

(iii) In all tests, concentrations must be given as the final concentrations during the treatment. Raw data, prior to transformation, should be provided. These should include actual quantities measured, e.g., neat numbers. For measurement of diffusion, the diameters of the discs and/or well should be indicated and the measurements should indicate whether the diameter of the discs and/or well was subtracted. Moreover, mention should be made as to whether the test chemical gave a sharp, diffuse, or double-zone of growth inhibition. If it is the latter, the investigator should indicate whether the inner or the outer zone was measured.

(iv) Viability data should be given as the actual plate counts with an indication of the dilution used and the volume plated or as derived titers (cells per ml). Transformed data alone in the absence of experimental data are not acceptable (i.e., ratios, differences, survival fraction).

(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 preferential inhibition or killing of the repair deficient strain. 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 preferential inhibition or killing of the repair deficient strain or a statistically significant and reproducible positive response at any one of the test points is considered not to interact with the genetic material of the organisms used in assay.

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

(4) *Test evaluation.* DNA damage tests in bacteria do not measure DNA repair *per se* nor do they measure mutations. They measure DNA damage which is expressed as cell killing or growth inhibition. A positive result in a DNA damage test in the absence of a positive result in another system is dif-

ficult to evaluate in the absence of a better data base.

(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) Bacterial strains used.
- (ii) Phase of bacterial cell growth at time of use in the assay.
- (iii) Media composition.
- (iv) Details of both the protocol used to prepare the metabolic activation system and its use in the assay.
- (v) Treatment protocol, including doses used and rationale for dose selection, positive and negative controls.
- (vi) Method used for determination of degree of cell kill.
- (vii) Dose-response relationship, if applicable.

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

(1) Ames, B.N., McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test," *Mutation Research*, 31:347-364 (1975).

(2) Kada, T., Sadie, Y., Tutikawa, K. "In vitro and host-mediated "rec-assay" procedures for screening chemical mutagens; and phloxine, a mutagenic red dye detected," *Mutation Research*, 16:165-174 (1972).

(3) Leifer, Z., Kada, T., Mandel, M., Zeiger, E., Stafford, R., Rosenkranz, H.S. "An evaluation of bacterial DNA repair tests for predicting genotoxicity and carcinogenicity: A report of the U.S. EPA's Gene-Tox Program," *Mutation Research*, 87:211-297 (1981).

(4) Slater, E.E., Anderson, M.D., Rosenkranz, H.S. "Rapid detection of mutagens and carcinogens." *Cancer Research*, 31:970-973 (1971).

§ 798.5955 Heritable translocation test in *Drosophila melanogaster*.

(a) *Purpose.* The heritable translocation test in *Drosophila* measures the induction of chromosomal translocations in germ cells of insects. Stocks carrying genetic markers on two or more chromosomes are used to follow the assortment of chromosomes in meiosis. The F₁ male progeny of treated parents

are individually mated to females and the F₂ progeny phenotypes are scored. The observed spectrum of phenotypes is used to determine the presence or absence of a translocation. This is usually indicated by a lack of independent assortment of genes on different chromosomes.

(b) *Definitions*—(1) Chromosome mutations are chromosomal changes resulting from breakage and reunion of chromosomes. Chromosomal mutations are also produced through nondisjunction of chromosomes during cell division.

(2) Reciprocal translocations are chromosomal translocations resulting from reciprocal exchanges between two or more chromosomes.

(3) Heritable translocations are reciprocal translocations transmitted from parent to the succeeding progeny.

(c) *Reference substances*. These may include, but need not be limited to, ethyl methanesulfonate or N-dimethylnitrosamine.

(d) *Test method*—(1) *Principle*. The method is based on the principle that balanced reciprocal chromosomal translocations can be induced by chemicals in the germ cells of treated flies and that these translocations are detected in the F₂ progeny using genetic markers (mutations). Different mutations may be used as genetic markers and two or more of the four chromosomes may be genetically marked for inclusion in this test.

(2) *Description*. Wild-type males are treated with chemical and bred with females of known genetic markers. The F₁ males are collected and individually bred with virgin females of the female parental stock. The resulting F₂ progeny are scored. Putative translocation carriers are confirmed with an F₃ cross.

