Determining Acceptable Risks: Experimental and Epidemiological Issues

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The extent to which results of experimental toxicology studies can be used to assess risk for humans varies. Administering high doses of chemicals to experimental animals may produce indirect adverse metabolic and nutritional effects, and metabolic pathways may differ at high and low doses. The rate of metabolism at high and low doses and the metabolic pathways of chemicals in different species are important for establishing the reasons for differences in species responses. Animal species best suited to serve as a surrogate for humans vary for different chemicals. For realistic risk assessments, more detailed information on the toxikokineti of chemicals in humans is needed. As data are developed to link early biochemical changes with future disease, it is important to determine their predictive value. Our present expectations of using various biomarkers to predict future health outcomes may be unrealistic. More baseline data are needed to determine which biomarkers are expressions of exposure and which are predictors of future disease. It is also unclear how lifestyle affects most biomarkers.

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Risk is the possibility of loss or injury, e.g., from losing money, having an accident, being exposed to harmful chemicals, or contracting an infectious disease. Populations living in different parts of the world and in different societies are affected by different types of risks to various degrees. In developing countries, infectious diseases, malnutrition, and infant mortality are primary concerns. In developed countries, chronic diseases, the ability to prolong life, and fear of violence have a high priority. Nonetheless, in the US, infant mortality is still relatively high and the prevalence of preventable communicable diseases is on the rise again. In this presentation, however, only risk related to exposure to man-made chemicals will be discussed.

For acute illnesses caused by chemicals, exposure is usually high and closely related to time of onset of the illness. Thus, diagnosing the illness and distinguishing it from other causes of disease is relatively easy, particularly if the nature of the exposure and the identity and effects of the chemical are known (1). However, making these types of correlations for chronic diseases is much more difficult. By their nature, chronic diseases are multifactorial and may have been initiated by events that occurred many years ago, events that may be difficult to reconstruct. For many chronic diseases, therefore, prevention is a much-emphasized, but difficult to achieve, goal.

Assessing Risk

It is now possible to measure chemicals in very low concentrations in food, water, air, and other environmental media. Once these concentrations have been determined, their significance must be interpreted. For many chemicals little information is available about their toxicity. Frequently, the dose that might cause an observable adverse effect in humans is not known. If toxicology data from experimental animals are available, acceptable levels of intake for humans are calculated by risk assessors, and environmental media standards are set. This approach frequently does not have a sound scientific basis and may over- or underestimate risk by several orders of magnitude. The results of these risk assessments are conveyed to the public without explaining the uncertainties inherent in such assessments. To reduce these uncertainties, an improved database and a better evaluation of the toxicodynamics of chemicals are needed.

Factors affecting uncertainties in risk assessment can be broadly divided into experimental and epidemiological issues.

Experimental Issues

Whether the results of experimental animal studies should be used for risk assessments depends on a variety of factors, the most important ones being: (a) the appropriateness of using experimental animal models to predict adverse effects in humans; (b) the use of relevant doses in experimental animal studies; (c) the accurate measurement of chemicals and their metabolites; and (d) the appropriate interpretation of biochemical and biological alterations.

Experimental Animal Models

The animal species most suited to serve as a surrogate for humans varies for different chemicals and for different end points. Enzyme systems in different species may vary or may be absent; e.g., because rat liver contains more epoxide hydroxylase than mouse liver does, the rat is less dependent on glutathione-S-transferase for the elimination of 1,3-butadiene epoxides. Therefore, given that the epoxides are the proximate carcinogens for 1,3-butadiene, rats are less susceptible to the carcinogenic effect of 1,3-butadiene than are mice. Apparently epoxide hydroxylase activity is greatest in monkeys and humans. Consequently, humans, if exposed to 1,3-butadiene, would be even less likely to develop cancer than rats and mice. A physiologically based toxikokinetic model could be developed for 1,3-butadiene that would account for the differences in metabolism between humans and experimental animals. Such an approach
should provide a more realistic assessment of the risk of 1,3-butadiene for humans (2-4).

Present risk assessments based on the animal data for 1,3-butadiene overestimate the risk of cancer for humans. To conduct more realistic risk assessments, detailed information on the toxicokinetics of 1,3-butadiene in humans would be helpful. Unfortunately, when exposure is assessed through human monitoring, either in workers or in accidental poisoning cases, little thought is usually given to studying the metabolism at that particular time. Much valuable information is thus lost because of the difficulty or impossibility of conducting these studies in volunteers.

