(v) Criteria for identifying micronucleated erythrocytes.

(vi) Dose-response relationship, if applicable.

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

(1) Cihak, R. ''Evaluation of benzidine by the micronucleus test,'' *Mutation Research*, 67: 383–384 (1979).

(2) Cole, R.J., Taylor, N., Cole, J., Arlett, C.F. ''Short-term tests for transplacentally active carcinogens. 1. Micronucleus formation in fetal and maternal mouse erythroblasts,'' *Mutation Research*, 80: 141–157 (1981).

(3) Kliesch, U., Danford, N., Adler, I.D. ''Micronucleus test and bone-marrow chromosome analysis. A comparison of 2 methods in vivo for evaluating chemically induced chromosomal alterations,'' *Mutation Research*, 80: 321–332 (1981).

(4) Matter, B., Schmid, W. ''Trenimon-induced chromosomal damage in bone-marrow cells of six mammalian species, evaluated by the micronucleus test,'' *Mutation Research*, 12: 417–425 (1971).

(5) Schmid, W. ''The micronucleus test,'' *Mutation Research, 31:9–15 (1975).*

(6) Schmid, W. ''The micronucleus test for cytogenetic analysis,'' *Chemical Mutagens, Principles and Methods for their Detection.* Vol. 4 Hollaender A, (Ed. A ed. (New York and London: Plenum Press, (1976) pp. 31–53.

(7) Heddle, J.A., Hite, M., Kurkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W., Salamone, M.F. ''The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency GeneTox Program,'' *Mutation Research*, 123: 61–118 (1983).

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19080, May 20, 1987; 52 FR 26150, July 13, 1987; 52 FR 34654, Sept. 14, 1987]

§ 798.5450 Rodent dominant lethal assay.

(a) *Purpose.* Dominant lethal (DL) effects cause embryonic or fetal death. Induction of a dominant lethal event after exposure to a chemical substance indicates that the substance has affected germinal tissue of the test species. Dominant lethals are generally accepted to be the result of chromosomal damage (structural and numerical anomalies) but gene mutations and toxic effects cannot be excluded.

(b) *Definition.* A dominant lethal mutation is one occurring in a germ cell which does not cause dysfunction of the gamete, but which is lethal to the fertilized egg or developing embryo.

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

(d) *Test method*—(1) *Principle.* Generally, male animals are exposed to the test substance and mated to untreated virgin females. The various germ cell stages can be tested separately by the use of sequential mating intervals. The females are sacrificed after an appropriate period of time and the contents of the uteri are examined to determine the numbers of implants and live and dead embryos. The calculation of the dominant lethal effect is based on comparison of the live implants per female in the treated group to the live implants per female in the control group. The increase of dead implants per female in the treated group over the dead implants per female in the control group reflects the post-implantation loss. The post-implantation loss is calculated by determining the ratio of dead to total implants from the treated group compared to the ratio of dead to total implants from the control group. Pre-implantation loss can be estimated on the basis of corpora lutea counts or by comparing the total implants per female in treated and control groups.

(2) *Description.* (i) Several treatment schedules are available. The most widely used requires single administration of the test substance. Other treatment schedules, such as treatment on five consecutive days, may be used if justified by the investigator.

(ii) Individual males are mated sequentially to virgin females at appropriate intervals. The number of matings following treatment is governed by the treatment schedule and should ensure that germ cell maturation is adequately covered. Females are sacrificed in the second half of pregnancy and the uterine contents examined to determine the total number

of implants and the number of live and dead embryos.

(3) *Animal selection*—(i) *Species.* Rats or mice are generally used as the test species. Strains with low background dominant lethality, high pregnancy frequency and high implant numbers are recommended.

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

(iii) *Number.* An adequate number of animals shall be used taking into account the spontaneous variation of the biological characteristics being evaluated. The number chosen should be based on the predetermined sensitivity of detection and power of significance. For example, in a typical experiment, the number of males in each group shall be sufficient to provide between 30 and 50 pregnant females per mating interval.

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

(4) *Control groups*—(i) *Concurrent controls.* Generally concurrent positive and negative (vehicle) controls shall be included in each experiment. When acceptable positive control results are available from experiments conducted recently (within the last 12 months) in the same laboratory these results can be used instead of a concurrent positive control.

(ii) *Positive controls.* Positive control substances shall be used at a dose which demonstrates the test sensitivity.

(5) *Test chemicals*—(i) *Vehicle.* When possible, test substances shall be dissolved or suspended in isotonic saline or distilled water. 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.* Normally, three dose levels shall be used. The highest dose shall produce signs of toxicity (e.g., slightly reduced fertility and slightly reduced body weight). However, in an initial assessment of dominant lethality a single high dose may be sufficient. Nontoxic substances shall be tested at 5g/kg or, if this is not practicable, then as the highest dose attainable.

(iii) *Route of administration.* The usual routes of administration are oral or by IP injection. Other routes may be appropriate.

(e) *Test performance.* (1) Individual males are mated sequentially at appropriate predetermined intervals to one or two virgin females. Females should be left with the males for at least the duration of one estrus cycle or alternatively until mating has occurred as determined by the presence of sperm in the vagina or by the presence of a vaginal plug.

(2) The number of matings following treatment should be governed by the treatment schedule and should ensure that germ cell maturation is adequately covered.

(3) Females should be sacrificed in the second half of pregnancy and uterine contents examined to determine the number of implants and live and dead embryos. The ovaries may be examined to determine the number of corpora lutea.

(f) *Data and report*—(1) *Treatment of results.* Data shall be tabulated to show the number of males, the number of pregnant females, and the number of nonpregnant females. Results of each mating, including the identity of each male and female, shall be reported individually. For each female, the dose level and week of mating and the frequencies of live implants and of dead implants shall be enumerated. If the data are recorded as early and late deaths, the tables shall make that clear. If preimplantation loss is estimated, it shall be reported. Preimplantation loss can be calculated as the difference between the number of corpora lutea and the number of implants or as a reduction in the average number of implants per female in comparison with control matings.

(2) *Statistical evaluation.* Data shall be evaluated by appropriate statistical methods. Differences among animals within the control and treatment groups shall be considered before making comparisons between treated and control groups.

(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 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