(i) *Illustrative example*. The following example serves to illustrate the method. Males carrying genes for red eye color on chromosomes II and III are bred with females of white eye color carrying alleles for brown (bw) on the second chromosome and scarlet (st) and pink (pp) on the third chromosome. The F₁ male progeny are bred with virgin females of the female parental stock and the resulting F₂ progeny are examined for eye color phenotypes. If there is no translocation in the F₁

male, then the resulting F₂ progeny will have four eye color phenotypes: red, white, orange, and brown. If the F₁ male carries a translocation between chromosomes II and III, only red and white eye phenotypes are obtained in the F₂ generation. This happens because the F₁ translocation heterozygote produces two balanced (carrying either the parental or the translocated configuration of markers) and two unbalanced gametes. The unbalanced gametes (carrying one normal and one translocated chromosome) are unable to develop into normal individuals in the F₂ generation.

(ii) [Reserved]

(3) *Drosophila stocks*. Wild-type males and females of the genotype bw:st:pp (white eyes) may be used in the heritable translocation test. Other appropriately marked *Drosophila* stocks may also be used.

(4) *Control groups*. (i) Concurrent positive and negative (vehicle) controls should be included in each experiment.

(ii) Negative (vehicle) controls should be included. The size of the negative (vehicle) control group should be determined by the availability of appropriate laboratory historical control data.

(iii) If the historical control data are of sufficient numbers, concurrent controls may not be necessary.

(5) *Test chemicals*—(i) *Vehicle*. Test chemicals should be dissolved in water. Compounds which are insoluble in water may be dissolved or suspended in appropriate vehicles (e.g., a mixture of ethanol and Tween-60 or 80), and then diluted in water or saline prior to administration. Dimethylsulfoxide should be avoided as a vehicle.

(ii) *Dose levels*. For the initial assessment of mutagenicity, it may be sufficient to test a single dose of the test substance. This dose should be the maximum tolerated dose or that which produces some indication of toxicity. If the test is being used to verify mutagenic activity, at least two additional exposure levels should be used.

(iii) *Route of administration*. Exposure may be oral, by injection or by exposure to gases or vapours. Feeding of the test compound may be done in sugar solution. When necessary, substances may be dissolved in 0.7 percent NaCl

solution and injected into the thorax or abdomen.

(e) *Test performance*—(1) *P1 mating*. (i) In the primary screen of a chemical, it is enough to sample one germ cell stage, either mature sperm or spermatids (for indirect acting mutagens). Other stages may be sampled if needed, i.e., when mature germ cells give a positive result and data from earlier germ cells are needed for the purpose of risk assessment. Thus, the treated males may be mated only once for a period of 3 days to sample sperm or transferred every 2 to 3 days to cover the entire germ cell cycle.

(ii) Mass matings may be performed because the control rate for translocations in the available literature is very low (near 0) and clustered events are extremely rare. Mated females may be aged for 2 weeks in order to recover an enhanced incidence of translocation due to the storage effect. The females are then allowed to lay eggs and F₁ males are collected for test mating.

(2) *F₁ mating*. F₁ males should be bred with virgin females of the parental female stock. Since each F₁ male represents one treated gamete of the male parent, the F₁ males have to be mated individually to virgin females. Each F₁ male should be mated to three females to ensure sufficient progeny.

(3) *Scoring the F₂ generation*. F₂ cultures (each representing 1 F₁ male tested) should be scored for the presence or absence of phenotype variations (linkage of markers) from the expected types. The test should be designed with a predetermined sensitivity and power. The number of flies in each group should reflect these defined parameters. The spontaneous mutant frequency observed in the appropriate control group will strongly influence the number of treated chromosomes that must be analyzed to detect substances which show mutation rates close to those of the controls. A positive test should be confirmed by F₃ mating trials.

(4) *Number of replicate experiments*. Replicate experiments are usually performed for each dose of the compound tested. If a chemical is a potent inducer of translocations, one experiment may be sufficient. Otherwise two

or three replicate experiments should be done.

(f) *Data and report*—(1) *Treatment of results*. Data should be tabulated to show the number of translocations and the number of fertile F₁ males at each exposure for each germ cell stage sampled.

(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 guideline 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-