurium his* D3052 revertants: the use of oligodeoxyribonucleotide colony hybridization, PCR, and direct sequencing in mutational analysis. *Environ. Mol. Mutag.* 18:224–30

71. Tindall KR, Whitaker RA. 1991. Rapid localization of point mutations in PCR products by chemical (HOT) modification. *Environ. Mol. Mutag.* 18:231–38

72. Maher VM, Yang J-L, Chen R-H, McGregor WG, Lukash L, et al. 1991. Use of PCR amplification of cDNA to study mechanisms of human cell mutagenesis and malignant transformation. *Environ. Mol. Mutag.* 18:239–44

73. Bell DA. 1991. Detection of DNA sequence polymorphisms in carcinogen metabolism genes by polymerase chain reaction. *Environ. Mol. Mutag.* 18:245–48

74. Cariello NF, Swenberg JA, De Bellis A, Skopek TR. 1991. Analysis of mutations using PCR and denaturing gradient gel electrophoresis. *Environ. Mol. Mutag.* 18:249–54

75. Hozier JC. 1991. A molecular geneticist's view of complex genomic lesions. *Environ. Mol. Mutag.* 18:255–58

76. Brandriff BF, Gordon LA, Trask BJ. 1991. DNA sequence mapping by fluorescence in situ hybridization. *Environ. Mol. Mutag.* 18:259–62

77. Davis LM. 1991. Molecular analysis of chromosomal rearrangements using pulsed field gel electrophoresis and somatic cell hybrids. *Environ. Mol. Mutag.* 18:263–69

78. Nicklas JA., Lippert MJ, Hunter TC, O'Neill JP, Albertini RJ. 1991. Analysis of human HPRT deletion mutations with X-linked probes and pulsed field gel electrophoresis. *Environ. Mol. Mutag.* 18:270–73

79. Zimmerman LJ, Fuscoe JC. 1991. Direct DNA sequencing of PCR products. *Environ. Mol. Mutag.* 18:274–76

80. McKusick VA. 1983. *Mendelian Inheritance in Man: Catalogs of Autosomal Dominant, Autosomal Recessive and X-linked Phenotypes.* Baltimore: Johns Hopkins Univ. Press

81. Russell LB. 1991. Factors that affect the molecular nature of germ-line mutations recovered in the mouse specific-locus test. *Environ. Mol. Mutag.* 18: 298–2

82. Lewis SE. 1991. The biochemical specific-locus test and a new multiple-endpoint mutation detection system: considerations for genetic risk assessment. *Environ. Mol. Mutag.* 18: 303–6

83. Dellarco VL, Rhomberg L, Shelby MD. 1990. Perspectives and future directions for genetic risk assessment. *Environ. Mol. Mutag.* 16:132–34

