

(3) *Neurotoxicity*—(i) *Required testing.* (1) An acute and subchronic functional observation battery shall be conducted with MBT in accordance with § 798.6050 of this chapter except for the revisions in paragraphs (d)(5) and (6) of § 798.6050.

(2) For the purpose of this section, the following provisions also apply:

(i) *Duration and frequency of exposure.* For acute study, animals shall be administered MBT over a period not to exceed 24 hours. For subchronic study, animals shall be dosed daily for at least 90 days.

(ii) *Route of exposure.* Animals shall be exposed to MBT orally.

(B)(1) An acute and subchronic motor activity test shall be conducted with IBT in accordance with § 798.6200 of this chapter except for the provisions in paragraphs (d)(5) and (6) of § 798.6200.

(2) For the purpose of this section the following provisions also apply:

(i) *Duration and frequency of exposure.* For acute study, animals shall be administered over a period not to exceed 4 hours. For subchronic study, animals shall be dosed daily for at least 90 days.

(ii) *Route of exposure.* Animals shall be exposed to MBT orally.

(C)(1) A subchronic neuropathology test shall be conducted with MBT in accordance with § 798.6400 of this chapter except for the provisions in paragraphs (d)(5) and (6) of § 798.6400.

(2) For the purpose of this section, the following provisions also apply:

(i) *Duration and frequency of exposure.* Animals shall be dosed daily for at least 90 days.

(ii) *Route of exposure.* Animals shall be exposed to MBT orally.

(ii) *Reporting requirements.* (A) The functional observation battery, motor activity, and neuropathology tests shall be completed and the final reports for each test submitted to EPA within 18 months of the effective date of the final rule.

(B) A progress report shall be submitted to EPA for the functional observation battery, motor activity, and neuropathology tests, respectively, 6 months after the effective date of the final rule.

(4) *Mutagenic effects—Chromosomal aberrations*—(i) *Required testing.* (A) A

dominant lethal assay shall be conducted with MBT in accordance with § 798.5450 of this chapter, using the oral route of administration.

(B) A heritable translocation assay shall be conducted with MBT in accordance with the test guideline specified in § 798.5460 of this chapter if MBT produces a positive result in the dominant lethal assay conducted pursuant to paragraph (e)(4)(i)(A) of this section and if, after a public program review, EPA issues a FEDERAL REGISTER notice or sends a certified letter to the test sponsor specifying that the testing shall be initiated.

(ii) *Reporting requirements.* (A) *Mutagenic effects—Chromosomal aberration testing of MBT* shall be completed and the final report submitted to EPA as follows: Dominant lethal assay, within 12 months after the effective date of this rule; heritable translocation assay, within 24 months after notification under paragraph (e)(4)(i)(B) of this section that the testing shall be initiated.

(B) For the dominant lethal assay, an interim progress report shall be submitted to EPA 6 months after the effective date of the final rule; for the heritable translocation assay, progress reports shall be submitted to EPA at 6-month intervals beginning 6 months after the date of EPA's notification of the test sponsor that testing shall be initiated until submission of the final report.

(f) *Effective date.* (1) The effective date of this final rule is October 21, 1988, except for paragraphs (a)(2), (d)(1)(i), (d)(2)(i)(B)(3), and (e)(3)(ii)(A) of this section. The effective date for paragraphs (a)(2), (d)(1)(i), (d)(2)(i)(B)(3), and (e)(3)(ii)(A) of this section is March 1, 1990.

(2) The guidelines and other test methods cited in this rule are referenced as they exist on the effective date of the final rule.

[53 FR 34530, Sept. 7, 1988; 53 FR 37393, Sept. 26, 1988, as amended at 55 FR 7326, Mar. 1, 1990; 58 FR 34205, June 23, 1993]

### § 799.2500 Mesityl oxide (MO).

(a) *Identification of test substance.* (1) Mesityl oxide (CAS No. 141-79-7) shall be tested in accordance with this section.

(2) Mesityl oxide of at least 97 per cent purity shall be used as the test substance.

