engineering for a high or low capacity of biotransformation. Also, tumor suppressor capacity or DNA repair capability can be negated in the animal to increase sensitivity in detecting carcinogens. For example, genetically modified mice in development include the p53+/–, Tg.AC, and Tg.rasH2 assays; they are to be used to supplement information gained in the conventional inbred strains and not to replace them.

Categories of Toxicity

A somewhat arbitrary system of toxicity ranking has evolved based on the median lethal dose of a substance. A substance with a median lethal dose of less than 1 mg/kg is considered to be extremely toxic, while various definitions of highly toxic, moderately toxic, and slightly toxic have been proposed. Generally, highly toxic substances have a median lethal dose of less than 50 mg/kg, moderately toxic have a median lethal dose of less than 500 mg/kg, and slightly toxic have a median lethal dose of greater than 500 mg/kg and up to approximately 5 g/kg, which approaches the practical limit of most dosing techniques.

Mixtures

Mixtures of poisons can be more toxic or less toxic than predicted from the toxicity of the individual components of the mixture. This phenomenon of increased toxicity of a mixture is known as *synergism*, and it results from an interaction of one component with the pharmacokinetics or pharmacodynamics of the second component; e.g., the first component might interfere with the elimination of the second component so that a given exposure of the second component produces higher concentrations in the body when applied in the mixture. *Antagonism* is the observation of less than predicted toxicity from a mixture, e.g., when one component induces a higher rate of inactivation of the second component, resulting in a higher concentration of a less toxic metabolite.

Drugs, pesticides, industrial chemicals, etc., when used in mixtures, or when giving simultaneous exposure, should be evaluated empirically for interactions to determine whether there is synergism or antagonism. When one component is nontoxic, this test is relatively simple; the nontoxic component can be administered at a high concentration with the complete range of doses of the toxic component. If the dosage–mortality line of the mixture differs significantly from the dosage–mortality line of the toxic component alone, then an interaction is indicated. If both components are toxic, then the test for an interaction is more complex. One approach is to prepare a mixture containing each component at its median lethal dose. Dilutions are

made and administered; then the observed dosage–mortality line is compared to a line predicted by adding the expected mortalities for the individual components at each dilution.

Toxicity, Hazard, and Risk

Toxicity and Hazard

For any substance, the term *hazard* can be used to describe the actual risk of poisoning. Thus, an estimate of toxicity is not a direct estimate of hazard. In fact, toxicity is only one variable to be considered in predicting how hazardous a substance will be during practical use. Another significant variable that must be considered is potential level of human exposure to the substance. This must be predicted based on factors such as the concentration and circumstances of use of the substance. While the intrinsic toxicity of a substance cannot be altered because it is a basic property of that substance, it is possible to reduce hazard of a toxic substance by reducing the practical risk of exposure. A simple example is the invention of childproof packaging of nonprescription drugs, which reduces the hazard associated with some drugs by making access to the drug more difficult. In another example, hazards posed by pesticides to the pesticide applicator have been reduced by preparing the pesticide in dissolvable polymer bags containing premeasured quantities designed to be dropped into the sprayer tank without opening. This innovation greatly reduced the risk of exposure to formulated pesticide concentrates by eliminating measuring and mixing by the applicator.

The Role of Laboratory Testing in Estimation of Hazard

Toxicological data from laboratory studies such as those described here are often used by regulatory agencies in the attempt to estimate the hazard to human health posed by a particular toxicant. Even though there may be issues in extrapolating from animal data to humans, and from higher to lower exposure levels, these studies are still extremely useful in estimating human hazard.

To help with both experimental design and interpretation of toxicological data, the mathematical tools of *statistics* are used. In terms of experimental design, statistics can help with issues such as randomization of subjects and choice of sample size. Then, because toxicological data sets are often quite large, *descriptive statistics* are useful to help summarize the data. Examples of descriptive statistics are the *mean*, *standard deviation* (SD), or *standard error of the mean* (SEM). The SEM is defined as

$$SEM = SD / \sqrt{N}$$

where N is the number of data points in the data set.

Statistics can also be used to help identify *differences* and *trends* in data sets. Normally distributed data that are continuous (such as weight, volume, etc.) may be analyzed using *parametric statistics*. *Nonparametric statistics* are used to analyze data sets that are not normally distributed, or that are made up of *discrete data* (data that occur only as integer values).