Different metabolic pathways may exist at high and low doses of a compound. Trichloroethylene, for example, at the doses humans might ordinarily encounter, is exhaled in greater proportion as the parent compound, rather than metabolized, by humans than by rats and mice. Quantitative differences in metabolism of this chemical also exist between rats and mice (5). Because the proximate carcinogen of trichloroethylene is a metabolite, humans would be less or not at all affected by the carcinogenic potential of trichloroethylene; they would excrete primarily the parent chemical and transform little or none into the proximate carcinogen. Mice, however, form more of the proximate carcinogen than rats and therefore are more likely to develop cancers—as experiments with rats and mice have shown. By determining why these species differences exist, potential risks to humans can be put into perspective.

In contrast, after acute methanol intoxication, humans and subhuman primates develop acidosis and ocular toxicity, whereas in nonprimate laboratory animals (e.g., dogs, rats, rabbits, and mice), acidosis and ocular toxicity are not observed. For methanol, therefore, the rhesus monkey and the pigtail macaque are the preferred animal models for evaluating human toxicity.

The metabolic sequence for methanol in all mammals is as follows: methanol → formaldehyde → formic acid → carbon dioxide + water + H+ In rats, rabbits, and guinea pigs, the initial oxidation of methanol to formaldehyde is mediated by a catalase–peroxidative system. In humans and nonhuman primates, however, methanol is oxidized via alcohol dehydrogenase. The rates of this initial metabolic step are similar in nonhuman primates and in rats. In the second step of methanol metabolism, formaldehyde is converted to formic acid, a step comprising two reactions: Formaldehyde is oxidized to S-formylglutathione, a process that requires reduced glutathione and is mediated by an NAD+–dependent formaldehyde dehydrogenase; then, S-formylglutathione is converted to formic acid, catalyzed by thiolase. It remains a matter of dispute as to which intermediate metabolite, formaldehyde or formic acid, is responsible for the delayed effects of methanol poisoning. However, formate accumulation in the blood of monkeys parallels the development of ocular disturbances and acidosis, supporting the presumption that formic acid is the prime suspect for the delayed effect and for ocular toxicity.

A folate-dependent pathway is responsible for metabolizing formic acid in nonhuman primates, rats, and other nonprimate species. However, rats use the pathway more efficiently than do nonhuman primates and humans, another explanation for the species-dependent nature of the acute toxicity of methanol. At high methanol doses, formate enters the folate-dependent pathway in humans and nonhuman primates at rates that exceed that pathway's capacity. A strong association exists between the efficiency of the formate metabolism and the hepatic concentration of tetrahydrofolate. Makar and Tephy (6) determined that rats fed a folate-deficient diet became acidotic and accumulated formate, similarly to nonhuman primates. Moreover, blocking the folate feedback loop with nitrous oxide (NO2) increased the toxicity of methanol (6-9). Black et al. (10) showed that the tetrahydrofolate concentration in monkey livers was 59% of that in rats, making monkeys more susceptible to the toxic effects of methanol through the accumulation of formate. Humans are similarly more susceptible. Thus, in this case, the rat would be a very poor predictor of human toxicity.

In the US, it has become common practice to select results obtained in the most sensitive species for risk assessments. A scientifically more correct approach would be to determine why differences in response exist between species and which species represents the best surrogate to predict adverse effects in humans. Differences in response to chemicals may be the result of differences in metabolism among various species and of differences of responses at high and low doses in the same species. The rate of metabolism at high and low doses in different species and the metabolic pathways of chemicals in humans are important tools in quantitative risk assessment.

Differences in concentration, binding, and distribution of the offending chemical in target cells at equilibrium may also modulate the susceptibility of different species or strains. The toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is mediated through apparently reversible binding to the arylhydrocarbon hydroxylase (Ah) receptor. Certain mouse strains are much less susceptible to the toxic effects of TCDD than others; e.g., a dose of 25 μg/kg of body weight per week produces liver toxicity and hepatic porphyria in C57BL/6 mice but not in DBA/2 mice (11). However, differences in the Ah receptor are the primary reason for the differences in toxicity between the two strains of mice. Quantitative differences in toxicity between species administered doses of TCDD may also result from differences in tissue distribution. At nontoxic doses, a proportionally larger amount of TCDD is stored in fatty tissue than in the liver, a target organ in many species (12). According to Carrier and Bordeur (13), TCDD and other halogenated polycyclic compounds in rats, monkeys, and humans show differences in toxicokinetics

