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positive result, one of which is a statistically significant dose-related increase in the number of dominant lethals. 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 dominant lethals 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) A positive DL assay suggests that under the test conditions the test substance may be genotoxic in the germ cells of the treated sex of the test species.

(ii) A negative result suggests that under the conditions of the test the test substance may not be genotoxic in the germ cells of the treated sex of the test species.

(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) Species, strain, age and weights of animals used, number of animals of each sex in experimental and control groups.

(ii) Test substance, vehicle used, dose levels and rationale for dosage selection, negative (vehicle) and positive controls, experimental observations, including signs of toxicity.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Methods used to determine that mating has occurred (where applicable).

(vi) Criteria for scoring dominant lethals including the number of early and late embryonic deaths.

(vii) Dose-response relationship, if applicable.

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

(1) Brewen, J.G., Payne, H.S., Jones, K.P., Preston, R.J. ''Studies on chemically induced dominant lethality. I. The cytogenetic basis of MMS-induced dominant lethality in post-meiotic germ cells'' *Mutation Research*, 33:239–250 (1975).

(2) Ehling, U.H., Machemer, L., Buselmaier, E., Dycka, D., Frohberg, H., Kratochvilova, J., Lang, R., Lorke, D., Muller, D., Pheh, J., Rohrborn, G., Roll, R., Schulze-Schencking, M., Wiemann, H. ''Standard protocol for the dominant lethal test on male mice. Set up by the Work Group ''Dominant lethal mutations of the ad hoc Committee Chemogenetics,'' *Archives of Toxicology*, 39:173–185 (1978).

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19081, May 20, 1987]

## § 798.5460 Rodent heritable translocation assays.

(a) *Purpose*. This test detects transmitted chromosomal damage which manifests as balanced reciprocal translocations in progeny descended from parental males treated with chemical mutagens.

(b) *Definitions*. (1) A heritable translocation is one in which distal segments of nonhomologous chromosomes are involved in a reciprocal exchange.

(2) Diakinesis and metaphase I are stages of meiotic prophase scored cytologically for the presence of multivalent chromosome association characteristic of translocation carriers.

(c) *Reference substances*. Not applicable.

(d) *Test method*—(1) *Principle*. When a balanced reciprocal translocation is induced in a parental male germ cell, the resulting progeny is translocation heterozygote.

(i) *Basis for fertility screening*. Male translocation heterozygotes may be completely sterile. This class consists of two types of translocations:

(A) Translocations between non-homologous chromosomes in which at least one of the breaks occurs close to one end of a chromosome.

(B) Those that carry multiple translocations. The majority of male translocation heterozygotes are semisterile—they carry one or (rarely) two translocations. The degree of semisterility is dependent upon the proportions of balanced and unbalanced

(duplication-deficiency) gametes produced in the ejaculate as a function of meiotic segregation. Balanced and unbalanced sperm are equally capable of fertilizing an egg. Balanced sperm lead to viable progeny. Unbalanced sperm result in early embryonic lethality.

(ii) *Basis for cytological screening*. The great majority of male translocation heterozygotes can be identified cytologically through analysis of diakinesis metaphase I spermatocytes. Translocation heterozygotes are characterized by the presence of multivalent chromosome association such as a ring or chain of four chromosomes held together by chiasmata in paired homologous regions. Some translocation carriers can be identified by the presence of extra long and/or extra short chromosomes in spermatogonial and somatic cell metaphase preparations.

(2) *Description*. Essentially, two methods have been used to screen for translocation heterozygosity; one method uses a mating sequence to identify sterile and semisterile males followed by cytological examination of suspect male individuals; the other method deletes the mating sequence altogether and all $F_1$ male progeny are examined cytologically for presence of translocation. In the former approach, the mating sequence serves as a screen which eliminates most fully fertile animals for cytological confirmation as translocation heterozygotes.

(3) *Animal selection*—(i) *Species*. The mouse is the species generally used, and is recommended.

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

(iii) *Number*. (A) The number of male animals necessary is determined by the following factors:

(*1*) The use of either historical or concurrent controls.

(*2*) The power of the test.

(*3*) The minimal rate of induction required.

(*4*) Whether positive controls are used.

(*5*) The level of significance desired.

(B) [Reserved]

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

(4) *Control groups*—(i) *Concurrent controls*. No concurrent positive or negative (vehicle) controls are recommended as routine parts of the heritable translocation assay. However, investigators not experienced in performing translocation testing shall include a substance known to produce translocations in the assay as a positive control reference chemical.

(ii) *Historical controls*. At the present time, historical control data must be used in tests for significance. When statistically reliable historical controls are not available, negative (vehicle) controls shall be used.

(5) *Test chemicals*—(i) *Vehicle*. When appropriate for the route of administration, solid and liquid test substances should be dissolved or suspended in distilled water or isotonic saline. Water-insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used shall neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test chemical should be employed.

(ii) *Dose levels*. At least two dose levels shall be used. The highest dose level shall result in toxic effects (which shall not produce an incidence of fatalities which would prevent a meaningful evaluation) or shall be the highest dose attainable or 5g/kg body weight.

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

(e) *Test performance*—(1) *Treatment and mating*. The animals shall be dosed with the test substances 7 days per week over a period of 35 days. After treatment, each male shall be caged with 2 untreated females for a period of 1 week. At the end of 1 week, females shall be separated from males and caged individually. When females give birth, the day of birth, litter size, and sex of progeny shall be recorded. All male progeny should be weaned, and all female progeny should be discarded.

