

Principles and Methods of Toxicology

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tration of the substance in a hyperbolic function relationship. If the concentration of the substance at the receptor site is dependent on the dose, then the response is dependent on the dose administered. This phenomenon is perhaps the simplest version of the receptor kinetic concept related to the dose response of a chemical. The kinetics of the receptor-substrate interaction may be more complicated, and different dose-response relationships could be drawn based on these complicated kinetics. Readers who are interested in different receptor-substance kinetics are referred to a detailed discussion by Ferdinard (21).

The quantal dose-response relationship is often difficult to conceptualize based on the receptor theory. However, quantal response can also be viewed as a graded response if the whole population is considered as an individual. This relationship can be best explained in terms of a probability distribution. For a particular response, members of a population, for example, all the rats in the world, respond differently to a particular stimulus such as a chemical insult. Some rats will be highly sensitive while some will be very resistant. If these different responses are distributed normally within the population (i.e., with most members of the population neither extremely sensitive nor resistant), the well-known "bell shaped" population distribution curve results.

If the probability of dose response is expressed in terms of cumulative response, a sigmoidal curve can be obtained as shown in Fig. 1. However, most biological response distributions are not exactly normal and tend to be skewed to the higher dose; i.e., extreme resistants have a larger "range of dose" to respond than the extremely sensitive portion of the population. In general, a logarithmic dose transformation can normalize the distribution (i.e., convert the skewed distribution to a normal distribution) (Fig. 2). After this logarithmic dose transformation, if the probability of the log dose-response is expressed cumulatively, the sigmoidal response curve is obtained (Fig. 2). How is this log-normal transformation related to a regular dose-response curve? Is there justification or basis for a log-dose transformation? To answer these questions, let us again look at Eq. (3). This equation can be rearranged to

$$E = \frac{[S]}{(k_2/k_1) + [S]}$$

which also can be rearranged to

$$E = \frac{k_1 [S]}{k_2 + k_1 [S]} \quad (4)$$

Over a certain concentration range, Eq. (4) will produce a curve very similar to the loga-

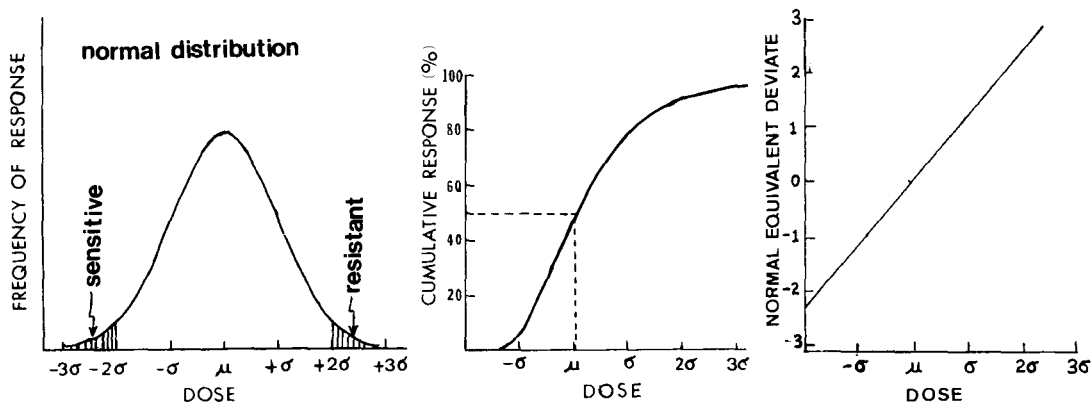


FIG. 1. Normal distribution of dose-response relationships: frequency of response, cumulative response, and cumulative response in terms of normal equivalent deviate.

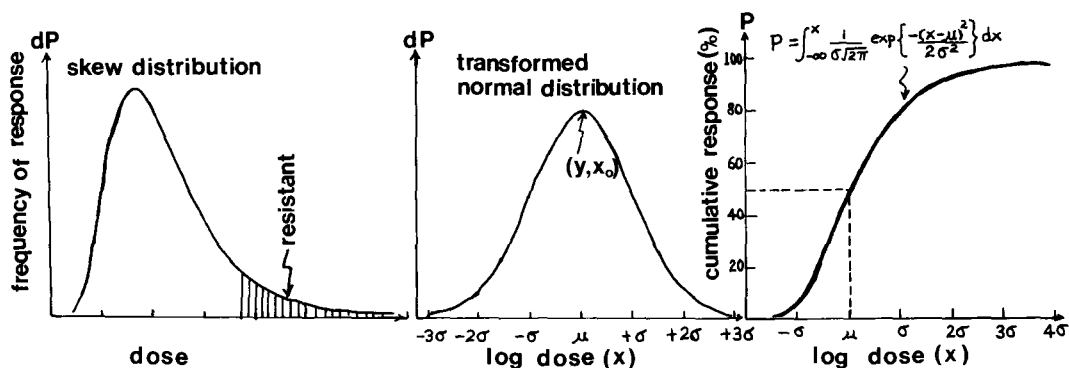


FIG. 2. Skew distribution of dose response can be normalized by log dose transformation.

