Chapter IV. Guidelines for Toxicity Tests

IV C 2. Acute Oral Toxicity Tests

Acute toxicity tests can provide preliminary information on the toxic nature of a material for which no other toxicology information is available. Such information can be used to:

- deal with cases of accidental ingestion of a large amount of the material (*e.g.*, for poison control information);

- determine possible target organs that should be scrutinized and/or special tests that should be conducted in repeated-dose toxicity tests; [1] and

- select doses for short-term and subchronic toxicity tests when no other toxicology information is available.

In most acute toxicity tests, each test animal is administered a single (relatively high) dose of the test substance, observed for 1 or 2 weeks for signs of treatment-related effects, then necropsied. Some acute toxicity tests (such as the "classical" LD_{50} test) are designed to determine the mean lethal dose of the test substance. The median lethal dose (or LD_{50}) is defined as the dose of a test substance that is lethal for 50% of the animals in a dose group. LD_{50} values have been used to compare relative acute hazards of industrial chemicals, especially when no other toxicology data are available for the chemicals. However, many important observations of toxicity are not represented by LD_{50} values or by slopes of dose-response curves for lethality. For example, information about morbidity and pathogenesis may have more toxicological significance than mortality, and these endpoints also should be evaluated in short term toxicity tests.

The Agency does not recommend that petitioners determine the median lethal dose (or LD_{50}) for direct food additives or color additives used in food. However, if a petitioner decides to conduct an acute oral toxicity test, alternative test protocols can provide useful information about the acute toxicity of a substance.[3] These protocols generally use fewer animals, and are thus more cost efficient, than tests designed to determine LD_{50}s.[2] The following guidelines should help the petitioner design acute oral toxicity tests when the petitioner has decided that such information is useful:

- The main focus of the acute toxicity test should be on observing the symptoms and recovery of the test animals, rather than on determining the median lethal dose (LD_{50}) of the substance.

- The rat often is used as the animal model in acute toxicity tests, but other species also may be used.

- Often only one sex is studied in an acute toxicity test; generally, the female is assumed to be more sensitive to the acute toxic effects of chemicals than the male.[1]

- Before deciding on the dose of a test compound that will be used in studying its acute toxicity, the compound's chemical and physical characteristics (including molecular weight, partition coefficient, and the toxicity of related chemicals) should be considered; otherwise, oral toxicity--including lethality--caused by relatively large doses of a chemical may have no biological relevance to the chemical's effects at lower doses. [1,5]

The following brief descriptions of oral toxicity tests may help the petitioner choose a test that meets his needs; detailed information about each type of test is available in the referenced material.

a. Limit Tests

To determine the acute toxicity of a new food additive that is not expected to be particularly toxic, 5 gm (or ml) of the compound/kg body weight of the test animal should be administered orally by gavage to several (perhaps 5) animals that have been fasted (overnight for rats, 4 hours for mice). Test animals should be observed closely for up to 14 days; symptoms of toxicity and recovery should be noted. Gross and histopathological examination of the test animals at the end of the study may help identify toxic effects on target organs. If no animals die as a result of this dose, there is no need to test higher dosages. The acute toxicity of the compound can then be expressed as being greater than 5 gm (or ml)/kg body weight of the test animal. This method is called the "limit test." In general, 5 gm or 5 ml of the test substance/kg body weight is the practical upper limit for the amount of test material that can be administered in one oral gavage dose to a rodent.

If there are deaths following administration of an acute dose of 5 gm/kg body weight, then a lower dose should be administered to several (perhaps 5) animals and the results evaluated as discussed above. For compounds expected to be acutely toxic at 5 gm/kg body weight, it would be wise to select a lower initial "limit" dose.

b. Dose-Probing Tests

Dose-probing acute toxicity protocols may have value when the petitioner has no preliminary information about the test substance that would help him select appropriate doses for toxicity studies. In a dose-probing acute toxicity test, one animal per each of 3 widely spaced dosages should be used and a sufficient observation period should follow administration of the doses. Subsequent toxicity studies may be based on the results of the dose-probing study. [1] Variations of dose-probing acute toxicity studies are described in the literature. [6,7] Other methods of determining appropriate doses for longer-term toxicity studies include a simple test wherein 3 or 4 doses are each administered to 1 or 2 test animals and the animals are observed for up to 14 days. If some of the animals die, one can estimate an approximate median lethal dose, termed ALD. [8]

c. Up-and-Down Tests

The "up-and-down" procedure involves dosing animals one at a time: First one animal at one dose, then another animal one or two days later at a higher dose (if the first animal survives) or a lower dose (if the first animal dies). This process continues until the approximate LD_{50} has been determined. One disadvantage to this test is the length of the study. Each animal should be observed for at least seven days after dosing so that delayed deaths can be recorded. However, this method usually requires only six or eight test animals as compared with the 40 to 50 test animals that may be used in the "classical" LD_{50} test. [9-11]

d. Pyramiding Tests

Pyramiding studies involve a minimum number of animals: Two animals are given successively increasing doses of the test substance on alternate days until an acutely toxic dose or some practical upward limit is reached. This test does not yield a lethality curve and often is used to assess acute toxicity in non-rodents. This test, although more like a short-term, repeated dose toxicity study than a true acute toxicity study, can provide useful preliminary information on the toxic nature of a new material for which no other toxicology information is available.

References

1. Gad, S.C. and Chengelis, C.P. (1988) In, Acute toxicity testing perspectives and horizons. (S.C. Gad and C.P. Chengelis eds.) The Telford Press. Caldwell, N.J. *pp. 2-4[a], 318[b], 156[c], 165-167[d], 159[e].*

2. Food and Drug Administration [FDA] (1988) LD_{50} Test Policy. *Fed.Reg. 53 (October 11 Issue):39650-39651*.

3. Litchfield, J.T., Jr., Wilcoxon, F. (1949) A simplified method of evaluating dose-effect experiments. *J.Pharmacol.Exp.Therap. 96:113*.

4. Anonymous. (1989) Comments on the LD_{50} and acute eye and skin irritation tests. In, a position paper reported by the Animals in Research Committee of the Society of Toxicology (SOT) and approved by the SOT Council. *Fund.Appl.Toxicol. 13:621-623*.

5. Zbinden, G. and Flury-Roversi, M. (1981) Significance of the LD50 test for the toxicological evaluation of chemical substances. *Arch.Toxicol. 47:77-99*.

6. Lorke, D. (1983) A new approach to practical acute toxicity testing. *Arch.Toxicol. 54:275-287*.

7. Schutz, E. & Fuchs, H. (1982). A new approach to minimizing the number of animals used in acute toxicity testing and optimizing the information of test results. *Arch.Toxicol.51:197-220*.

8. Deichmann, W.B. and LeBlanc, T.J. (1943) Determination of the approximate lethal dose with about six animals. *J.Indust. Hygiene Toxicol. 25:415-417*.

9. Bruce, R.D. (1985) An up-and-down procedure for acute toxicity testing. *Fund.Appl.Toxicol. 5:151-157*.

10. Gad, S.C., Smith, A.C., Cramp, A.L., Gavigan, F.A., and Derelanko, M.J. (1984) Innovative Designs and Practices for Acute Systemic Toxicity Studies. *Drug Chem.Toxicol. 7:423-434*.

11. Muller, H. and Kley, H.P. (1982) Retrospective study on the reliability of an "approximate LD_{50}," determined with a small number of animals. *Arch.Toxicol. 51:189-196*.