Acute toxicity test with terrestrial animals

(a) Test Animals Generally, rats and mice are selected for determining the relative potency of various toxicants, because they are economical, readily available and easy to handle. In certain cases, (i) when the rate and pattern of biotransformation in human-beings is known to be significantly different from these rodents, and (ii) when LD_{50} for rats and mice are markedly different; some non-rodent species may be selected .

Sometimes, the tests are performed against insects to determine relative efficacy of some poisons (*e.g.* pesticides) later to be used for the suppression of their population. The LD_{50} values are often evaluated for both the sexes and also for adult and young animals because of differences in susceptibility. After selection of the test species, the individuals are acclimated to the laboratory conditions generally for one week prior to the beginning of the test.

(b) Route of Administration The organisms may be exposed to toxicants by any of the following routes:

(i) topically, where the test chemical is directly applied on certain parts of the body of organisms, *e.g.* dorsal thoracic region of insects,

(ii) the toxicant is mixed with the feed and fed to the organisms,

(iii) highly volatile chemicals are exposed in vapour form to evaluate inhalation toxicity,

(iv) the animal may be dipped in the test solution for a while and taken out, and

(v) toxicant may be administered by other parenteral routes, generally in the form of injections.

The mode of administration is often decided as per need and purpose of the test, test organism and nature of chemical whose toxicity is to be determined. Toxicants easily reach the blood circulation through parenteral routes and cause prompt action.

(c) ***Doses and number of animals*** For the evaluation of $LD_{50,}$ four or five doses of test chemicals are exposed to the test animals. The doses should be selected (after exploratory test) in such a way that there should be at least one dose which causes more than 50 % mortality, but less than 90% mortality and another dose which causes nearly 50% mortality, and a third dose preferably causing more than 10% mortality of test organisms.

For the more precision in LD_{50} value :

(i)there should be smaller ratio between successive doses, and

(ii) the number of individuals per dose should be more (*e.g.* 20).

Generally, 10-20 animals are exposed per dose of test chemical and 4 or 5 doses of a chemical are selected. For determining LD_{50} for large animals (such as dogs) generally much smaller number of them is used.

(d) Observation Following administration of test chemical in/on the body of organisms, changes in their normal behaviour are recorded. Mortality is recorded after every 24 hours until the end of the test. The litter and dead individuals are removed from the test media/cage at the earliest possible in order to avoid contamination.

The caging pattern, number of individuals per cage, environmental temperature, relative humidity are important characteristics affecting the toxicity of chemicals, besides certain parameters related to the organisms.

Calculation of LC_{50} or LD_{50}

On the basis of data obtained from careful observation of mortality (at different doses/concentrations) either from aquatic toxicity test or from tests on terrestrial animals, LC_{50} or LD_{50} values may be calculated by either of the two methods:

(a) Graphical interpolation method To calculate time dependent LC_{50} or LD_{50} values, a graph is plotted between the doses/concentrations of toxicant and percent mortality of organisms observed at each dose/concentration. The obtained curve is known as *dose-mortality curve* or *concentration -mortality curve* (17.1).

FIGURE 17.1 from page 204

Later, the exact dose or concentration is read at 50% mortality and this is reported as LD_{50} or LC_{50} for a particular exposure duration under certain set of laboratory conditions.

(b) Statistical method Based on the data obtained from acute lethality test, time-dependent LC_{50} or LD_{50} values and their 95% confidence limits can be calculated by any of a variety of statistical methods (Litchfield and Wilcoxon, 1949; Goulden,1959; Swaroop, 1957; Finney, 1971). But, most widely used methods are probit analysis method (Finney, 1971) and Litchfield and Wilcoxon's method. In probit analysis method, the logarithmic values are read for each concentration/dose exposed and probit values are read for the percent mortality. Then a graph is plotted between logarithmic doses/concentrations and the probit mortalities. The curve so obtained is termed as *probit-mortality curve* (Figure 17.2)

FIGURE 17.2 from page 205

From this curve, the values of expected probits are read and then corrected probits are calculated by the formula given by Finney (1971) to finally calculate LC_{50} or LD_{50} values after a number of steps. In case of mortality in control groups, the data are corrected by Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\%\text{ test mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}} \times 100$$

2. CHRONIC TOXICITY TEST

Chronic toxicity test is designed to investigate the effects of exposure to a chemical over prolonged periods either to entire life-cycle or to a particular stage or to most sensitive stage. Chronic toxicity tests are helpful in: (i) understanding the mechanism of toxicity of chemicals, (ii) deriving MATC, no observed effect levels and presumable safe levels of toxicants, and (iii) assessing the most sensitive stage in the life-cycle of an organism.

Chronic toxicity test is designed to examine the effect of long-term exposure of animals to selected chemicals. The exposure of chemical is determined on the basis of acute toxicity data. The animals are then subjected to selected levels of toxicants for prolonged periods (normally more than 3 months) and desired parameters are investigated after certain period of exposure. The basic objectives of chronic toxicity tests may be: (i) To study adverse effects of chemicals on the structures and functions of organs, tissues and cells after prolonged periods of exposure. (ii) To study the secondary effects of toxicants, such as carcinogenicity, teratogenicity and mutagenicity. (iii) To evaluate no effect levels or safe levels of toxicants.

As the chronic exposures cover prolonged periods of animal life, these tests indicate the doses or concentrations of toxicants that interfere with normal growth, development and attainment of reproductive potential of test organisms. Generally, the doses/ concentrations of toxicants producing chronic effects are lower than those producing more readily observable acute effects, such as mortality of organisms. Therefore, chronic toxicity tests provide a more sensitive measure of toxicity of chemical than acute toxicity tests.

The basic test design is similar to acute toxicity tests. The selection of animal, test chemical, exposure system, mode of administration or exposure may be decided much in the same way. The dose/concentration of chemical aimed to be exposed to test animals for long -term exposure are much lower than acute dose/ concentration. Since chronic toxicity tests include prolonged periods of exposure, in case of static exposures, the test solutions are normally replaced after every twenty four hour by the respective concentrations of test chemicals. The feeding is also necessary during long-term experiments and uneaten food materials are immediately taken out from the test solutions in order to avoid contamination. In case of exposure of terrestrial animals, the exposed doses of toxicants are repeated after certain interval.

After desired periods of exposure, the desired parts of the body or tissues are taken out to examine alterations in selected parameters following standard and established methods. Generally, three categories of tests are commonly used to predict the chronic effects of toxic chemicals on aquatic organisms.

(i) The toxicants are exposed for the whole life cycle of test organisms to measure the effects of chronic exposure to a chemical on survival, growth, reproduction and other parameters over one or more generations of a population of test organisms.

(ii) Tests on most sensitive life-stages are performed to examine the effects of chronic exposure to a chemical on survival and growth of toxicologically most sensitive life-stages of a species.

(iii) Effects of toxicants on various physiological parameters of test organisms are studied in functional tests.

The data from all these tests are used to estimate chronic toxicity thresholds.

(i) Life-cycle test In these tests, groups of test animals are exposed to a series of concentrations of toxicant. The test is initiated either with eggs, larvae or juveniles and continued until the test organisms reproduce. It may continue for several generations as per objectives of the test. The concentration of toxicants may range from those having significant adverse effects on survival, growth and reproduction to at least one causing significant adverse effects on these parameters. The test species are limited to those who can complete their life-cycle under laboratory conditions.

(ii) Tests with sensitive life-stages The life-cycle toxicity test involves considerable time and expense, especially in case of vertebrates. Therefore, the test methods have been developed to perform experiments on most sensitive life-stages of test organisms to predict chronic toxicity thresholds. Generally, the early life-stages of fish are more sensitive to test chemicals, hence these stages are selected for this type of test. Apart from saving time and expense, these tests can be performed with a large number of species than life-cycle tests. Thus, the estimates of these tests can be applied for a much wider variety of species from wide range of habitats and trophic levels as compared to life-cycle toxicity tests. The estimates of such tests do not differ significantly from those estimated from life-cycle toxicity tests (Mc Kim, 1985). Thus, these tests are reliable also.

