

## § 798.5200

40 CFR part 792, subpart J, and paragraph (h) of this section, the following specific information shall be reported:

(i) Strain, age and weight of animals used; numbers of animals of each sex in experimental and control groups.

(ii) Test chemical vehicle, doses used, rationale for dose selection, and toxicity data, if available.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Number of loci screened for both treated and spontaneous data.

(vi) Criteria for scoring mutants.

(vii) Number of mutants found/locus.

(viii) Loci at which mutations were found.

(ix) Use of concurrent negative and positive controls.

(x) Dose-response relationship, if applicable.

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

(1) Personal communication from Susan E. Lewis, Ph.D. to Dr. Michael Cimino, U.S. EPA, OPPT, October 5, 1989.

(2) Johnson, F.M., G.T. Roberts, R.K. Sharma, F.Chasalow, R. Zweidinger, A. Morgan, R.W. Hendren, and S.E.Lewis. "The detection of mutants in mice by electrophoresis: Results of a model induction experiment with procarbazine." *Genetics* 97:113-124 (1981).

(3) Johnson, F.M. and S.E. Lewis. "Mutation rate determinations based on electrophoretic analysis of laboratory mice." *Mutation Research* 82:125-135 (1981a).

(4) Johnson, F.M. and S.E. Lewis. "Electrophoretically detected germinal mutations induced by ethylnitrosourea in the mouse." *Proceedings of the National Academy of Sciences* 78:3138-3141 (1981b).

(5) Lewis, S.E., C. Felton, L.B. Barnett, W. Generoso, N. Cacheiro, and M.D. Shelby. "Dominant visible and electrophoretically expressed mutations induced in male mice exposed to ethylene oxide by inhalation." *Environmental Mutagenesis* 8:867-872 (1986).

(h) *Additional requirements.* Testing facilities conducting the mouse biochemical specific locus test in accordance with this section shall, in addition to adhering to the provisions of §§ 792.190 and 792.195 of this chapter, obtain, adequately identify, and retain for at least 10 years, acceptable 35-mm photographs (and their negatives) of the stained isoelectric-focussing columns and the stained starch-gels obtained following analyses of blood and

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kidney preparations, respectively, from mutant mice, their siblings, and their parents.

[55 FR 12641, Apr. 5, 1990]

### § 798.5200 Mouse visible specific locus test.

(a) *Purpose.* The mouse visible specific locus test (MSLT) may be used to detect and quantitate mutations in the germ line of a mammalian species.

(b) *Definitions.* (1) A visible specific locus mutation is a genetic change that alters factors responsible for coat color and other visible characteristics of certain mouse strains.

(2) The germ line is the cells in the gonads of higher eukaryotes which are the carriers of the genetic information for the species.

(c) *Reference substances.* Not applicable.

(d) *Test method—(1) Principle.* (i) The principle of the MSLT is to cross individuals who differ with respect to the genes present at certain specific loci, so that a genetic alteration involving the standard gene at any one of these loci will produce an offspring detectably different from the standard heterozygote. The genetic change may be detectable by various means, depending on the loci chosen to be marked.

(ii) Three variations of the method currently exist for detecting newly arising point mutations in mouse germ cells:

(A) The visible specific locus test using either 5 or 7 loci.

(B) The biochemical specific locus test using up to 20 enzymes.

(C) The test for mutations at histocompatibility loci.

(iii) Of the three tests, the visible specific locus test has been most widely used in assessing genetic hazard due to environmental agents. It is the method described in this guideline.

(2) *Description.* For technical reasons, males rather than females are generally treated with the test agent. Treated males are then mated to females which are genetically homozygous for certain specific visible marker loci. Offspring are examined in the next generation for evidence that a new mutation has arisen.

(3) *Animal selection*—(i) *Species and strain*. Mice shall be used as the test species. Male mice shall be either (C<sub>3</sub>H×101)F<sub>1</sub> or (101×C<sub>3</sub>H)F<sub>1</sub> hybrids. Females shall be T stock virgins.

(ii) *Age*. Healthy sexually mature animals shall be used.

(iii) *Number*. A decision on the minimum number of treated animals should take into account the spontaneous variation of the biological characterization being evaluated. Other considerations should include:

(A) The use of either historical or concurrent controls.

(B) The power of the test.

(C) The minimal rate of induction required.

(D) The use of positive controls.

(E) The level of significance desired.

(iv) *Assignment to groups*. Animals shall be randomized and assigned to treatment and control groups.

(4) *Control groups*—(i) *Concurrent controls*. The use of positive or spontaneous controls is left to the discretion of the investigator. However, any laboratory which has had no prior experience with the test shall, at its first attempt, produce a negative control sample of 20,000 and a positive control, using 100 mg/kg 1-ethyl-nitrosourea, in a sample of 5,000 offspring.

(ii) *Historical controls*. Long term, accumulated spontaneous control data of 43/801,406 are available for comparative purposes.

