

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of heritable translocations. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of heritable translocations or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the heritable translocation test in *Drosophila* indicate that under the test conditions the test substance causes chromosome damage in germ cells of this insect.

(ii) Negative results indicate that under the test conditions the test substance does not cause chromosomal damage in *D. melanogaster*.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) *Drosophila* stock used in the assay, age of insects, number of males treated, number of F₂ cultures established, number of replicate experiments.

(ii) Test chemical vehicle, treatment and mating schedule, exposure levels, toxicity data, dose and route of exposure.

(iii) Positive and negative (vehicle) controls.

(iv) Historical control data, if available.

(v) Number of chromosomes scored.

(vi) Criteria for scoring mutant chromosomes.

(vii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guide-

line the following references should be consulted:

(1) Wurgler, F.E., Sobels, F.H., Vogel, E. "Drosophila as assay system for detecting genetic changes," *Handbook of mutagenicity test procedures*. Eds. Kilby, B.J., Legator, M., Nichols, W., Ramel, C. (Amsterdam: Elsevier/North Holland Biomedical Press, 1979) pp. 335-374.

(2) [Reserved]

Subpart G—Neurotoxicity

§ 798.6050 Functional observational battery.

(a) *Purpose.* In the assessment and evaluation of the potential human health effects of substances, it may be necessary to test for neurotoxic effects. Substances that have been observed to cause neurotoxic signs (e.g., convulsions, tremors, ataxia) in other toxicity tests, as well as those having a structural similarity to known neurotoxicants, should be evaluated for neurotoxicity. The functional observational battery is a noninvasive procedure designed to detect gross functional deficits in young adults resulting from exposure to chemicals and to better quantify neurotoxic effects detected in other studies. This battery of tests is not intended to provide a detailed evaluation of neurotoxicity. It is designed to be used in conjunction with neuropathologic evaluation and/or general toxicity testing. Additional functional tests may be necessary to assess completely the neurotoxic potential of a chemical.

(b) *Definitions.* (1) Neurotoxicity is any adverse effect on the structure or function of the central and/or peripheral nervous system related to exposure to a chemical substance.

(2) A toxic effect is an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance.

(c) *Principle of the test method.* The material is administered by an appropriate route to laboratory rodents. The animals are observed under carefully standardized conditions with sufficient frequency to ensure the detection of behavioral and/or neurologic abnormalities, if present. Various functions that could be affected by neurotoxicants are assessed during each observation period.

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain*. The laboratory rat or mouse is recommended. Although information will generally be lacking, whenever possible the choice of species should take into consideration such factors as the comparative metabolism of the chemical and species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies. The potential for combined studies should also be considered. Standard strains should be used.

(ii) *Age*. Young adult animals (at least 42 days old for the rat or mouse) shall be used.

(iii) *Sex*. (A) Equal numbers of animals of each sex are required for each dose level.

(B) The females shall be nulliparous and nonpregnant.

(2) *Number of animals*. At least eight animals of each sex should be used at each dose level and should be designated for behavioral testing. If interim sacrifices are planned, the number should be increased by the number of animals scheduled to be sacrificed before the end of the study. Animals shall be randomly assigned to treatment and control groups.

(3) *Control groups*. (i) A concurrent (“sham” exposure or vehicle) control group is required. Subjects shall be treated in the same way as for an exposure group except that administration of the test substance is omitted.

(ii) Concurrent or historic data from the laboratory performing the testing shall provide evidence of the ability of the procedures used to detect major neurotoxic endpoints such as limb weakness or paralysis (e.g., acrylamide), CNS stimulation (e.g., β , β' -iminodipropionitrile) autonomic signs (e.g., physostigmine).

(iii) A satellite group may be treated with the high dose level for the duration of exposure and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate duration, normally not less than 28 days.

(4) *Dose levels and dose selection*. At least 3 doses, equally spaced on a log scale (e.g., $\frac{1}{2}$ log units) over a range of at least 1 log unit shall be used in addition to a zero dose or vehicle adminis-

tration. The data should be sufficient to produce a dose-effect curve.

