Chapter IV. Guidelines for Toxicity Tests

IV C 8. *In Utero* Exposure Phase for Addition to Carcinogenicity Study with Rodents

An *in utero* exposure phase should be added to one of two recommended carcinogenicity studies with rodents (see **Chapters IV C 6** and **7**). In general, the *in utero* phase should be added to the carcinogenicity bioassay with rats, because the rat is the recommended species for reproduction studies (see **Chapter IV C 9**) and the Agency has a larger database on carcinogenicity bioassays with *in utero* exposure in rats than in mice. The Agency recommends including an *in utero* exposure phase in carcinogenicity bioassays for direct food additives and color additives used in food because human fetuses will generally be exposed to these additives during *in utero* development.

a. Experimental Animals

i. Species and Strain Selection

This guideline is for use with the rat or mouse; if other species are used, modifications of this guideline will be necessary. Strains selected should not have low fecundity and should be sensitive to teratogens and embryotoxins.

ii. Age

All test and control parental animals should be weaned and acclimated before treatment begins.

iii. Number

The number of animals per sex recommended in the guideline to which the *in utero* phase is to be added should serve as a guide for determining the number of animals/group for mating. One male and one female per litter is preferred; no more than two males and two females per litter should be included in any group. For example, if the petitioner decides that each group in the combined chronic toxicity/carcinogenicity bioassay should contain 70 animals per sex, at least 70 litters/group should be produced in the *in utero* phase. Thus, for this example the number of parental animals per sex for the *in utero* phase should be sufficient to ensure at least 70 litters per group.

iv. Caging and Animal Maintenance

Animals should be single-caged for this phase, except during mating and lactation. Food and water should be provided ad libitum. The animals' diet should meet all nutritional requirements to support pregnancy in the test species. Special attention should be paid to diet composition when the test material itself is a nutrient, because such material may have to be incorporated into the diet at levels which may interfere with normal nutrition. Under these circumstances, an additional control group fed basal diet may be necessary.

b. Dose Selection, Treatment Period, and Method of Dosing

i. Duration of Treatment

The parental animals (P) should receive the test substance for a minimum of four weeks prior to mating. Exposure should be continued throughout pre-mating, mating, gestation, and lactation until weaning of the F_1 animals.

ii. Route of Administration

The test compound or vehicle should be administered using the route which most closely approximates the pattern of human exposure (diet or drinking water). Oral intubation (gavage) may be appropriate in instances where human exposure is via a bolus dose or when it is essential for the animal to receive a specified amount of the test compound. The use of gavage may also be required when analysis of the agent in the diet is not possible, when the agent is not stable in the diet, or when the agent is not palatable. The maximum volume of solution that can be given by gavage in one dose depends on the test animal's size; for rodents, this should not exceed 1 ml/100 g body weight. If the test substance must be given in divided doses, all doses should be administered within a 6-hour period.

iii. Selection of Dose Levels

In general, the doses selected should be those that are recommended in the guideline to which the *in utero* phase has been added. However, as a result of maternal or fetal toxicity, it may be necessary to use lower doses during the *in utero* phase of chronic feeding studies in order to produce sufficient offspring for the post-weaning phase. Data justifying this protocol modification should be provided; it is strongly recommended that selections of doses for *in utero* phases of chronic feeding studies be based on the results of pilot studies. Results from metabolism and pharmacokinetic studies should also provide guidance in selecting an appropriate dosage regimen.

iv. Mating Procedures

For each mating, one or two females should be placed with one male. The following morning, each female should be examined for the presence of sperm in the vaginal lavage or the presence of a sperm plug. The day when sperm are found is considered day 0 of gestation. Sibling matings should be avoided.

v. Standardizing the Number of Pups per Litter

Standardization of the number of pups per litter through culling is optional. Litters may be standardized to 10 or 8 based on historical litter size for the strain. It is recommended that standardization be performed on postnatal day 4 by reducing all litters of more than 10 (or 8) to 10 (or 8) in a random manner. If possible, the retained litter-mates should consist of equal numbers of males and females; excess males or females should be randomly selected out. Random selection is important to guard against the human tendency to keep the most fit animals in the study.

vi. Selection of F_1 Animals

One animal per sex per litter should be randomly selected.

c. Clinical Observations

i. Parental Animals

Parental animals should be observed carefully at least twice daily. Relevant behavior changes and all signs of toxicity, including mortality, should be recorded. Dams should be weighed immediately before the first dose of the test compound is administered, and weekly during gestation and lactation.

Optimally, animals should be weighed daily if the test compound is administered by gavage. Weekly measurements of food consumption should be made.

ii. F_1 Animals

These animals should be observed carefully at least twice daily. Observations of general appearance and the presence of dead pups should be recorded. Pups should be counted on days 0 (birth), 4, 7, 14, and 21 of lactation. Pups should be weighed as a litter on days 0 (birth), 4 (before and after culling, if appropriate), 7, and 14, but should be weighed individually on day 21. Number of pups per sex should be recorded on days 4 (before and after culling, if appropriate), 7, and 14; the sex of individual pups should be recorded on day 21.

d. Other Recommendations

i. Termination of P and F_1 Animals not Selected for the Post-Weaning Phase

These animals should be killed after weaning of the F_1 animals. If toxic signs or reproductive toxicity are observed, these animals should be subject to a complete gross necropsy.

ii. Data Reporting

Litter mates should be identified. Other data should be recorded as described for the toxicity test guideline used for the post-weaning phase (see **Chapter IV C 9**).