rithmic function $E = k_1 \log (k_2[S] + 1)$ (8). Therefore, there may be justification for the log-transformation beside simply a mathematical convenience.

Since a sigmoidal curve is more difficult to analyze than a straight line, many experts feel that further transformation of the log-dose response hyperbolic function is necessary to obtain a "straight-line" function curve. Perhaps the most widely used transformation is the normal equivalent deviate (NED) or the similar Probit transformation (3,8–10,16,22,30). This technique involves the log-dose transform and the transformation of the cumulative response probability to the NED or Probit. After both the probability and the dose are transformed, their transformed values are directly related to each other. A brief derivation of this "straight-line" direct function relationship between the log-dose and NED or probit will be presented later in this chapter.

Median Lethal Dose (LD₅₀) and Its Determination

Definition

The LD₅₀, in its simplest form, is the dose of a compound that causes 50% mortality in a population. A more precise definition has been provided by the OECD panel of experts as "the statistically derived single dose of a substance that can be expected to cause death

in 50% of the animals" (34). In other words, an LD₅₀ of a compound is not a constant, as it has been treated by many toxicologists; rather, it is a statistical term designed to describe the lethal response of a compound in a particular population under some discrete set of experimental conditions.

Significance of the LD₅₀ Value

The numeric value of the LD₅₀ has been used to classify and to compare toxicity among chemicals. The extent of involvement of the LD₅₀ in safety evaluation has almost reached a level of abuse. Although determining the LD₅₀ under a set of experimental conditions can provide valuable information about the toxicity of a compound, the numeric LD₅₀ *per se* is not equivalent to acute toxicity. One must always remember that lethality is only one of many indices in assessing acute toxicity. The slope (response/dose) of the dose-response curve, the time to death, the pharmacotoxic signs, and the pathological findings are all vital or even more critical than the LD₅₀ in the evaluation of acute toxicity. Therefore, defining acute toxicity based only on the numeric value of an LD₅₀ is dangerous.

As pointed out in a previous paragraph, lethality is a quantal response, and the probability of a cumulative response is relative to dose in a hyperbolic (sigmoidal) function. The cumulative probability of response is directly

ded to the standard deviates of a log-dose population (Fig. 1). Therefore, the slope of the log dose-response curve will indicate the relationship between the change of dose and the lethal response. This relationship is perhaps more important in risk assessment than the numeric value of LD_{50} , because more insight about the intrinsic toxic characteristics of a compound is available. Sometimes, the slope can give a clue to the mechanism of toxicity. For example, a steep slope may indicate rapid onset of action or faster absorption. A large margin of safety is predicted when a compound has a flat slope, i.e., only a small increase in response with a large increase in dose. With the slope, it is often possible to extrapolate the response to a low dose, e.g., LD_{10} , LD_1 , or even to a no observe effect level. Knowing the slope is especially important when comparing a set of compounds. Two compounds may have identical LD_{50} values but different slopes and thus have quite different toxicologic characteristics depending on the range of doses. Parallel dose-response curves may indicate a similar mechanism of toxicity, kinetic pattern, and probably similar prognosis. However, neither the LD_{50} nor the slope can absolutely reveal a specific mechanism.

Determination of LD_{50}

Many methods are available for the determination of the LD_{50} . They can be grouped into two categories, the "normal-population assumption" and the "normal population assumption free" methods. The former usually can be analyzed by graphic procedures.

The "population normality assumption free" methods are represented by the Thompson's moving average interpolation (39,41) and the "up and down" method (6,7,12). The former method is widely accepted, and convenient tables (16) are available for estimation of the value of the LD_{50} with confidence limits when either zero or 100% mortality incidences are observed. However, there are some restrictions for using this method, i.e., four

doses at equal log-dose intervals and the numbers of animals per dose level must be equal. (The reader can find details of this method in Chapter 9). The "up and down" or the "Pyramid" method is designed to estimate the LD_{50} with a small number of samples. It has an economical advantage because fewer animals are needed, but the test may be time consuming and require excessive test material. Because of the advantage of using only a few animals, this method is popular when the test has to be conducted in large animals such as cows or sheep or expensive animals such as monkeys.