(i) The highest dose shall produce (A) clear behavioral effects or (B) life-threatening toxicity.

(ii) The data from the lower doses must show either (A) graded dose-dependent effects at 2 dose levels or (B) no effects at 2 dose levels, respectively.

(5) *Duration and frequency of exposure*. The duration and frequency of exposure will be specified in the test rule.

(6) *Route of exposure*. The test substance shall be administered by the route specified in the test rule. This route will usually be the one most closely approximating the expected route of human exposure. The exposure protocol shall conform to that outlined in the appropriate acute or subchronic toxicity study guideline under subpart B or subpart C of this part.

(7) *Combined protocol*. Subjects used for other toxicity studies may be used if none of the requirements of either study are violated by the combination.

(8) *Study conduct*. (i) All animals in a given study should be observed carefully by trained technicians who are blind with respect to the animals' treatments. Standard procedures to minimize observer variability shall be followed. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of inter-observer reliability is required. All animals should be observed prior to initiation of exposure. Subsequent observations should be made with sufficient frequency to ensure the detection of behavioral and/or neurologic abnormalities, if present. At minimum, observations at 1 hour, 6 hours, 24 hours, 7 days, and 14 days and monthly thereafter are recommended. In a subchronic study, subsequent to the first exposure all observations should be made before the daily exposure. The animals should be removed from the home cage to a standard arena for observation. Effort should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables that can affect behavior are sound level, temperature, humidity, lighting, odors, time of day, and environmental distractions.

Explicit, operationally defined scales for each function should be used. The development of objective quantitative measures of the observational endpoints specified is encouraged.

(ii) The following is a minimal list of observations that shall be noted:

(A) Any unusual responses with respect to body position, activity level, coordination of movement, and gait.

(B) Any unusual or bizarre behavior including, but not limited to, headflicking, head searching, compulsive biting or licking, self-mutilation, circling, and walking backwards.

(C) The presence of:

(1) Convulsions.

(2) Tremors.

(3) Increased levels of lacrimation and/or red-colored tears.

(4) Increased levels of salivation.

(5) Piloerection.

(6) Pupillary dilation or constriction.

(7) Unusual respiration (shallow, labored, dyspneic, gasping, and retching) and/or mouth breathing.

(8) Diarrhea.

(9) Excessive or diminished urination.

(10) Vocalization.

(D) Forelimb/hindlimb grip strength. The procedure described by Meyer et al. (1979), under paragraph (f)(9) of this section is recommended.

(E) Sensory function. A simple assessment of sensory function (vision, audition, pain perception) shall be made. Marshall et al. (1971) under paragraph (f)(8) of this section have described a neurologic exam for this purpose; these procedures are also discussed by Deuel (1977), under paragraph (f)(4) of this section. Irwin (1968) under paragraph (f)(7) of this section described a number of reflex tests intended to detect gross sensory deficits, including the visual placing response, Preyer reflex, and tail pinch. Many procedures have been developed for assessing pain perception (e.g., Anker, 1974 under paragraph (f)(1) of this section; D'Amour and Smith 1941 under paragraph (f)(3) of this section; Evans 1971 under paragraph (f)(6) of this section).

(e) *Data reporting and evaluation.* In addition to the reporting requirements specified under 40 CFR part 792 subpart J the final test report must include the following information.

(1) *Description of system and test methods.* (i) A detailed description of the procedures used to standardize observation, including the arena and operational definitions for scoring observations.

(ii) Positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used. Historic data may be used if all aspects of the experimental protocol are the same, including personnel.

(2) *Results.* The following information must be arranged by test group dose level.

(i) In tabular form, data for each animal must be provided showing:

(A) Its identification number.

(B) Its body weight and score on each sign at each observation time, the time and cause of death (if appropriate).

(ii) Summary data for each group must include:

(A) The number of animals at the start of the test.

(B) The number of animals showing each observation score at each observation time.

(C) The percentage of animals showing each abnormal sign at each observation time.

(D) The mean and standard deviation for each continuous endpoint at each observation time.

(3) *Evaluation of data.* The findings of a functional observational battery should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlative histopathological findings. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence and severity, of any neurotoxic effects. The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design and needed adjustments for multiple comparisons.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Anker, S.I. "New hot plate tests to quantify antinociceptive and narcotic antagonist activities," *European Journal of Pharmacology*, 27: 1-4 (1974).

(2) Coughenour, L.L., McLean, J.R. and Parker, R.B. "A new device for the rapid

measurement of impaired motor function in mice," *Pharmacology, Biochemistry and Behavior*, 6: 351-353 (1977).

(3) D'Amour, F.E., Smith, D.L. "A method for determining loss of pain sensation," *Journal of Pharmacology and Experimental Therapeutics*, 72: 74-79 (1941).

(4) Deuel, R.K. "Determining sensory deficits in animals," *Methods in Psychobiology* Ed. Myers R.D. (New York: Academic Press, 1977) pp. 99-125.

(5) Edwards, P.M., Parker, V.H. "A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats," *Toxicology and Applied Pharmacology*, 40: 589-591 (1977).

(6) Evans, W.O. "A new technique for the investigation of some analgesic drugs on reflexive behavior in the rat," *Psychopharmacologia*, 2: 318-325 (1961).

(7) Irwin, S. "Comprehensive observational assessment: Ia. A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse," *Psychopharmacologia*, 13: 222-257 (1968).

(8) Marshall, J.F., Turner, B.H., Teitelbaum, P. "Sensory neglect produced by lateral hypothalamic damage," *Science*, 174: 523-525 (1971).

(9) Meyer, O.A., Tilson, H.A., Byrd, W.C., Riley, M.T. "A method for the routine assessment of fore- and hindlimb grip strength of rats and mice," *Neurobehavioral Toxicology*, 1: 233-236 (1979).

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§ 798.6200 Motor activity.

(a) *Purpose*—(1) *General*. In the assessment and evaluation of the toxic characteristics of a substance, determination of the effects of administration of the substance on motor activity is useful when neurotoxicity is suspected.

(2) *Acute Motor Activity Test*. The purpose of the acute motor activity test is to examine changes in motor activity occurring over a range of acute exposure levels. These changes may then be evaluated in the context of changes occurring in other organ systems. This test is an initial step in determining the potential of a substance to produce acute neurotoxicity and may be used to screen members of a class of substances for known neurotoxicity, and/or to establish a dosage regimen prior to the initiation of subchronic neurotoxicity testing.

(3) *Subchronic Motor Activity Test*. The purpose of the subchronic motor activity test is to determine whether the repeated administration of a suspected

neurotoxicant results in changes in motor activity. These changes may be evaluated in the context of changes occurring in other organ systems. This test is an initial step in determining the potential of a substance to produce subchronic neurotoxicity.

(b) *Definitions*. (1) Neurotoxicity is the adverse effect on the structure or function of the central and/or peripheral nervous system related to exposure to a chemical substance.

(2) Motor activity is any movement of the experimental animal.

(3) A toxic effect is an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance.

(c) *Principle of the test method*. The test substance is administered to several groups of experimental animals, one dose being used per group. Measurements of motor activity are made. The exposure levels at which significant changes in motor activity are produced are compared to those levels which produce toxic effects not originating in the central and/or peripheral nervous system.

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain*. Testing shall be performed in a laboratory rat or mouse. The choice of species should take into consideration such factors as the comparative metabolism of the chemical and species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies, the potential for combined studies, and the availability of other toxicity data for the species.

(ii) *Age*. Young adult animals (at least 42 days old for rat or mouse) should be used.

(iii) *Sex*. (A) Equal numbers of animals of each sex are required for each dose level for the motor activity test.

(B) The females shall be nulliparous and nonpregnant.

(2) *Number of animals*. Animals shall be randomly assigned to test and control groups. Each test or control group must be designed to contain a sufficient number of animals at the completion of the study to detect a 40 percent change in activity of the test groups relative to the control group with 90 percent power at the 5 percent level. For most designs, calculations can be