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Measuring Toxicity and Assessing Risk

Introduction

Toxicology is the science of poisons and has as its focus the study of the adverse effects of chemicals on living organisms. Although almost any substance in sufficient quantities (even water) can be a poison, toxicology focuses primarily on substances that can cause these adverse effects when administered in relatively small quantities. Knowledge of the relative toxicity of substances is fundamental to all applications of toxicology, from development of a new drug to the modeling of the effects of an environmental pollutant. This chapter describes approaches used by toxicologists to determine the toxicity of a substance. We consider principles of the dose vs. response relationship, methods used to evaluate toxicity in laboratory animals, and subsequent statistical analyses for quantitation of toxicity. We also discuss the use of toxicity data in assessing the risks of exposure to potentially hazardous substances.

Chemistry of Toxicants

Knowledge of the chemistry of a poison is of primary importance because it is a major determinant in the solubility and reactivity of the substance. Chemistry can dictate the *vehicle* used to administer the substance in testing, and can predict to some extent the duration of the test, as well as any potential products of biotransformation (metabolism). For small molecules, knowledge of chemistry is relatively simple to gain by examining the chemical formula and performing tests such as an octanol/water partitioning experiment to find the relative lipid solubility. However, the coming generation of drugs will be composed of many large polymeric compounds such as proteins and nucleic acids, which are more challenging to study chemically. There is also a trend to return to botanical products, which are often highly complex mixtures with multiple active ingredients. This can complicate the administration and interpretation of testing for toxicity.

Toxicity Testing Methods

A wealth of information can be gathered from toxicity testing by carefully observing animals during and following exposure. These data may provide evidence for the mode of action of the substance and provide clues as to which physiological system, organs, and tissues could be affected.

Although specific protocols for toxicity testing have been developed by various regulatory agencies (such as FDA and EPA), they share many characteristics in common. Of course, in any study, the handling and treatment of animals must be humane and must be the same for all animals in the study, whether they are in treated or control groups. Animals may be tagged for identification, using either simple numbered, metal *ear tags*, or more sophisticated devices such as *electronic transponding implants*. Animals must be housed in clean, comfortable conditions with access to adequate food and water. Typically, animals are housed in conventional, box-type cages, although in some cases specialized cages such as metabolism cages may be used. These cages are equipped with a separator for collection and measurement of urine and feces, so that consumption of food and water can be measured more accurately.

In a typical study, *body weight* of the test animals is measured either daily or periodically. Animals are observed for *behavior* (comparing behavior of treated with control animals) and *symptomology* (such as tremors or convulsions, for example). During the exposure period, animals are monitored closely for symptoms of poisoning, as well as timing of appearance of those symptoms, which might suggest the mechanism of poisoning of the substance. A slow onset of poisoning, for example, might suggest a *bioactivation* of the substance to a more toxic *metabolite*, or product, which accumulates as the *parent substance* is converted.

Following an exposure period, the animals are sacrificed and *necropsy* is performed. This is a procedure in which the treated and control animals are dissected and organs are weighed and examined for toxic effects in gross morphology and physiology. Sections of tissue samples may be sliced on a *microtome* and examined under the microscope for evidence of *histopathology* (which is any abnormality in cell or tissue). Tissue samples may also be analyzed for the presence of biochemical indicators of pathology.

Factors to Be Considered in Planning Toxicity Testing

There are several questions that must be answered in determining the toxicity of a chemical substance. Among these are:

1. Through what physiological route does exposure occur (in other words, how does the substance get into the body)?
2. How much of the substance is necessary to produce toxicity?
3. Over what period of time does exposure occur?

Toxicity testing attempts to answer these questions and thus provide practical information about the risks involved in exposure to potentially toxic compounds.