The "population normality assumption" method is represented by the "probit analysis" approach, which can either be by graphic means (27) or by mathematical calculations (22). Since the probit analysis is widely used in evaluating acute toxicity data, the principles will be discussed briefly. This method involves the transformation of both the cumulative response probability and the dose.

When the dose is transformed into a log dose (x), the frequency of response versus "log doses" follows a normal distribution (Fig. 2), which can be expressed mathematically as

$$dP = \frac{1}{\sigma\sqrt{2\pi}} \exp \left\{ \frac{-(x - \mu)^2}{2\sigma^2} \right\} \quad (5)$$

where σ^2 and μ are the variance and the mean of the population, respectively, and P is the probability corresponding to each value of x (Fig. 2). The LD_{50} is defined as the log dose that can produce 50% mortality in a population (i.e., $P=0.5$ or 50% cumulative response). Let x_0 be the log LD_{50} ; then $P=0.5$ will correspond to the area under the log-normal distribution curve from $-\infty$ to x_0 ; or $P=0.5$ will correspond to the intergration of Eq. (5) from $-\infty$ to x_0 : That is,

$$P=0.5 = \int_{-\infty}^{x_0} \frac{1}{\sigma\sqrt{2\pi}} \exp \left\{ \frac{-(x - \mu)^2}{2\sigma^2} \right\} dx \quad (6)$$

The solution of Eq. (6) is $x = \mu$, the "true mean" or the median of the log-normal distri-

when inhalation exposure is not expected to occur because of the physical properties of the chemical, inhalation toxicity testing may not be needed. Such a case is not uncommon if respirable particles cannot be generated even under the most favorable laboratory conditions. Nonetheless, for all practical purposes, oral, dermal, skin, and eye tests should be considered in the initial acute investigation. These four tests are often sufficient for regulatory purposes, although increasing concerns are also placed on the inhalation and skin sensitization studies.

Principles and Methodologies of Acute Oral Toxicity Studies

Principles

The test substance, undiluted or diluted with appropriate solvents or suspending vehicles, is given to several groups of animals by gavage with a feeding needle or by gastric intubation. A vehicle control group is included if needed but generally is not necessary if the toxicity of the vehicle is known. Clinical signs, morbidity, and mortality are observed at specific intervals. Animals that die or become extremely moribund during the study are subjected to necropsies. Animals that survive the test period are killed and necropsied at the end of the observation period. Tissues may be saved for histopathologic examination to facilitate the understanding of the acute toxicity of the compound. In order to increase the reproducibility of the study, all experimental conditions and procedures should be standardized, and the study should be conducted according to "generally recognized good laboratory practices" (18,32).

Animals

Species.

It has been documented that responses caused by a compound often vary greatly among different species. Ideally, all toxicity tests should, therefore, be conducted with an animal which will elicit compound related

toxic responses similar to those which occur in man, i.e., an animal which metabolizes the compound identically to man and has the same susceptible organ system(s). Under such ideal conditions, the animal data then may be extrapolated to man. Unfortunately, finding such an ideal animal is difficult, if not impossible.

A less ideal approach is to conduct acute toxicity studies in a variety of animal species under the assumption that if the toxicity of a compound is consistent in all the species tested, there is a greater chance that such a response may also occur in man. Even though the response in different species is not consistent, it is generally considered better to err on the safe side with the risk assessment being based on the most sensitive species unless there is justification that such responses are less likely to occur in humans, for example, because of dissimilarity in metabolism between the less sensitive animal species and man. While these are logical assumptions and generally quite reliable, the danger of underestimating or overestimating the responses in humans still exists. Therefore, there is no absolute criterion for selecting a particular animal species. However, priority should be given to species with metabolism or other physiological and biochemical parameters similar to man. Animal species should also be selected on the basis of convenience, economical factors, and the existing data base for the animal. Most commonly, rats, mice, rabbits, and guinea pigs are chosen for acute toxicity studies.

Other animal variations.

Acute toxicity even within a particular species can vary with health conditions, age, sex, genetic makeup, body weight, differences in absorption, distribution, metabolism, and excretion of the compound, and the influence of hormones (13). A conscious investigator should be aware of the possible interaction of chemical treatment with these parameters. For example, immature animals may lack an effective drug metabolizing enzyme system; this may contribute to the higher toxicity of the compound in the immature animal if an