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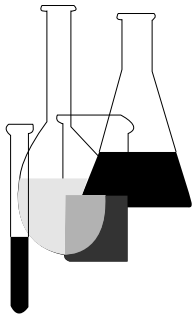
Health Effects Test Guidelines

OPPTS 870.5380 In Vivo Mammalian Cytogenetics Tests: Spermatogonial Chromosomal Aberrations



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 870.5380 In vivo mammalian cytogenetics tests: Spermatogonial chromosomal aberrations.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are the OPPT 40 CFR 798.5380 In Vivo Mammalian Cytogenetics Tests: Spermatogonial Chromosomal Aberrations and OPP 84–2 Mutagenicity Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982.

(b) **Purpose.** The purpose of the in vivo mammalian spermatogonial cell assay for chromosomal aberrations is to identify those agents that reach the mammalian gonad and induce structural chromosomal aberrations in spermatogonial mitoses. It is recognized that other assays and endpoints are useful for addressing the questions of gonadal exposure and induced genetic effects. This guideline, however, is restricted to the detection of chromosomal aberrations in spermatogonial cells.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Spermatogonia are the mitotically dividing cells which are the premeiotic progenitors of haploid gametes in the male gonad.

Structural chromosomal aberrations are changes in the morphology of the chromosome which are detectable during the metaphase stage of cell division. Aberration types can be observed as deletions, intrachanges or interchanges. These include breakage and reunion events which are expressed as chromatid-type aberrations, which involve single chromatids, or chromosome-type aberrations, which involve both sister chromatids.

(d) **Test method**—(1) **Principle**. The method relies upon testicular cell preparations from animals which have been exposed to the test substance by an appropriate route of administration and sacrificed at selected intervals after treatment. Prior to sacrifice, animals are treated with a spindle inhibitor such as colchicine in order to accumulate metaphase cells. In general, cells are examined at the first mitosis after exposure to the test agent. Chromosome preparations are made, stained and microscopically analyzed.

(2) **Animal selection**—(i) **Species and strain.** Although other mammalian species are useful for spermatogonial analyses, mice are the most widely used. This guideline emphasizes the use of the mouse as the test animal. At this time, there is no basis for recommending a specific strain.

(ii) **Age**. Young, sexually mature animals should be used.

(iii) **Number and sex**. At least eight male animals per treatment and control group are recommended. For those agents that may cause mortality, sufficient animals should be treated to ensure that there is a minimum of five survivors per group.

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

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

(ii) **Positive controls.** A compound known to produce chromosomal aberrations in spermatogonia should be employed as the positive control. When feasible, the positive control should be administered in the same vehicle and by the same route as the test chemical. In view of the animal-to-animal variability in response that may occur in this assay, it is suggested that the positive control be run at more than one dose level.

(4) **Test chemicals**—(i) **Vehicle**. When appropriate, solid and liquid substances should be dissolved or suspended in distilled water or isotonic saline. Water-insoluble chemicals may be dissolved or suspended in other appropriate vehicles. The vehicle used should not interfere with the test compound nor produce toxic effects. DMSO has been observed to induce adverse effects with certain substances, and is not recommended as a solvent. However, if it is used, it should not be used at toxic concentrations. Fresh preparations of the test compound should be employed.

(ii) **Dose levels.** The use of three dose levels is recommended. Dose selection should be made on the basis of range-finding experiment(s) that establish the maximum tolerated dose (MTD), which should be the highest dose tested. The MTD may be defined as the lowest dose that produces signs of overt toxicity or a significant suppression of mitoses. Alternatively, some high proportion (e.g. 50 to 80 percent) of the LD50 may be used as indicative that an acceptable MTD was achieved. Other doses should be selected to cover reasonable intervals on a dose response curve. For example, the second dose may be approximately 50 percent of the high dose and the third dose no less than 25 percent of the high dose. The highest dose for freely soluble nontoxic chemicals should be 5,000 mg/kg. Chemicals in suspension should be tested to the maximum amount feasible. Range-finding data should be based upon the species, strain, route of administration and treatment schedule to be used in the mutagenicity assay proper.

(iii) **Route of administration.** The usual routes of administration are intraperitoneal injection or oral gavage. Other routes may be acceptable; if other routes are used, they should be justified.

(iv) **Treatment schedule.** It has been routine practice in chromosomal aberration studies to administer the test chemical once only. However, based on toxicological information, a repeated treatment schedule may be employed. Under a multiple-treatment schedule, the spermatogonial chromosomal aberration assay may be run as part of subchronic studies. In at least one treatment group the treatment should induce some sign of toxicity in the animals. Other multiple treatment protocols, e.g. two to five treatments separated by 24 h, may be employed.

(e) **Test performance**—(1) **Treatment and sampling times**. (i) Two harvest times are recommended after single dose administration. Since the cell cycle time for mouse spermatogonial cells is estimated to be between 24 to 30 h, it is recommended that the first harvest be at 24 to 30 h after treatment and the second approximately 24 h after the first.

(ii) These harvest times should allow the detection of chromosomal aberrations induced by test agents which either do not affect or which delay the cell cycle. The 24– to 30–h harvest time would be expected to sample cells which, in the absence of delay or in the presence of slight delay, had completed at least one cell cycle. The second harvest time would be expected to detect metaphase cells which under nondelayed conditions had progressed through approximately two cell cycles. If there is cell cycle delay caused by the test agent, the second harvest time would be expected to yield variable ratios of first and second generation metaphase cells.

