

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

(4) *Test evaluation.* (i) Positive results from the *S. typhimurium* reverse mutation assay indicate that, under the test conditions, the test substance induces point mutations by base changes or frameshifts in the genome of this organism.

(ii) Negative results indicate that under the test conditions the test substance is not mutagenic in *S. typhimurium*.

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

(i) Bacterial strain used.

(ii) Metabolic activation system used (source, amount and cofactor); details of preparations of S-9 mix.

(iii) Dose levels and rationale for selection of dose.

(iv) Positive and negative controls.

(v) Individual plate counts, mean number of revertant colonies per plate, standard deviation.

(vi) 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) de Serres, F.J., Shelby, M.D. "The *Salmonella* mutagenicity assay: recommendations," *Science* 203:563-565 (1979).

(3) Prival, M.J., Mitchell, V.D. "Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavin mononucleotide and hamster liver S-9," *Mutation Research* 97:103-116 (1982).

(4) Vogel, H.J., Bonner, D.M. "Acetylornithinase of *E. coli*: partial purification and some properties," *Journal of Biological Chemistry*. 218:97-106 (1956).

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19078, May 20, 1987]

§ 798.5275 Sex-linked recessive lethal test in *Drosophila melanogaster*.

(a) *Purpose.* The sex-linked recessive lethal (SLRL) test using *Drosophila melanogaster* detects the occurrence of mutations, both point mutations and small deletions, in the germ line of the insect. This test is a forward mutation assay capable of screening for mutations at about 800 loci on the X-chromosome. This represents about 80 percent of all X-chromosome loci. The X-chromosome represents approximately one-fifth of the entire haploid genome.

(b) *Definitions.* (1) Lethal mutation is a change in the genome which, when expressed, causes death to the carrier.

(2) Recessive mutation is a change in the genome which is expressed in the homozygous or hemizygous condition.

(3) Sex-Linked genes are present on the sex (X or Y) chromosomes. Sex-linked genes in the context of this guideline refer only to those located on the X-chromosome.

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

(d) *Test method—(1) Principle.* Mutations in the X-chromosome of *D. melanogaster* are phenotypically expressed in males carrying the mutant gene. When the mutation is lethal in the hemizygous condition, its presence is inferred from the absence of one class of male offspring out of the two that are normally produced by a heterozygous female. The SLRL test takes advantage of these facts by means of specially marked and arranged chromosomes.

(2) *Description.* Wild-type males are treated and mated to appropriate females. Female offspring are mated individually to their brothers, and in the next generation the progeny from each separate dose are scored for phenotypically wild-type males. Absence of these males indicates that a sex-linked recessive lethal mutation has occurred in a germ cell of the P₁ male.

(3) *Drosophila stocks.* Males of a well-defined wild type stock and females of the Muller-5 stock may be used. Other appropriately marked female stocks

with multiple inverted X-chromosomes may also be used.

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

(ii) *Positive controls*. Examples of positive controls include ethyl methanesulfonate and N-nitroso-dimethylamine.

(iii) *Other positive controls*. Other positive control reference substances may be used.

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

(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 is sufficient to test a single dose of the test substance for screening purposes. This dose should be the maximum tolerated dose, or that which produces some indication of toxicity, or shall be the highest dose attainable. For dose-response purposes, at least three additional dose levels should be used.

(iii) *Route of administration*. Exposure may be oral, by injection or by exposure to gases or vapors. 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) *Treatment and mating*. Wild-type males (3 to 5 days old) shall be treated with the test substance and mated individually to an appropriate number of virgin females from the Muller-5 stock or females from another appropriately marked (with multiply-inverted X-chromosomes) stock. The females shall be replaced with fresh virgins every 2 to 3 days to cover the entire germ cell cycle. The offspring of these females are scored for lethal effects cor-

responding to the effects on mature sperm, mid or late stage spermatids, early spermatids, spermatocytes and spermatogonia at the time of treatment.