(2) *Testing for translocation heterozygosity*. When males are sexually mature, testing for translocation heterozygosity shall begin. One of two methods shall be used; the first method involves mating, determining those $F_1$ progeny which are sterile or

semisterile and subsequent cytological analysis of suspect progeny; the other method does not involve mating and determining sterility or semisterility; all progeny are examined cytologically.

(i) *Determination of sterility or semisterility*—(A) *Conventional method.* Females are mated, usually three females for each male, and each female is killed at midpregnancy. Living and dead implantations are counted. Criteria for determining normal and semisterile males are usually established for each new strain because the number of dead implantations varies considerably among strains.

(B) *Sequential method.* Males to be tested are caged individually with females and the majority of the presumably normal males are identified on the basis of a predetermined size of 1 or 2 litters. Breeding pens are examined daily on weekdays beginning 18 days after pairing. Young are discarded immediately after they are scored. Males that sire a litter whose size is the same as or greater than the minimum set for a translocation-free condition are discarded with their litter. If the litter size is smaller than the predetermined number, a second litter is produced with the same rule applying. Males that cannot be classified as normal after production of a second litter are tested further by the conventional method or by cytological confirmation of translocation.

(ii) *Cytological analysis.* For cytological analysis of suspected semisteriles, the air-drying technique is used. Observation of at least 2 diakinesis-metaphase 1 cells with mutivalent association constitutes the required evidence for the presence of a translocation. Sterile males are examined by one of two methods, those with testes of normal size and sperm in the epididymis are examined by the same techniques used for semisteriles. Animals with small testes are examined by squash preparations or, alternatively, by examination of mitotic metaphase preparations. If squash preparations do not yield diakinesis-metaphase 1 cells, analysis of spermatogonia or bone marrow for the presence of unusually long or short chromosomes should be performed.

(f) *Data and report*—(1) *Treatment of results.* (i) Data shall be presented in tabular form and shall include the number of animals at risk, the germ cell stage treated, the number of partial steriles and semisteriles (if the fertility test is used), the number of cytogenetically confirmed translocation heterozygotes (if the fertility test is used, report the number of confirmed steriles and confirmed partial steriles), the translocation rate, and either the standard error of the rate or the upper 95 percent confidence limit on the rate.

(ii) These data shall be presented for both treated and control groups. Historical or concurrent controls shall be specified, as well as the randomization procedure used for concurrent controls.

(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 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 assay indicate that under the test conditions the test substance causes heritable chromosomal damage in the test species.

(ii) Negative results indicate that under the test conditions the test substance does not cause heritable chromosomal damage in the test species.

(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) Species, strain, age, weight and number of animals of each sex in each group.

(ii) Test chemical vehicle, route and schedule of administration, toxicity data.

(iii) Dosing regimen, doses tested and rationale for dosage selection.

(iv) Mating schedule, number of females mated to each male.

(v) The use of historical or concurrent controls.

(vi) Screening procedure including the decision criteria used and the method by which they were determined.

(vii) Dose-response relationship, if applicable.

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

(1) Generoso, W.M., Bishop, J.B., Goslee, D.G., Newell, G.W., Sheu, G-J, von Halle, E. ''Heritable translocation test in mice,'' *Mutation Research*, 76:191–215 (1980).

(2) [Reserved]

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19081, May 20, 1987]

## § 798.5500 Differential growth inhibition of repair proficient and repair deficient bacteria: "Bacterial DNA damage or repair tests."

(a) *Purpose*. Bacterial DNA damage or repair tests measure DNA damage which is expressed as differential cell killing or growth inhibition of repair deficient bacteria in a set of repair proficient and deficient strains. These tests do not measure mutagenic events *per se*. They are used as an indication of the interaction of a chemical with genetic material implying the potential for genotoxicity.

(b) *Definition*. Test for differential growth inhibition of repair proficient and repair deficient bacteria measure differences in chemically induced cell killing between wild-type strains with full repair capacity and mutant strains deficient in one or more of the enzymes which govern repair of damaged DNA.

(c) *Reference substances*. These may include, but need not be limited to, chloramphenicol or methyl methanesulfonate.

(d) *Test method*—(1) *Principle*. The tests detect agents that interact with cellular DNA to produce growth inhibition or killing. This interaction is recognized by specific cellular repair systems. The assays are based upon the use of paired bacterial strains that differ by the presence of absence of specific DNA repair genes. The response is expressed in the preferential inhibition of growth or the preferential killing of the DNA repair deficient strain since it is incapable of removing certain chemical lesions from its DNA.

(2) *Description*. Several methods for performing the test have been described. Those described here are:

(i) Tests performed on solid medium (diffusion tests).

(ii) Tests performed in liquid culture (suspension tests).

(3) *Strain selection*—(i) *Designation*. At the present time, *Escherichia coli polA* (W3110/p3478) or *Bacillus subtilis rec* (H17/M45) pairs are recommended. Other pairs may be utilized when appropriate.

(ii) *Preparation and storage*. Stock culture preparation and storage, growth requirements, method of strain identification and demonstration of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(4) *Bacterial growth*. Good microbiological techniques should be used to grow fresh cultures of bacteria. The phase of growth and cell density should be documented and should be adequate for the experimental design.

(5) *Metabolic activation*. Bacteria should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. The most commonly used system is a cofactor supplemented postmitochondrial fraction prepared from the livers of rodents treated with enzyme inducing agents. The use of other species, tissues or techniques may also be appropriate.

(6) *Control groups*—(i) *Concurrent controls*. Concurrent positive, negative, and vehicle controls should be included in each assay.

(ii) *Negative controls*. The negative control should show nonpreferential growth inhibition (i.e., should affect