(iii) A single harvest is recommended after multiple dose treatment. This harvest should be approximately one cell cycle (24 to 30 h) after administration of the last dose. It is sufficient to harvest both negative and positive controls at only one time following treatment.

(2) **Administration of spindle inhibitor**. Approximately 3 to 5 h before they are sacrificed, animals should be injected intraperitoneally with an appropriate dose of a spindle inhibitor (e.g. colchicine) to arrest cells in metaphase.

(3) **Preparation of slides.** Immediately after sacrifice, testicular samples should be obtained. Spermatogonial cells are exposed to hypotonic solution and fixed. Some methods use acid or enzyme steps to increase the yield of spermatogonial cells for analysis. The cells should then be spread on slides and stained. Chromosome preparations should be made following standard procedures.

(4) **Confirmatory experiments.** Good scientific practice suggests that every experiment should be verified in an independent repeat assay. However, assuming that the procedures described above have been properly conducted, there is no absolute requirement for a repeat assay. In the case of a marginal or equivocal response, or of a positive response at a single dose, a repeat assay should be required. In the repeat assay, modification

of study parameters (e.g. dose level, number of doses, and sacrifice time) should be considered.

(5) **Analysis.** Slides should be coded so that the persons evaluating the assay are unaware of the identity of treatment and control groups. Cells from all animals surviving treatment should be scored. Where possible, at least 50 spermatogonial cells per animal should be scored. When it is not feasible to score 50 cells from an animal, the maximum practical number of cells should be scored. Scoring less than 50 cells per animal must be justified.

(f) **Data and report**—(1) **Treatment of results.** Individual animal data should be presented in tabular form for each treatment and control group. For each animal, the number of cells scored, the numbers and types of structural aberrations, the aberrations per cell and the percent of cells with aberrations should be listed. In addition to the individual animal data, group means and standard deviations should be presented. Group data should be based upon the mean frequencies per animal. Gaps (achromatic lesions) should be recorded separately and should not be included in the calculation of aberration frequencies.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods.

(3) **Interpretation of results.** There are several degrees of response in a mutagenicity assay. Although most assays will give clearly positive or negative results, in some tests the data set will preclude making a definitive judgement about the activity of the test agent. These equivocal or questionable responses will occur regardless of protocol employed or the number of times the assay is repeated. For these chemicals, it is essential that all available information on the test agent be evaluated when making judgments about mutagenic activity or lack thereof in a specific assay.

(i) There are several criteria for determining a positive response. The two most common criteria will be discussed here, although it is recognized that there are others which also define a valid positive response. One criterion is a statistically significant dose-related increase in the number of spermatogonia with chromosomal aberrations. Another criterion is based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) If these or other acceptable criteria for a positive response are not met, and assuming that a definitive judgement can be made about the lack of genetic activity, the test agent is considered to be nonmutagenic in this assay.

(iii) In all instances, scientific judgement should enter into the evaluation of test results.

(4) **Test evaluation.** (i) A positive spermatogonial aberration assay indicates that, under the test conditions, the substance induces a significant increase in the frequency of chromosomal aberrations in the gonad of the test species.

(ii) Negative results indicate that under the test conditions the substance does not produce a significant increase in the frequency of chromosomal aberrations in the gonad of the test species.

(5) **Test report.** In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, and under paragraph (g)(1) of this guideline, the following specific information should be reported:

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

(ii) Identity and purity of the test chemical, vehicle, dose levels used, rationale for dose selection, and route of administration.

(iii) Identity of spindle inhibitor, its concentration, and duration of treatment.

(iv) Rationale for and description of treatment and sampling schedules, toxicity data, negative and positive controls.

(v) Details of the protocol used for slide preparation and staining of cells.

(vi) Criteria for scoring aberrations.

(vii) The statistical methods used.

(viii) Dose-response relationship, if applicable.

(ix) Historical control data, if available, for both positive and negative controls.

(x) Results obtained and statistical analysis.

(g) **References**. The following references should be consulted for additional background material on this test guideline.

(1) Ashby, J. et al., Overview of the study in relation to protocol design of the rodent bone-marrow micronucleus assay. *Mutation Research* 234:223–248 (1990).

(2) Adler, I.D. and Brewen, J.G., Effect of chemicals on chromosome aberration production in male and female germ cells. In *Chemical Mutagens, Principles and Methods in their Detection*. Eds. F.J. de Serres, A. Hollaender. Plenum, New York (1982) pp. 1–35.

(3) Adler, I.D., Cytogenetic tests in mammals, *Mutagenicity Testing, A Practical Approach.* Eds. S. Venitt, J.M. Parry, (Oxford: IRL Press, 1984) pp. 275–306.

(4) Albanese, R. et al. Mammalian germ cell cytogenetics. Report of the UKEMS subcommittee of guidelines for mutagenicity studies, Part II, 1984. pp. 145–172.

(5) Brewen, J.G. and Preston, R.J. Analysis of chromosome aberrations in mammalian germ cells, *Chemical Mutagens: Principles and Methods for Their Detection*, Vol 5. Eds. A. Hollaender, F.J. de Serres, (New York: Plenum Press, 1978) pp. 127–150.

(6) Preston, R.J. et al., Mammalian in vivo and in vitro cytogenetics assays: Report of the Gene-Tox Program. *Mutation Research* 87:143–188 (1981).