1 Nonstandard abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; Ah, arylhydrocarbon hydroxylase; and NOAEL, "no observed adverse effect level."
between species. These differences are related to the dose and the affinity of these chemicals for binding sites in the liver. These authors gave the following explanation for this phenomenon: “Below hepatic saturation the contribution of liver load to total body burden increases as body burden increases, tending towards second-order kinetics.” At saturation, body burden eliminations follow zero-order kinetics; as the dose increases, the intestinally absorbed fraction of TCDD decreases. Furthermore, as the administered dose increases, a larger proportion of the total dose is found in the liver (14). The alteration in the ratio of the liver-to-fat concentration is the most sensitive marker for dose dependence and for dose-dependent protein binding of TCDD in the liver. Because of the limited TCDD binding capacity of the liver, the liver-to-fat concentration ratio reaches a maximum and is predicted to decrease at higher body burdens, where hepatic binding to induced proteins approaches saturation. The body burden for half-maximal sequestration of TCDD in the liver in humans was estimated by Carrier (15) to be 0.5–1.5 μg/kg of body wt. The liver/fat concentration ratio varies for different isomers of this class of chemicals. More highly chlorinated isomers tend to have ratios higher than TCDD itself; they also are less toxic. The binding protein constant in the liver is lower for the more highly chlorinated dioxins.

Andersen et al. (16) developed a receptor-mediated, physiologically based pharmacokinetic model for the tissue distribution and enzyme-inducing properties of TCDD, which they used to examine the tissue disposition of TCDD as well as the induction of a TCDD-binding protein and of cytochrome P4501A1 in rats.

In humans, the amount of Ah receptor in lung, skin, tonsils, and liver is appreciably less than in the murine model, suggesting that humans would be less, rather than more, susceptible to the adverse effects of TCDD, (17–20). The relative distribution between various tissues seems to be proportionally different at equivalently administered doses in different animal species (12). Humans have a greater tendency to concentrate TCDD in adipose tissue. Because of the differences in the amount of the Ah receptor and the affinity of TCDD for the Ah receptor in humans, binding of TCDD to the receptor is more labile, facilitating the migration of TCDD into adipose tissue away from vital organs. Therefore, an administered dose that does not produce adverse effects in laboratory animals would also not produce adverse effects in humans. In fact, humans tolerate somewhat higher administered doses and are about two orders of magnitude less sensitive to TCDD than some laboratory animal species.

The Ah receptor is also important for a broad range of other chemicals. To use these more sophisticated models and incorporate information about receptors into risk assessments in the future necessitates a greater effort to characterize such receptors in human and experimental animal tissues. Concentrations of the chemicals and their metabolites in paired tissue samples such as liver, lung, and adipose tissue in humans and laboratory an-

imals at steady states would be useful for comparing target tissue concentrations between species at toxic and nontoxic doses. Moreover, although target tissue concentrations may be quite similar between species, the doses necessary to reach such target tissue levels may be different, given interspecies differences in characteristics of the receptors and in the distribution of the chemicals. The characteristics of the Ah receptor of different hosts and differences in the amount of the Ah receptor in different tissues of the same host also modulate general toxicity and target organ toxicity. The degree of chlorination and the position of the chlorine atoms of other chlorinated dibenzodioxins will affect tissue distribution.

High-Dose Experimental Animal Studies

To uncover adverse effects produced by chemicals and to identify target organ toxicity, investigators usually administer high doses of chemicals to animals. Humans, however, are generally exposed to much lower doses, particularly if the exposure is environmental rather than occupational. In the absence of human data, results from such animal studies are used to calculate an acceptable dose for humans. For noncancer end points, some laboratory animal studies may be available that provide a “no observed adverse effect level” (NOAEL). In the US, this result is divided by uncertainty factors to extrapolate from animals to humans. The uncertainty factors usually consist of multiples of 10 to account for the heterogeneity of human populations, species variation, whether the lowest dose was a NOAEL, and whether the study in the laboratory animal species was a lifetime study. Thus, the use of inadequate data could result in an uncertainty factor of 10,000, more because little is known about a given chemical than because of the inherent toxicity of the chemical.

In these extrapolations, indirect effects at high doses that would be absent at low doses are not considered. An example is depletion in the liver of glutathione, whose nucleophilic thiol is important for the detoxification of electrophilic metabolites and oxidizing agents (21), e.g., bromobenzene and acetaminophen. Oxalates and certain sulfonamides may, under certain circumstances, be present in urine in such high concentrations that they precipitate, form crystals and stones, and produce secondary impairment of the kidney. Long-term treatment of animals with diuretics may lead to a loss of potassium, resulting in potassium-depletion nephropathy. At lower doses, these effects would not occur and may not be relevant for humans.

