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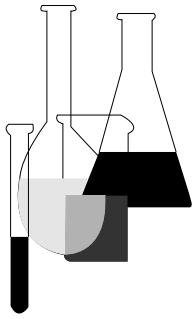
Health Effects Test Guidelines

OPPTS 870.5385
In Vivo Mammalian Cytogenetics Tests: Bone Marrow Chromosomal Analysis



"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.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 870.5385 In vivo mammalian cytogenetics tests: Bone marrow chromosomal analysis.

(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 material used in developing this harmonized OPPTS test guideline is OPPT 40 CFR 798.5385 In Vivo Mammalian Bone Marrow Cytogenetics Tests: Chromosomal Analysis; OPP 84–2 Mutagenicity Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD guideline 475 Genetic Toxicology: In Vivo Mammalian Bone Marrow Cytogenetics Tests—Chromosomal Analysis.

(b) **Purpose.** The in vivo bone marrow cytogenetic test is a mutagenicity test for the detection of structural chromosomal aberrations. Chromosomal aberrations are generally evaluated in first posttreatment mitoses. With the majority of chemical mutagens, induced aberrations are of the chromatid type but chromosome type aberrations also occur.

(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.

Chromatid-type aberrations are damage expressed as breakage of single chromatids or breakage and/or reunion between chromatids.

Chromosome-type aberrations are changes which result from damage expressed in both sister chromatids at the same time.

(d) **Test method**—(1) **Principle.** Animals are exposed to test chemicals by appropriate routes and are sacrificed at sequential intervals. Chromosome preparations are made from bone marrow cells. The stained preparations are examined and metaphase cells are scored for chromosomal aberrations.

(2) **Description.** The method employs bone marrow of laboratory rodents which have been exposed to test chemicals. Prior to sacrifice, animals are further treated with a spindle inhibitor, (e.g., colchicine or Colcemid ®) to arrest the cells in *c*-metaphase. Chromosome preparations from the cells are stained and scored for chromosomal aberrations.

(3) **Animal selection**—(i) **Species and strain.** Any appropriate mammalian species may be used. Examples of commonly used rodent species are rats, mice, and hamsters.

(ii) **Age.** Healthy young adult animals should be used.

(iii) **Number and sex.** At least five female and five male animals per experimental and control group should be used. Thus, 10 animals would be sacrificed per time per group treated with the test compound if several test times after treatment are included in the experimental schedule. The use of a single sex or smaller number of animals should be justified.

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

(4) **Control groups**—(1) **Concurrent controls.** (i) Concurrent positive and negative (vehicle) controls should be included in the assay.

(ii) **Positive controls.** A single dose positive control showing a significant response at any one time point is adequate. A compound known to produce chromosomal aberrations in vivo should be employed as the positive control.

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

(ii) **Dose levels.** For an initial assessment, one dose of the test substance may be used, the dose being the maximum tolerated dose (to a maximum of 5,000 mg/kg) or that producing some indication of cytotoxicity (e.g., partial inhibition of mitosis) or should be the highest dose attainable (to a maximum of 5,000 mg/kg). Additional dose levels may be used. For determination of dose-response, at least three dose levels should be used.

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

(iv) **Treatment schedule.** In general, test substances should be administered once only. However, based on toxicological information a repeated treatment schedule may be employed.

(e) **Test performance**—(1) **Generally the test may be performed in two assays.** (i) Animals should be treated with the test substance once at the selected doses. Samples should be taken at three times after treatment. For rodents, the central sampling interval is 24 h. Since cell cycle kinetics can be influenced by the test substance, one earlier and one later sampling interval adequately spaced within the range of 6 to 48 h should be applied. Where the additional dose levels are tested in a subsequent experiment, samples should be taken at the predetermined most sensitive interval or, if this is not established, at the central sampling time. If the

most sensitive interval is known and documented with data, only this one time point should be sampled.

(ii) If a repeated treatment schedule is used at the selected doses, samples should be taken 6 and 24 h after the last treatment; other sampling times may be used if justified. Where the additional dose levels are tested in a subsequent experiment, samples should be taken at the predetermined most sensitive interval or, if this is not established, at 6 h after the last treatment.

(2) **Administration of spindle inhibitor.** Prior to sacrifice, animals should be injected IP with an appropriate dose of a spindle inhibitor (e.g., colchicine or Colcemid®) to arrest cells in *c*-metaphase.

(3) **Preparation of slides.** Immediately after sacrifice, the bone marrow should be obtained, exposed to hypotonic solution, and fixed. The cells should then be spread on slides and stained. Chromosome preparations should be made following standard procedures.

(4) **Analysis.** The number of cells to be analyzed per animal should be based upon the number of animals used, the negative control frequency, the predetermined sensitivity, and the power chosen for the test. Slides should be coded before microscopic analysis.

(f) **Data and report**—(1) **Treatment of results.** Data should be presented in tabular form for both cells and animals. Different types of structural chromosomal aberrations should be listed with their numbers and a mean frequency per cell for each animal in all treated and control groups. Gaps (achromatic lesions) should be recorded separately and not included in the total abberration frequency. Differences among animals within each group should be considered before making comparisons between treated and control groups.

(2) **Statistical evaluation.** Data should 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 structual chromosomal aberrations or abnormal metaphase figures. Another criterion may be based upon detection of a reproducible and statistically significant positive response for a 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 chromosomal aberrations or abnormal metaphase figures 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 in vivo bone marrow cytogenetics assay indicate that under the test conditions the test substance induces chromosomal aberrations in the bone marrow of the test species.

(ii) Negative results indicate that under the test conditions, the test substance does not induce chromosomal aberrations in the bone marrow 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 should be reported:

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

(ii) Test chemical vehicle, dose levels used, rationale for dose selection.

(iii) Route of administration, treatment and sampling schedules, toxicity data, negative and positive controls.

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

(v) Details of the protocol used for chromosome preparation, number of cells scored per animal, type and number of aberrations given separately for each treated and control animal.

(vi) Mitotic index, where applicable.

(vii) Criteria for scoring aberrations.

(viii) Number and frequency of aberrant cells per animal in each treatment and control groups.

(ix) Total number of aberrations per group.

(x) Number of cells with aberrations per group.

(xi) Dose-response relationship, if applicable.

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

(1) Adler, I.D. et al., In vivo cytogenetic effects of trimethyl phosphate and of TEPA on bone marrow cells of male rats, *Mutation Research* 13:263–273 (1971).

(2) Evans, H.J. Cytological methods for detecting chemical mutagens, *Chemical Mutagens: Principles and Methods for Their Detection,* Vol. 4. Ed. A. Hollaender. Plenum, New York and London. (1976) pp. 1–29.

(3) Kilian, J.D. et al. A collaborative study to measure intralaboratory variation with the in vivo bone morrow metaphase procedure. *Handbook of mutagenicity test procedures.* Eds. Kilby, B.J., Legator, M. Nichols, C., Ramel, D. Elsevier/North Holland Biomedical Press, Amsterdam (1977) 243–260.

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