24. Additional testing by other routes may be required if the pesticide is determined to be a prenatal developmental toxicant after oral dosing.

25. May be combined with the 2–generation reproduction study in rodents by utilizing a second mating of the parental animals in either generation.

26. Required to support products intended for food uses and to support products intended for nonfood uses if use of the product is likely to result in significant human exposure over a portion of the human life span in terms of frequency, magnitude or duration of exposure.

27. An information-based approach to testing is preferred, which utilizes the best available knowledge on the chemical (hazard, pharmacokinetic, or mechanistic data) to determine whether a standard guideline study, an enhanced guideline study, or an alternative study should be conducted to assess potential hazard to the developing animal, or in some cases to support a waiver for such testing. Registrants should submit any alternative proposed testing protocols and supporting scientific rationale to the Agency prior to study initiation.

28. Study required using a weight-of-evidence approach considering:

(i) The pesticide causes treatment-related neurological effects in adult animal studies (*i.e.*, clinical signs of neurotoxicity, neuropathology, functional or behavioral effects).

(ii) The pesticide causes treatment-related neurological effects in developing animals, following pre- and postnatal exposure (*i.e.*, nervous system malformations or neuropathy, brain weight changes in offspring, functional or behavioral changes in the offspring).

(iii) The pesticide elicits a causative association between exposures and adverse neurological effects in human epidemiological studies.

(iv) The pesticide evokes a mechanism that is associated with adverse effects on the development of the nervous system (e.g., SAR relationship to known neurotoxicants, altered neuroreceptor or neurotransmitter responses).

29. The use of a combined study that utilizes the 2–generation reproduction study in rodents as a basic protocol for the addition of other endpoints or functional assessments in the immature animal is encouraged.

30. At a minimum, an initial battery of mutagenicity tests with possible confirmatory testing is required. Other relevant mutagenicity tests that may have been performed, plus a complete reference list must also be submitted.

31. Choice of assay using either:

(i) Mouse lymphoma L5178Y cells, thymidine kinase (tk) gene locus, maximizing assay conditions for small colony expression or detection;

(ii) Chinese hamster ovary (CHO) or Chinese hamster lung fibroblast (V79) cells, hypoxanthine-guanine phosphoribosyl transferase (hgprt) gene locus, accompanied by an appropriate *in vitro* test for clastogenicity; or

(iii) CHO cells strains AS52, xanthine-guanine phosphoribosyl transferase (xprt) gene locus.

32. The micronucleus rodent bone marrow assay is preferred; however, rodent bone marrow assays using metaphase analysis (aberrations) are acceptable.

33. Required when chronic or carcinogenicity studies are required. May be required if significant adverse effects are seen in available toxicology studies and these effects can be further elucidated by metabolism studies.

34. May be required if the product's use will result in exposure to domestic animals through, but not limited to, direct application.

35. A risk assessment assuming that dermal absorption is equal to oral absorption must be performed to determine if the study is required, and to identify the doses and duration of exposure for which dermal absorption is to be quantified.

36. A 1–year non-rodent study (*i.e.*, 1–year dog study) would be required if the Agency finds that a pesticide chemical is highly bioaccumulating and is eliminated so slowly that it does not achieve steady state or sufficient tissue concentrations to elicit an effect during a 90–day study. EPA would require the appropriate tier II metabolism and pharmacokinetic studies to evaluate more precisely bioavailability, half-life, and steady state to determine if a longer duration dog toxicity study is needed.

### § 158.510 Tiered testing options for nonfood pesticides.

For nonfood use pesticides only, applicants have two options for generating and submitting required toxicology (§ 158.500) and human exposure (§ 158.1020, § 158.1070, and § 158.1410) studies. Applicants are to select one of the following:

(a) Acute, subchronic, chronic, and other toxicological studies on the active ingredient must be submitted together. The specific makeup of the set of toxicology study requirements is based on the anticipated exposure to the pesticide as determined by the Agency. If hazards are identified based upon review of these studies, specific exposure data will be required to evaluate risk.

(b) Certain toxicological and exposure studies must be submitted simultaneously with the toxicology data submitted in a tiered system. Exposure data must be submitted along with first tier toxicology data. The requirement for additional second and third level toxicology testing will be determined by the Agency based on the results of the first tiered studies.

(1) The required first-tier toxicology studies consist of:

(i) Battery of acute studies.

(ii) A subchronic 90–day dermal study or a subchronic 90–day inhalation study.

(iii) An acute and subchronic neurotoxicity screening battery in the rat.

(iv) Prenatal developmental toxicity studies in both the rat and rabbit.

(v) Reproduction and fertility studies in rats.

(vi) Battery of mutagenicity studies.

(vii) Immunotoxicity study.

(2) The conditionally required second-tier studies include:

(i) Subchronic 90–day feeding studies in both the rodent and nonrodent.

(ii) Dermal penetration study.

(3) The conditionally required third-tier studies include:

(i) Chronic feeding studies in the rodent.

(ii) Carcinogenicity.

(iii) Metabolism study.

(iv) Additional mutagenicity testing.

## Subpart G— Ecological Effects

### § 158.630 Terrestrial and aquatic nontarget organisms data requirements table.

(a) *General.* Sections 158.100 through 158.130 describe how to use this table to determine the terrestrial and aquatic nontarget data requirements for a particular pesticide product. Notes that apply to an individual test including specific conditions, qualifications, or exceptions to the designated test are listed in paragraph (e) of this section.

(b) *Use patterns.* (1) The terrestrial use pattern includes products classified under the general use patterns of terrestrial food crop, terrestrial feed crop, and terrestrial nonfood crop. The aquatic use pattern includes products classified under the general use patterns of aquatic food crop and aquatic nonfood use patterns. The greenhouse use pattern includes products classified under the general use patterns of greenhouse food crop and greenhouse nonfood crop. The indoor use pattern includes products classified under the general use patterns of indoor food and indoor nonfood use.

(2) Data are also required for the general use patterns of forestry and residential outdoor use.

(3) In general, for all outdoor end-uses, including turf, the following studies are required: Two avian oral $LD_{50}$, two avian dietary $LC_{50}$, two avian reproduction studies, two freshwater fish $LC_{50}$, one freshwater invertebrate $EC_{50}$, one honeybee acute contact $LD_{50}$, one freshwater fish early-life stage, one freshwater invertebrate life cycle, and three estuarine acute $LC_{50}/EC_{50}$ studies -- fish, mollusk and invertebrate. All other outdoor residential uses, *i.e.*, gardens and ornamental will not usually require the freshwater fish early-life stage, the freshwater invertebrate life-cycle, and the acute estuarine tests.

(c) *Key.* R=Required; CR=Conditionally required; NR=Not required; TGAI=Technical grade of the active ingredient; TEP=Typical end-use product; PAI=Pure active ingredient; EP=end-use product. Commas between the test substances (*i.e.*, TGAI, TEP) indicate that data may be required on the TGAI or the TEP depending on the conditions set forth in the test note.

(d) *Table.* The following table shows the data requirements for nontarget terrestrial and aquatic organism. The table notes are shown in paragraph (e) of this section.