(2) *F₁ matings*. Heterozygous F₁ females from the above crosses shall be allowed to mate individually (i.e., one female per vial) with their brothers. In the F₂ generation, each culture shall be scored for the absence of wild-type males. If a culture appears to have arisen from an F₁ female carrying a lethal in the parental X-chromosome (i.e., no males with the treated chromosome are observed), daughters of that female with the same genotype shall be tested to ascertain if the lethality is repeated in the next generation.

(3) *Number of matings*. (i) 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 analysed to detect substances which show mutation rates close to those of the controls.

(ii) Test results should be confirmed in a separate experiment.

(f) *Data and report*—(1) *Treatment of results*. Data shall be tabulated to show the number of chromosomes tested, the number of nonfertile males and the number of lethal chromosomes at each exposure concentration and for each mating period for each male treated. Numbers of clusters of different size per male shall be reported.

(2) *Statistical evaluation*. Data shall be evaluated by appropriate statistical techniques.

(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 sex-lined recessive lethals. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

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

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

(4) *Test evaluation.* (i) Positive results in the SLRL test in *D. melanogaster* indicate that under the test conditions the test agent causes mutations in germ cells of this insect.

(ii) Negative results indicate that under the test conditions the test substance is not mutagenic 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 shall be reported.

(i) *Drosophila* stock used in the assay, age of insects, number of males treated, number of sterile males, number of F₂ cultures established, number of F₂ cultures without progeny.

(ii) Test chemical vehicle, treatment and sampling schedule, exposure levels, toxicity data, negative (vehicle) and positive controls, if appropriate.

(iii) Criteria for scoring lethals.

(iv) Number of chromosomes tested, number of chromosomes scored, number of chromosomes carrying a lethal mutation.

(v) Historical control data, if available.

(vi) Dose-response relationship, if applicable.

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

(1) Sobels, F.H., Vogel, E. "The capacity of *Drosophila* for detecting relevant genetic damage," *Mutation Research* 41:95-106 (1976).

(2) Wurgler F.E., Sobels F.H., Vogel E. "*Drosophila* as assay system for detecting genetic changes," *Handbook of mutagenicity test procedures*. Eds. Kilbey, B.J., Legator, M., Nichols, W., Ramel, C., (Amsterdam: Elsevier/North

Holland Biomedical Press, 1977) pp. 335-373.

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19079, May 20, 1987]

§ 798.5300 **Detection of gene mutations in somatic cells in culture.**

(a) *Purpose.* Mammalian cell culture systems may be used to detect mutations induced by chemical substances. Widely used cell lines include L5178Y mouse lymphoma cells and the CHO and V-79 lines of Chinese hamster cells. In these cell lines the most commonly used systems measure mutation at the thymidine kinase (TK), hypoxanthine-guanine-phosphoribosyl transferase (HPRT) and Na⁺/K⁺ ATPase loci. The TK and HPRT mutational systems detect base pair mutations, frameshift mutations, and small deletions; the Na⁺/K⁺ ATPase system detects base pair mutations only.

(b) *Definitions.* (1) A forward mutation assay detects a gene mutation from the parental type to the mutant form which gives rise to a change in an enzymatic or functional protein.

(2) Base pair mutagens are agents which cause a base change in the DNA.

(3) Frameshift mutagens are agents which cause the addition or deletion of single or multiple base pairs in the DNA molecule.

(4) Phenotypic expression time is a period during which unaltered gene products are depleted from newly mutated cells.

(c) *Reference substances.* These may include, but need not be limited to, ethyl methanesulfonate, N-nitroso-dimethylamine, 2-acetylaminofluorene, 7,12-dimethylbenzanthracene or hycanthone.

(d) *Test method*—(1) *Principle.* Cells are exposed to test substance, both with and without metabolic activation, for a suitable period of time and subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection. Cells deficient in thymidine kinase (TK) due to the forward mutation TK⁺→TK⁻ are resistant to the cytotoxic effects of pyrimidine analogues such as bromodeoxyuridine (BrdU), fluorodeoxyuridine (FdU) or