Accurate Measurement of Chemicals and Metabolites

Some chemicals are metabolized and excreted rapidly, such that it is frequently not possible to determine whether a person has been exposed to a chemical by measuring its concentration in body fluids and tissues. For the more persistent chemicals, quantities measured in biological specimens can give information about the degree of exposure. Such chemicals include the organic halogenated persistent chemicals such as chlorinated
and brominated dibenzodioxins, naphthalenes, biphenyls, chlorinated hydrocarbon pesticides, and heavy metals. These chemicals are ubiquitous, and trace amounts of them are found in tissues in the general population. Their identification in human tissues, therefore, does not necessarily indicate any special exposure or that this finding has clinical relevance.

Furthermore, the chemical analysis of biological specimens for these chemicals is difficult. Inexperienced laboratories may report erroneous results because their specimen cleanup is not satisfactory, the laboratory has been contaminated with the chemicals to be analyzed, or the analytical method they use is not sufficiently specific. Sample collection may also introduce errors. If specimens are obtained from workers in a factory, contamination of the specimen may result because the chemicals to be measured are affected by the factory environment. Samples may also be inappropriately preserved, resulting in errors of quantification. Quantification of chemicals in urine presents a special problem unless 24-h urines are collected. For early morning specimens or "spot" samples, quantification can be improved if creatinine and specific gravity are also determined. For some chemicals creatinine is a better measure; for others, specific gravity may be more important. Before the results of the chemical analyses are interpreted, laboratory performance should be reviewed. Collaboration between the laboratory and the person(s) collecting the specimens is imperative.

Appropriate Interpretation of Biochemical Alterations

Many sophisticated tests are now conducted in experimental animals and in humans to measure biochemical and physiological changes. It is frequently not clear whether these changes are early warning signs of disease in the individual with these abnormalities or whether such changes suggest that a given group of animals or humans is at a higher risk to develop a specific disease in the future. It is also not clear, after cessation of exposure, whether and to what extent such changes are repaired over time. For instance, at present we do not know whether high amounts of DNA adducts are associated with the development of cancer in specific individuals. This would appear to depend on the type of adducts and on the cells' ability to repair the damage. The rate of cell proliferation and cell division, slow or rapid, is age-, species-, and organ-specific and would affect this process. At the moment we know little about these various mechanisms, although much speculation has been advanced.

A simple example of a frequently performed clinical laboratory test illustrates this point. Above-normal concentrations of cholesterol in serum may or may not be a risk factor for cardiovascular disease. What is important is the interrelationship between total cholesterol and high- and low-density lipoproteins. The concentrations of these various serum lipid fractions show genetic variations and age and sex variations. Serum lipid concentrations also differ in racially different populations (22). Low concentrations of high-density lipoprotein may be a risk factor for cardiovascular disease mainly in populations with a high-fat diet (23). Thus, the ability of an individual to increase the concentration of high-density lipoproteins in conjunction with a high-fat diet is a deciding factor in protection against cardiovascular disease. An extreme example supports this point. Patients with Tangier disease, a rare disorder of lipoprotein metabolism characterized by extremely low concentration of high-density lipoprotein and low total cholesterol and low-density lipoprotein, may still develop severe atherosclerosis even though their total cholesterol concentrations are much lower than those of the general population (24). This example illustrates that interpreting the results of measurements of specific biomarkers or individual biochemical changes in isolation, and interpreting their significance for disease without accounting for other modulating factors and for the ability of biological systems to compensate, will result in erroneous risk predictions.

Our expectations that most biomarkers will predict future health outcomes may be unrealistic. Too little is known about them at present to make them useful in risk assessment and in risk-management decisions. More baseline data are needed to interpret results of such monitoring efforts intelligently and to determine with any degree of certainty which biomarkers are expressions of exposure and which are expressions of future disease. Normal background values for biomarkers in the population may vary with age, nutrition, well-being or disease, lifestyle, and use of medications.

The results of biomarker measurements in the presence of disease are more easily interpretable and can give useful information about the progression of a particular disease. For instance, measuring gene rearrangements through molecular pathology tests is clinically useful for forming a more specific diagnosis of certain types of leukemia and for monitoring leukemia patients during and after therapy, confirming remissions, and detecting relapses early (25).