(5) *Test chemicals*—(i) *Vehicle*. When possible, test chemicals should be dissolved or suspended in distilled water or isotonic saline buffered appropriately, if needed, for stability. Water-insoluble chemicals shall be dissolved or suspended in appropriate vehicles. The vehicle used shall neither interfere with the test compound nor produce major toxic effects. Fresh preparations of the test chemical should be employed.

(ii) *Dose levels*. Usually, only one dose level need be tested. This should be the highest dose tolerated without toxic effects, provided that any temporary sterility induced due to elimination of spermatogonia is of only moderate duration, as determined by a return of males to fertility within 80 days after treatment. For evaluation of dose-re-

sponse, it is recommended that at least two dose levels be tested.

(iii) *Route of administration*. Acceptable routes of administration include gavage, inhalation, admixture with food or water, and IP or IV injections.

(e) *Test performance*—(1) *Treatment and mating*. Hybrid F<sub>1</sub> (C<sub>3</sub> H×101 or 101×C<sub>3</sub> H) male mice shall be treated with the test substance and immediately mated to virgin T stock females. Each treated male shall be mated to a fresh group of 2 to 4 virgin females each week for 7 weeks, after which he shall be returned to the first group of females and rotated through the seven sets of females repeatedly. This mating schedule generally permits sampling of all postspERMATOGONIAL stages of germ cell development during the first 7 weeks and rapid accumulation of data for exposed spermatogonial stem cells thereafter. Repeated mating cycles should be conducted until the entire spermatogonial cycle has been evaluated and enough offspring have been obtained to meet the power criterion of the assay.

(2) *Examination of offspring*. (i) Offspring may be examined at (or soon after) birth but must be examined at about 3 weeks of age at which time the numbers of mutant and nonmutant offspring in each litter shall be recorded.

(ii) Nonmutant progeny should be discarded. Mutant progeny shall be subjected to genetic tests for verification.

(f) *Data and report*—(1) *Treatment of results*. Data shall be presented in tabular form and shall permit independent analysis of cell stage specific effects and dose dependent phenomena. The data shall be recorded and analyzed in such a way that clusters of identical mutations are clearly identified. The individual mutants detected shall be thoroughly described. In addition, concurrent positive and negative control data, if they are available, shall be tabulated so that it is possible to differentiate between concurrent (when available) and long-term accumulated mutation frequencies.

(2) *Statistical evaluation*. Data shall 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 specific locus mutations. 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 specific locus mutations 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 MSLT indicate that under the test conditions the test substance induces heritable gene mutations in the test species.

(ii) Negative results indicate that under the test conditions the test substance does not induce heritable gene mutations in the test species.

(5) *Test report.* In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, and paragraph (h) of this section, the following specific information shall be reported:

(i) Strain, age and weight of animals used, number of animals of each sex in experimental and control groups.

(ii) Test chemical vehicle, doses used and rationale for dose selection, toxicity data.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Time of examination for mutant progeny.

(vi) Criteria for scoring mutants.

(vii) Use of concurrent or negative controls.

(viii) Dose response relationship, if applicable.

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

(1) Russell, L.B., Shelby, P.B., von Halle, E., Sheridan, W., Valcovic, L. The mouse specific locus test with agents other than radiations: interpretation of data and recommendations for future work: A report of the U.S. EPA's Gene-Tox Program," *Mutation Research*, 86:329-354 (1981).

(2) [Reserved]

(h) *Additional requirements.* Testing facilities conducting the mouse visible specific locus test in accordance with this section shall, in addition to adhering to the provisions of §§ 792.190 and 792.195 of this chapter, obtain, and retain for at least 10 years, acceptable 35-mm color photographs (and their negatives) demonstrating the visible mutations observed in mutant animals and the lack of such mutations in their siblings and parents.

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19078, May 20, 1987; 55 FR 12643, Apr. 5, 1990]

#### § 798.5265 The salmonella typhimurium reverse mutation assay.

(a) *Purpose.* The *Salmonella typhimurium* histidine (his) reversion system is a microbial assay which measures his<sup>-</sup> → his<sup>+</sup> reversion induced by chemicals which cause base changes or frameshift mutations in the genome of this organism.

(b) *Definitions.* (1) A reverse mutation assay in *Salmonella typhimurium* detects mutation in a gene of a histidine requiring strain to produce a histidine independent strain of this organism.

(2) Base pair mutagens are agents which cause a base change in the DNA. In a reversion assay, this change may occur at the site of the original mutation or at a second site in the chromosome.

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

(c) *Reference substances.* These may include, but need not be limited to, sodium azide, 2-nitrofluorene, 9-aminoacridine, 2-aminoanthracene, congo red, benzopurpurin 4B, trypan blue or direct blue 1.

(d) *Test method—(1) Principle.* Bacteria are exposed to test chemical with and without a metabolic activation system and plated onto minimal medium. After a suitable period of incubation, revertant colonies are counted and compared to the number of spontaneous revertants in an untreated and/or vehicle control culture.

(2) *Description.* Several methods for performing the test have been described. Among those used are: