II. TOXICITY TESTS AGAINST HIGHER ANIMALS

The aim of toxicity determination in higher animals is to evaluate safety limits to man. For this purpose generally rats, mice, pigs and rabbits are taken as test animals. While selecting the test animals it should

be kept in mind that the animals must be healthy and of genetic composition. They should also be of same sex, age, level and rearing conditions.

The chemicals which are evaluated for determination of toxicity are generally classified according to their LD_{50}.

Extremely toxic – LD50 less than one
Highly toxic – 1 to 50
Moderately toxic – 50 to 500
Slightly toxic – 500 to 5000
Practically non–toxic – 5000 to 15000
Relatively harmless – more than 15000

1. Acute toxicity:

Acute toxicity tests are conducted to obtain data on the following aspects:

i. Acute oral toxicity:

There are four major methods of oral administration of toxicant (a) adding toxicant to the diet of the animal (b) application of toxicant directly into the stomach with the help of a stomach tube or in capsule (c) intravenous injection and (d) intraperitoneal injection. In injection method 15–20% vegetable oil or polyethylene glycol alongwith emulsifier and isotonic saline are mixed with toxicant. In no case the volume should exceed 0.5 ml. The speed of injection should also be slow.

ii. Acute dermal toxicity:

Dermal toxicity test is important in order to test the safety of toxicant for workers. In this test the toxicant is applied on the shaved area of the test animal around the abdomen or back which is then covered by cotton gauze.

(iii) Acute inhalation toxicity:

The tests for inhalation toxicity are conducted by two methods:

a. the animal is kept in a closed chamber and is exposed to toxicant in the form of sprays of aerosols.

b. the test animal is kept in a chamber which is constantly supplied with an air current carrying the toxicant. According to Federal Hazardous Substance Act inhalation toxicant is a substance that produces death within 14 days in half of a group of white rats when in-

haled continuously for a period of one hour or less at an atmospheric concentration of more than 200 ppm but not more than 2000 ppm or more than 2 mg but not more than 200 mg/litre of mist or dust.

2. Chronic toxicity

There are three objectives for studying chronic toxicity: True chronic toxicity effect on the organs and tissues, secondary effects such as carcinogenic (liver carcinoma), teratogenic and mutagenic (chromosomal aberrations) and no effect.

In true chronic toxicity study the purpose is to find out any undesirable and harmful effect on the test animal such as illness, gross morphological and physiological changes. To study the secondary effects the period of administration is kept 3 to 18 months and the doses are chosen in such a way that they should be slightly higher than those which are expected to be present on food stuff for human consumption. The 'no effect' tests are conducted in the same manner as in the case of true and secondary chronic effects but on a very sensitive animal species. It is based on the assumption that human susceptibilities to toxicants are equivalent to those found in most sensitive animal species.

i. Tolerance: It is defined as the maximum concentration in ppm of pesticide residue that is permitted in or on food at a specified stage of the harvesting, storage, transport, marketing or preparation of food upto the final point of consumption. The tolerance is infact never greater than permissible level. When a chemical is safe it would not require any tolerance. On the other hand, if the residue of any pesticide has carcinogenic effect on the organism then no residue of such pesticide will be allowed i.e. the tolerance for such pesticide will be zero. The term has now been replaced by Maximum Residual Limit.

ii. Food factor: It is average fraction of total diet made by the food or class of food under question. For example, if the total food consumed by a man per day is 2 kg. and the treated food (containing 1 ppm pesticide) be 500 gm the food factor will be $500 \div 2000 = 0.25$.

iii. Acceptable Daily Intake (ADI): It is the daily dose of a chemical which during an entire life-time, appears to be without appreciable risk on the basis of all facts known at that time. It is expressed in mg/kg body weight/day.

iv. Maximum Residual Limit (MRL): The MRL is the concentration of a residue in or on a food when first offered for consumption. It is expressed in ppm of fresh weight of the food and is calculated from the

(i) food factor (ii) acceptable daily intake and (iii) average weight of the consumer.

For example if the total food (TF) consumed by a man per day is 2 kg. in which the contaminated food (CF) is 0.5 kg., pesticide present in contaminated food (PCF) is 1 ppm ADI is 0.1 kg/kg/day and the body weight of man is 70 kg., then the MRL is calculated as follows:

$$\text{Food Factor (FF)} = \frac{CF}{TF}$$

$$= \frac{0.5}{2}$$

$$= 0.25$$

Total daily intake of pesticide = PCF x FF x TF
= 1 x 0.25 x 2
= 0.5 mg.

Average body weight of man = 70 kg.

Daily intake of pesticide in mg/kg = 0.5 ÷ 70
= 0.007 mg/kg/day

Since the daily intake is less than AI (0.1 mg/kg/day) it shows that the quantity of contaminated food taken by man daily will not harm at 1 ppm level of pesticidal contamination. The MRL is the level upto which this pesticidal contamination of 1 ppm can be raised in the food so that the daily intake of pesticide (in this case –0.007 mg) does not exceed the ADI. From ADI the quantity of pesticide/day/man is calculated as follows:

ADI = 0.1 mg/kg/day

ADI/day/man = 0.1 x 70
= 7 mg

$$\text{Maximum Residual Limit} = \frac{\text{ADI/day/man}}{CF} = \frac{7}{0.5} = 14 \text{ ppm}$$

$$= \frac{7}{5,00,000} \times 10,00,000$$

= 14 ppm

In this case the pesticidal contamination can be raised from 1 ppm to 14 ppm without any harmful effect to man provided that the

quantity of contaminated food in the daily diet of the man remains 500 gm.

Since the MRL can not be fixed on the basis of experiment conducted on human beings, the information has to be obtained from the results based on test animals. Hence, a safety factor, ideally 100 fold is incorporated while fixing MRL for each food commodity.

The situation is made more comfortable by the following considerations:

(a) The MRL is based on the assumption that a pesticide is used on the whole quantity of food in question, whereas this will rarely be the case.

(b) A substantial loss of residue will occur during storage, processing, cooking thus leading to much more lower level at the time of consumption.

(c) The MRL is related to the level of ADI of the pesticide in question and the value is assessed on the assumption that the residue will be consumed daily over the entire life time.