Tests such as *Student's t-test* (a parametric test) or the *Mann–Whitney U test* (a nonparametric test) test the hypothesis that two sets of data are *significantly different*. These tests deliver a *p value* that tells you the probability that the differences between the two groups are simply due to random chance. General scientific consensus states that if there is a 5% or less chance that the differences between the two sets of data are due to chance (in other words, a *p* value less than or equal to 0.05), then the difference can be termed significant. Multiple groups can be compared using *analysis of variance* (ANOVA) tests, of which there are both parametric and nonparametric forms. If the results of an ANOVA indicate a significant difference, a variety of tests known as *post hoc tests* may be used to further analyze where the differences lie.

One final use of statistics is in the analysis of trends. Tools such as *linear regression analysis* can help determine the relationship between two variables such as, for example, dose and response. A statistic called the *correlation coefficient* (also known as r^2) measures the accuracy with which the data fit the hypothesized linear relationship.

It can be quite difficult to extrapolate from laboratory studies to real-world situations, which is one reason why the processes of risk assessment and management are often fraught with controversy. Although laboratory animals can serve as models for humans in toxicological testing, species differences do exist. Also, many scientists have criticized the practice of using very high doses of toxicants during laboratory testing and then attempting to apply the results to a situation where human exposure levels are actually very low. Therefore, data must be interpreted with caution.

Epidemiological Data

Along with laboratory data, data from *epidemiological* studies are also used in risk estimation. These studies examine the relationships between exposure to a toxicant (usually accidental or voluntary exposure) and either disease *incidence* (the rate at which new cases of the disease appear in a human population) or disease *prevalence* (the number of existing cases of the disease at a particular point in time).

Epidemiological studies do have some drawbacks. Because of variability in genetic and environmental factors between individual humans, it can be extremely difficult to be sure that differences in disease incidence or prevalence between exposed and control groups are really due to the factor being tested and not to some other *confounding factor* (a factor that can cause a difference between the groups, but is not the factor being tested). Also, exposure levels may be difficult to estimate (particularly if exposure to the

toxicant occurred some time in the past). To maximize reliability of results, exposed and control groups are often matched as closely as possible for potential confounding factors such as age, sex, lifestyle factors, working conditions, or living conditions. Also, the larger the number of individuals participating in the study, the easier it is to detect small differences between the exposed and control groups.

Recently, the technique of *meta-analysis* has been added to the tools of epidemiologists. This technique involves combining results from different studies in order to acquire the statistical power necessary to determine whether two groups (generally exposed and control) differ with respect to development of disease. A meta-analysis, however, is only as good as the data that it is based on, and there are many disagreements as to how to select which studies to include. Even in published papers, data are not always complete, and in fact, there may be some bias inherent in the pool of available published papers, as negative results may be less publishable than positive results.

Finally, one additional caveat must be kept in mind in terms of epidemiological studies (and, of course, of laboratory studies as well). This is the important concept that *correlation is not causation*. Even if a so-called risk factor is shown to be positively associated with an increased risk of disease, it does not necessarily mean that the risk factor causes the disease.

Risk Assessment and Risk Management

In the process of *risk assessment*, hazard is weighed against benefit as regulatory decisions are made concerning potentially toxic substances. The National Academy of Sciences/National Research Council published a report in 1983 outlining the steps involved in risk assessment and risk management. They identify four main components of the process of risk assessment: (1) *hazard identification*, where it is determined whether a substance is a potential health hazard; (2) *dose–response evaluation*, where the dose–response relationship is quantified; (3) *exposure assessment*, where potential exposure levels are estimated; and (4) finally, this information is merged in the process of *risk characterization*, where effects on the exposed population are estimated. Descriptions of risk are often phrased in terms of the chances of contracting a particular disease during a lifetime of exposure to a particular toxicant at a given level of exposure.

Risk assessment is then followed by *risk management*, which is the process by which regulatory decisions are made concerning health risks. Risk management takes not only risk assessment results but also other political, social, and economic factors into account when making decisions about regulating potential toxicants. Government agencies involved in risk management include the *Occupational Safety and Health Administration* (OSHA), the *Food and Drug Administration* (FDA), and the *Environmental Protection Agency* (EPA).