Epidemiological issues

The epidemiological issues for chemical exposures are the same as for other epidemiological studies. Two approaches to epidemiological studies are prevalent: determining whether a given exposure has resulted in illness in a population, and determining whether a given illness has been produced by exposure to a chemical. Both approaches have their problems.

Exposure

At the outset, one must determine whether exposures were sufficiently high to have resulted in adverse health effects. This frequently becomes a judgment call because, for many chemicals, adverse human health effects are unknown. However, information from occupational exposures may be helpful. If sufficient numbers of workers have been exposed to much higher concentrations of a given chemical than the general public without experiencing ill effects, then one may reasonably assume that the general public is not adversely affected.
Once a local source of a chemical has been identified, such as solvents in well water used as drinking water, the extent of exposure of individuals, the probable daily dose, and whether that dose makes a significant contribution to overall exposure from other sources should be determined (26). If the general population receives similar exposures, then epidemiology studies are not feasible because no unexposed comparison group exists.

The measurement of chemicals of concern in body tissues and fluids gives important information about the dose individuals have received. However, unless some chemicals are measured immediately after exposure, they will not be detected because of their rapid excretion. Sometimes metabolites are retained slightly longer than the parent chemical, e.g., p-nitrophenol, a metabolite of parathion [phosphorothioic acid o,o-diethyl-o-(4-nitrophenyl) ester]. Some chemicals, e.g., halogenated polycyclic compounds and heavy metals, are not as easily excreted and therefore accumulate in the body. Exposure assessments for these can be based on measurements in body fluids and tissues. For some halogenated polycyclic chemicals, half-lives in humans are on the order of years, and past exposures can still be assessed at a later date. Again, such body burdens must be compared with body burdens in the general population to determine whether a given person has had a higher exposure. When making such comparisons, the arithmetic means of the "exposed" group may be higher than those of the comparison group, but the individual values may overlap. Such results should not be construed as representing differences in exposure and dose between the two groups. Instead, such observations merely suggest that the data are skewed. Frequently, log transformation of the data or calculation of geometric means will illustrate this point. Such differences may occur because the two populations were not matched well by age, smoking habits, liver function, or some other factor affecting the toxicokinetics of a particular chemical. In some studies, the control or comparison population was small or may not have been a truly random sample (27). As larger numbers of people are studied, the distribution range of the chemical concentrations measured usually widens and fewer outliers are observed.

Physiological factors may also affect the concentrations of chemicals in tissues and body fluids. For instance, the concentrations of lipid-soluble chemicals will be higher in serum in nonfasting specimens and in people with higher concentrations of serum lipid. They will be lower in the serum of neonates than in their mothers' serum because of the lower lipid concentrations in the blood of neonates. These factors should be considered when data are compared.

Indirect exposure measures such as induction or inhibition of enzymes, chromosome breaks, sister chromatid exchange, DNA adduct formation, lipid peroxidation, and formation of porphyrins are more difficult to evaluate. They are nonspecific and, unless careful medical and exposure histories are taken, the data cannot be interpreted. These markers can be affected by smoking, alcohol consumption, use of specific medications, exposure to x-ray irradiation, chemotherapy, and the presence of underlying disease or congenital abnormalities. For many of these end points, insufficient information is available from different age groups of the general population and of specific ethnic groups. It is also not clear how nutrition affects them. Finally, no prospective studies have been conducted to determine what the health outcomes are in individuals with "abnormal" values for these various markers.

Disease

Very few chronic diseases have been identified as having been caused by chemicals alone; the mesotheliosas, and asbestosis and other pneumoconioses are some that have. A few studies have demonstrated that certain diseases are more prevalent after exposure to specific chemicals, e.g., chronic myelogenous leukemia after exposure to benzene and bladder cancer after exposure to β-naphthylamine or 2-methylchboroaniline. However, these illnesses may also occur without these exposures, and in specific cases it may be difficult to determine whether a given exposure was causally associated with a specific disease. For some chronic diseases, the incidence and (or) prevalence in the general population is not very well known. Furthermore, because clinical and pathological criteria for classifying diseases and criteria for reporting diseases continue to evolve, trend analysis for some diseases is difficult.

Because of the many uncertainties and confounders affecting epidemiology studies, the scientific community is frequently divided on whether results of specific studies are overinterpreted or flawed. Under such circumstances, careful and objective analysis of all data, and perhaps additional studies, may eventually lead to more conclusive and convincing results.

References