Routes of Exposure

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See also:
Toxicokinetics *Ch. 2, p. 16*

Various means of administration or *routes of exposure* are used in toxicity testing. Oral toxicity is of primary concern when considering a substance that might be ingested in food, such as the residue, or a pesticide or food additive, or taken orally as a drug. Dosing through the mouth is technically described as the *peroral* or *per os* (po) method. In some cases, the substance to be tested may be added directly to the animal's food or water. Alternatively, it may be dissolved in water, vegetable oil, or another *vehicle* (depending on the solubility of the test substance) and introduced directly into the esophagus or stomach through use of a curved needle-like tube (a process called *gavage*). Dermal administration may be considered for a substance that might be handled by workers, such as paints, inks, and dyes, or for cosmetics applied to the skin. The test substance is painted onto the skin, covered with a patch of gauze held with tape, and plastic is wrapped around the body to prevent ingestion of the substance. Finally, respiratory administration should be considered in testing industrial solvents or cosmetics applied in an aerosol spray.

Toxicity may also be assessed by direct injection of the substance, using a syringe and needle. *Intraperitoneal* (ip) *injections* are made into the body cavity; *intramuscular* (im) *injections* are placed into a large muscle of the hind leg; *subcutaneous* (sc) *injections* are placed just beneath the skin; *intravenous* (iv) *injections* are made directly into a large vein. Data derived from these injections are especially useful in estimating doses for investigations of drugs that eventually may be injected by an analogous method in human patients.

Determining the Responses to Varying Doses of a Substance

Several terms are used to describe levels of exposure to toxicants. The terms *dose* and *dosage* have been used nearly interchangeably, although dose commonly refers to the amount of a chemical administered, and dosage refers to the amount of chemical administered per unit body weight of the recipient. Thus, a dose of a drug might be expressed in milligrams, while the dosage would be expressed as milligram per kilogram of body weight. In toxicity

testing most chemical amounts are calculated and administered as dosages, which allows better standardization of the amount of chemical received, and allows a better basis for comparison of effects between individuals and species of widely varying body size. In respiratory exposures, exposure levels are usually measured by the concentration of the substance in the environment (in parts per million).

Quantitative toxicology involves challenging test animals with the substance to be evaluated, which is applied in an ordered series of doses. The dose is controlled by the toxicologist; therefore, it is considered to be the independent variable. Response of the animals may be measured in many different ways, and is generally dependent on the dose applied (i.e., it is the dependent variable).

Responses can be scored and related to dose in order to determine the dose vs. response relationship. One response considered in toxicology is the death of the animal. This is scored as a *quantal value*, alive (no response: 0) or dead (response: 1), and recorded as *mortality*. A dose producing mortality is a lethal dose of the substance. In other experiments, the observed response may be a *continuous variable* that can be measured in each subject. Examples of continuous variables include consumption of oxygen, time to onset of convulsions, degree of inhibition of an enzyme, and loss of weight.

A basic principle of toxicology is that response varies proportionally to a geometric, not arithmetic, increase in dose. This means that to test a substance that produces responses in a small proportion of animals at 1 to 2 mg/kg, a geometric dosing range (1, 2, 4, 8, and 16 mg/kg) would be used rather than an arithmetic range (1, 2, 3, 4, and 5 mg/kg). Because of this, graphs relating dose and response are generally plotted with the response value on the y-axis and the logarithm of the dose on the x-axis.

Timing of Exposure

Often, the first of many considerations in toxicity testing is to assess the acute toxicity of the chemical. *Acute toxicity* is the toxicity that results from a single exposure to the substance. Typically, animals are dosed with a single dose and then observed for up to 14 days. One example of an acute toxicity test is the LD_{50}, which will be discussed next in this chapter. *Subacute toxicity testing* measures the response to substances that are delivered through repeated or continuous exposure over a period that generally does not exceed 14 days; *subchronic toxicity testing* involves repeated or continuous exposure over a period of 90 days. The final category of exposure is *chronic toxicity testing*, which refers to repeated or continuous exposures that last for more than 90 days. To ensure sufficient challenge, animals are often exposed to the maximum tolerated dose, the greatest dose that neither kills nor causes incapacitating symptoms. While very high doses are used so that any chronic toxicity of the test compound will be observable, some experts consider that effects seen at large doses may be due to massive physical damage or *mito-*