Chapter IV. Guidelines for Toxicity Tests

IV C 7. Combined Chronic Toxicity/Carcinogenicity Studies with Rodents

Carcinogenicity studies (bioassays) in two rodent species (usually rats and mice) are recommended for substances in Concern Level III (see **Chapter IV C 6**). One of the carcinogenicity studies (preferably in rats) should be combined with a chronic rodent toxicity study into a single, effective long-term study. Guidelines for the combined study are described in this chapter.

The Agency acknowledges that it is sometimes difficult to set appropriate dose levels for a combined chronic toxicity/carcinogenicity study with rodents. However, when pre-chronic studies permit reasonable estimates of toxicity in longer-term studies, the combined approach is recommended.

Unless specific exceptions are noted below, general recommendations for toxicity studies (see **Chapter IV B 1**) and for reporting the results of toxicity studies (see **Chapter IV B 2**) apply to combined chronic toxicity/carcinogenicity studies with rodents.

a. Experimental Animals

i. Age

In carcinogenicity studies without *in utero* exposure, dosing of rodents should begin as soon as possible after weaning and acclimation, and before they are 6 weeks old. In carcinogenicity studies with *in utero* exposure, dosing of rodents should begin at weaning.

ii. Species and Strains

In selecting rodent species and strains for combined chronic toxicity and carcinogenicity studies, it is important to consider the test animals' general sensitivity to carcinogenic chemicals and the responsiveness of particular organs and tissues of test animals to carcinogenic stimuli. Preference should generally be given to species and strains with low incidences of spontaneous tumors.

At this time, there is no scientific basis for selecting among inbred, out-bred or hybrid rodent strains for carcinogenicity studies. Instead, the important consideration is that test animals come from well-characterized and healthy colonies. A thorough understanding of the normal patterns of tumor development (background tumor incidence) throughout the lifespan of untreated test animals (historical and concurrent controls) is critical to the evaluation of the results of combined chronic toxicity and carcinogenicity studies in rodents. It should be noted that strains that are not inbred often have unpredictable background tumor incidences. Because recent information suggests there is decreased survivability for some strains of rats (see **Chapter IV C 6 a**), test animals should be selected that are likely to achieve the recommended duration of this study.

Rats generally are used for combined chronic toxicity and carcinogenicity studies; however, other rodent species may be used. If possible, the strain selected for this study should be susceptible to the carcinogenic or toxic effects of the class of substances to which the test compound belongs, unless the background tumor incidence in that strain is so high that a meaningful assessment of the effects of the test substance could not be made.

iii. Number and Sex

Ideally experimental and control groups should have a sufficient number of animals at the beginning of the experiment to ensure that at least 25 rodents per sex per group survive to the end of the study. [Additional information on the subject of survivorship is contained in **Chapter IV C 6 a**.]

Satellite groups of test animals should be used to evaluate the chronic toxicity of the test substance; satellite experimental and control groups should consist of at least 10 rodents per sex.

If interim necropsies (other than those involving satellite animals) are planned, the total number of rodents of each sex per group should be increased by the number scheduled to be killed before completion of the study; at least 10 rodents per sex per group should be available for interim necropsy.

b. Administration of the Test Substance

i. Duration of Testing

Animals should be exposed to the test substance 7 days per week for 104 consecutive weeks (two years). If an *in utero* phase is added to this study, duration of dosing should be 104 consecutive weeks (two years) post-weaning.

Satellite groups of test and control animals used to assess the chronic toxicity of the test substance should be retained in the study for at least 12 months (one year).

ii. Dosed Groups

Information from subchronic toxicity studies should be used to identify dose levels of the test substance for combined chronic toxicity and carcinogenicity studies. At least three dose levels should be used (one dose level per group). No dose used in these studies should cause an incidence of fatalities high enough to prevent meaningful evaluation of the data from the studies.

a) Assessment of the Carcinogenicity of the Test Substance:

High Dose: The high dose should be the maximum tolerated dose (MTD). [Additional information on the selection of the MTD is discussed in **Chapter IV C 6 b**.

Low Dose: The low dose level should not interfere with normal growth, development, and lifespan of test animals, nor should it produce any other signs of toxicity. In general, the low dose should not be less than 10% of the high dose.

Intermediate Dose: The intermediate dose level should be between the high and low doses of the test substance. The exact dose chosen as the intermediate dose may depend on the pharmacokinetic properties of the test substance.

Optional Fourth Dose: If significant differences exist in the pharmacokinetic or metabolic profiles of the test substance administered at high and low doses, an optional (fourth) dose level may be included in the study. This dose level should be the highest dose that produces a pharmacokinetic or metabolic profile similar to profiles obtained at low doses. The number of test animals in the optional group should be selected to provide approximately the same sensitivity for the detection of carcinogenic effects as the high-dose group provides.

b) Assessment of the Chronic Toxicity of the Test Substance: Satellite control and dosed groups are included in the study to assess the chronic toxicity of the test substance. The highest dose for satellite

animals should produce toxicity so that a toxicological profile of the test substance can be obtained. The lowest dose level for satellite animals should not cause any toxicity.

c. Observations and Clinical Tests

i. Observations of Test Animals

Body weight should be recorded weekly for all test animals throughout the study. Food consumption (or water consumption if the test substance is administered in the drinking water) should be measured every week during the combined chronic toxicity/carcinogenicity study; petitioners also should attempt to quantify spillage of food by test animals. Petitioners should use this information to calculate intake of the test substance for each week of the combined study.

ii. Clinical Testing

Ophthalmological Examination: This examination should be performed on all animals at the beginning of the study, every three months thereafter, and at the end of the study.

Hematology: Erythrocyte counts and total and differential leukocyte counts for all test animals in the principal experimental and control groups should be made before dosing, at 3, 6, 12, and 18 months during the study, and immediately prior to terminal necropsy.

Hematology tests also should be conducted on all rodents in the satellite groups of experimental and control animals. Hematology samples should be taken before dosing, at 3-month intervals during the study, and immediately before interim necropsy.

Clinical Chemistry: Clinical chemistry tests should be performed on at least 10 animals per sex in each principal experimental and control group before dosing, at 3, 6, 12, and 18 months during the study, and at the end of the study.

Clinical chemistry tests also should be conducted on all rodents in the satellite groups of experimental and control animals. Blood samples should be taken before dosing, at 3-month intervals during the study, and immediately before interim necropsy.

Urinalyses: Microscopic analysis of urine sediment and determination of specific gravity of urine samples are recommended before dosing, at 3, 6, 9, 12, and 18 months during the study, and at the end of the study. These tests should be performed on at least 10 animals of each sex in each principal experimental and control group in the study.

Urinalyses should also be conducted on all rodents in the satellite groups of experimental and control animals. Urine samples should be collected before dosing, at 3-month intervals during the study, and immediately before interim necropsy.

d. Necropsy and Histopathology Examination

See **Chapter IV B 1 e** for appropriate tissues and organs.