84. Crow JF, Denniston C. 1981. Evaluation of genetic risks. In *Measurement of*

Risks, ed. GG Berg, HD Maillie. New York: Plenum
85. US Environmental Protection Agency. 1986. Environmental Protection Agency; guidelines for mutagenicity risk assessment. *Fed. Regist.* 51:34006–12
86. Sofuni T. 1993. Japanese guidelines for mutagenicity testing. *Environ. Mol. Mutag.* 21:2–7
87. Kirkland DJ. 1993. Genetic toxicology testing requirements: official and unofficial views from Europe. *Environ. Mol. Mutag.* 21:8–14
88. Auletta AE, Dearfield KL, Cimino MC. 1993. Mutagenicity test schemes and guidelines: US EPA Office of Pollution Prevention and Toxics and Office of Pesticide Programs. *Environ. Mol. Mutag.* 21:38–45
89. Department of National Health and Welfare. 1993. The assessment of mutagenicity. *Environ. Mol. Mutag.* 21:15–37
90. Dellarco VL, Farland WH. 1990. Introduction to the US Environmental Protection Agency's genetic risk assessment on ethylene oxide. *Environ. Mol. Mutag.* 16:83–84
91. Dellarco VL, Generoso WM, Sega GA, Fowle JR, Jacobson-Kram D. 1990. Review of the mutagenicity of ethylene oxide. *Environ. Mol. Mutag.* 16:85–103
92. Rhomberg L, Dellarco VL, Siegel-Scott C, Dearfield KL, Jacobson-Kram D. 1990. Quantitative estimation of the genetic risk associated with the induction of heritable translocations at low-dose exposure: ethylene oxide as an example. *Environ. Mol. Mutag.* 16:104–25
93. Generoso WM, Cain KT, Cornett CV, Cacheiro NLA, Hughes LA. 1990. Concentration-response curves for ethylene-oxide-induced heritable translocations and dominant lethal mutations. *Environ. Mol. Mutag.* 16:126–31
94. US Environmental Protection Agency. 1986. Guidelines for carcinogen risk assessment. *Fed. Regist.* 51:33992–4000
95. Huff JE, Haseman J, Rall D. 1991. Scientific concepts, value, and significance of chemical carcinogenesis studies. *Annu. Rev. Pharmacol. Toxicol.* 31: 621–52
96. KS Crump & Co. 1985. *Selecting the number of stages in linearized multistage analysis and assessing goodness-of-fit.* Prepared for the Environmental Protection Agency, Washington, DC. Ruston, LA: Crump & Co.
97. Haseman JK, Huff JE. 1987. Species correlation in long-term carcinogenicity studies. *Cancer Lett.* 37:125–37
98. Tennant RW, Margolin BH, Shelby MD, Zeiger E, Spalding J, et al. 1987. Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science* 236:933–41
99. Grasso P, Sharratt M, Cohen AJ. 1991. Role of persistent, non-genotoxic tissue damage in rodent cancer and relevance to humans. *Annu. Rev. Pharmacol. Toxicol.* 31:253–87
100. Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, et al. 1994. A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 264:436–40
101. Culotta E, Koshland Jr. DE. 1994. p53 sweeps through cancer research. *Science* 262:1958–61
102. Knudson AG. 1993. All in the (cancer) family. *Nat. Genet.* 5:103–4
103. Pardee AB. 1989. G1 events and regulation of cell proliferation. *Science* 246: 603–8
104. Laskey RA, Fairman MP, Blow JJ. 1989. S phase of the cell cycle. *Science* 246: 609–14
105. McIntosh JR, Koonce MP. 1989. Mitosis. *Science* 246:622–28
106. Hartwell LH, Weinert TA. 1989. Checkpoints: controls that ensure the order of cell cycle events. *Science* 246:629–34
107. Pines J. 1991. Cyclins: wheels within wheels. *Cell Growth Differ.* 2:305–10
108. Ohtsubo M, Roberts JM. 1993. Cyclin-dependent regulation of G1 in mammalian fibroblasts. *Science* 259:1908–12
109. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, et al. 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell* 75:817–25
110. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. 1993. The p21 cdk-interacting protein cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 75:805–16
111. Adam D, Dimitrijevic N, Schartl M. 1993. Tumor suppression in *Xiphophorus* by an accidentally acquired promoter. *Science* 259:816–19
112. Hinds PW, Dowdy SF, Eaton EN, Arnold A, Weinberg RA. 1994. Function of a human cyclin gene as an oncogene. *Proc. Natl. Acad. Sci. USA* 91:709–13
113. Papadopoulos N, Nicolaides NC, Wei Y-F, Ruben SM, Carter KC, et al. 1994. Mutation of a mutL homolog in hereditary colon cancer. *Science* 263:1625–29
114. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, et al. 1994. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 368:258–61
115. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, et al. 1993. The

human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 75:1027–38
116. Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Parsons R, et al. 1993. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 75: 1215–25
117. Parsons R, Li G-M, Longley MJ, Fang W-H, Papadopoulos N, et al. 1994. Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* 75:1227–36
118. Fearon ER, Vogelstein B. 1990. A genetic model for colorectal tumorigenesis. *Cell* 61:759–67
119. Hollstein M, Sidransky D, Vogelstein B, Harris CC. 1991. p53 mutations in human cancers. *Science* 253:49–53
120. Aguilar F, Harris CC, Sun T, Hollstein M, Cerutti P. 1994. Geographical variation of p53 mutational profile in nonmalignant human liver. *Science* 264: 1317–19
121. Reitz RH, Mendrala AL, Guengerich FP. 1989. In vitro metabolism of methylene chloride in human and animal tissues: use in physiologically based pharmacokinetic models. *Toxicol. Appl. Pharmacol.* 97:230–46
122. Hattis D. 1990. Pharmacokinetic principles for dose-rate extrapolation of carcinogenic risk from genetically active agents. *Risk Anal.* 10(2):303–15
123. Scheuplein RJ, Shoaf SE, Brown RN. 1990. Role of pharmacokinetics in safety evaluation and regulatory considerations. *Annu. Rev. Pharmacol. Toxicol.* 30:197–18
124. McKone TE, Howd RA. 1992. Estimating dermal uptake of nonionic organic chemicals from water and soil: I. Unified fugacity-based models for risk assessments. *Risk Anal.* 12:543–57
125. O'Flaherty EJ, Scott W, Schreiner C, Beliles RP. 1992. A physiologically based kinetic model of rat and mouse gestation: Disposition of a weak acid. *Toxicol. Appl. Pharmacol.* 112:245–56
126. Conolly RB, Andersen ME. 1991. Biologically based pharmacodynamic models: tools for toxicological research and risk assessment. *Annu. Rev. Pharmacol. Toxicol.* 31:503–23
127. Hattis D, White P, Koch P. 1993. Uncertainties in pharmacokinetic modeling for perchloroethylene: II. Comparison of model predictions with data for a variety of different parameters. *Risk Anal.* 13:599–610
128. Schreiber JS. 1993. Predicted infant exposure to tetrachloroethene in human breastmilk. *Risk Anal.* 13:515–24
129. Dankovic DA, Bailer AJ. 1994. The impact of exercise and intersubject variability on dose estimates for dichloromethane derived from a physiologically based pharmacokinetic model. *Fund. Appl. Toxicol.* 22:20–25
130. California Comparative Risk Project. 1994. *Toward the 21st Century: Planning for the Protection of California's Environment.* Berkeley, CA: Calif. Comp. Risk Project. 642 pp.