(iii) Functional test The organisms respond to sub-lethal levels of toxicants physiologically and behaviourally. These organismic responses may be ascribed to: (a) the functional responses occur, which as a result of action of toxicants, and / or (b) the functional responses may occur as adaptive responses of organisms to the toxicants exposed.

These functional responses (sub-lethal effects) may be studied in the laboratory employing a variety of analytical procedures. The functional responses of organisms observed following exposure to sub-lethal levels of toxicants are generally divided into: (i) Behavioural responses, (ii) Physiological responses, (iii) Biochemical responses, and (iv) Histopathological responses.

The behaviour represents in integrated response of test organisms consequent upon complex biochemical and physiological changes. Toxicants induced behavioural changes indicate their effects on internal homeostasis. The behavioural end-points may thus be used as sensitive indicators of sub-lethal effects of toxicants. The common behavioural responses of aquatic organisms receiving considerable attention are locomotion and swimming, attraction-avoidance, predator-prey relationship, aggression, territoriality and learning. To record the behavioural responses of organisms separate tests are performed which are termed as behavioural tests. Detailed account on behavioural tests has been given later in this section.

The commonly studied physiological and biochemical responses are respiration and oxygen consumption, studies on the activity of enzymes, clinical chemistry and haematology. The histological changes may significantly modify the function of tissues and organs. Thus, histological studies following chronic exposures to toxicants are also useful. Hence, it may be inferred that the functional tests employing behavioural, physiological, biochemical and histological parameters are on one hand useful for

evaluating the environmental hazards of chemicals and on the other hand, they may provide valuable information on the mode of action of toxicants.

Maximum Acceptable Toxicant Concentration (MATC)

The chronic toxicity tests are also useful in determining the maximum acceptable toxicant concentration (MATC) and presumable sale concentrations. *MATC is threshold concentration that produces statistically significant adverse effects, but for the death of the organisms exposed.* It is a hypothetical concentration and is bounded by a range at lower end by the highest concentration in the chronic test, which produces no effect (no observed effect concentration, NOEC) and at higher end by the lowest concentration that produces statistically significant effect (lowest observed effect concentration, LOWC). MATC may thus be represented as:

NOEC < MATC < LOEC

The concept of application factor (AF) has been proposed with an aim to correlate acute toxicity and chronic toxicity of chemical to aquatic organisms. It is *unitless* and chemical specific value derived by dividing chronic toxic concentration with that of acute toxic concentration. In practice, application factor is calculated by dividing the limits (NOEC and LOEC) of MATC by 96 hour LC_{50} obtained from acute toxicity test. The application factor is reported as a concentration range. For example, if MATC range is >0.5-<1.0 mg/l and 96 h-LC_{50} is 10 mg/l,

$$AF = \frac{MATC}{96h\ LC_{50}} = \frac{\geq 0.5 - < 1.0\ mg/l}{10\ mg/l} = 0.05 - 0.1$$

The application factor is chemical specific, its value determined for one species may be applied to other species as well. This hypothesis has been considered very useful. Consequently, application factor may be used to derive estimates of chronic toxicity without performing chronic toxicity tests. This saves time and cost of chronic toxicity tests.

The application factor determined for a chemical from acute and chronic toxicity tests against one species will be the same for other species. Thus, by performing only acute toxicity tests, chronic toxicity thresholds (*e.g.* MATC) for another species may be estimated on the basis of following formula:

$$MATC = AF \times 96h\ LC_{50}$$

The acute and chronic toxicity data including the concept of application factor may be of immense use in deriving presumably safe levels of toxicants. The presumable safe levels of toxicants may be calculated on the basis of formula proposed by Hart *et al.* (1946) given as under :

$$C = (48\ h\ LC_{50} \times AF) / S^2$$

where C = presumable safe concentration of toxicant, AF = application factor, and $S = 24\ h\ LC_{50} / 48h\ LC_{50}$.

In recent approaches, the concept of application factor has been replaced by short-term, early life-stage or more sensitive stage tests, which provide estimates of chronic toxicity of chemical without performing chronic tests. The tests with early life-stages are useful in predicting the chronic toxicity of a number of chemicals against a wide variety of organisms.