(b) *Persons required to submit study plans, conduct tests, and submit data.* (1) All persons who manufacture or process or intend to manufacture or process MO from the effective date of this rule, February 3, 1986, to the end of the reimbursement period shall submit letters of intent to conduct testing or exemption applications, study plans, and/or shall conduct tests, and submit data as specified in this section, subpart A of this part, and part 790 of this chapter.

(2) Persons subject to this section are not subject to the requirements of § 790.50(a)(2), (5), and (6) and (b), and § 790.87(a)(1)(ii) of this chapter.

(3) Persons who notify EPA of their intent to conduct tests in compliance with the requirements of this section must submit plans for these tests no later than 30 days before the initiation of each of those tests.

(4) In addition to the requirements of § 790.87(a)(2) and (3) of this chapter, EPA will conditionally approve exemption applications for this rule if EPA has received a letter of intent to conduct the testing from which exemption is sought and EPA has adopted test standards and schedules in a final Phase II test rule.

(c) *Health effects testing—(1) Subchronic inhalation toxicity—(i) Required testing.* A 90-day subchronic inhalation toxicity test shall be conducted with MO.

(ii) *Test standard.* Inhalation subchronic toxicity testing shall be conducted with MO in accordance with § 798.2450 of this chapter, except for the provisions of § 798.2450 (d)(1)(i) and (d)(11)(i)(A).

(iii) For the purposes of this section the following provisions also apply:

(A) *Animal Selection—species and strain.* The rat shall be used. Commonly used laboratory strains should be employed. The tester should provide justification/reasoning for its selection.

(B) *Clinical examinations.* Certain hematological determinations shall be carried out at least three times during the test period: just prior to initiation of dosing (base line data), after approximately 30 days on test, and just

prior to terminal sacrifice at the end of the test period. Hematology determinations which shall be appropriate to all studies include the following: Hematocrit, hemoglobin concentration, erythrocyte count, total and differential leucocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(iv) *Reporting requirements.* (A) The subchronic testing shall be completed and the final results submitted to the Agency within 15 months of the effective date of the final Phase II test rule.

(B) Progress reports shall be provided every 6 months beginning 6 months after the effective date of the final Phase II test rule.

(2) *Mutagenic effects—chromosomal aberrations—(i) Required testing.* (A) An *in vitro* cytogenetic test shall be conducted with MO.

(B) An *in vivo* cytogenetic test shall be conducted for MO if the *in vitro* cytogenetic test conducted pursuant to paragraph (c)(2)(i)(A) of this section produces a negative result.

(C) A dominant lethal assay shall be conducted for MO if it produces a positive result in the *in vivo* or *in vitro* cytogenetics test conducted pursuant to paragraphs (c)(2)(i)(A) and (B) of this section.

(D) A heritable translocation assay shall be conducted for MO if it produces a positive result in the dominant lethal assay conducted pursuant to paragraph (c)(2)(i)(C) of this section.

(ii) *Test standard.* (A) (1) The *in vitro* mammalian cytogenetics test shall be conducted with MO in accordance with § 798.5375 of this chapter except for the provisions in § 798.5375 (d)(3)(i) and (d)(6)(ii).

(2) For the purposes of this section the following provisions also apply:

(i) *Type of cells used in the assay.* MO shall be tested in established cell lines. The cell line or strain used shall be checked for *Mycoplasma* contamination and for karyotype stability.

(ii) *Exposure concentrations.* At least 3 concentrations of the test substance over a range adequate to define the response shall be tested. The highest test concentration tested with and without metabolic activation shall be 5 milligrams per milliliter or that dose which

ows evidence of cytotoxicity or reduced mitotic activity.

(B)(1) The *in vivo* mammalian bone marrow cytogenetics test: Chromosomal analysis shall be conducted with MO in accordance with §798.5385 of this chapter except for the provisions in §798.5385(d)(5) (ii) and (iii).

(2) For the purposes of this section the following provisions also apply:

(i) *Dose levels.* Three dose levels shall be used. The highest dose tested shall be the maximum tolerated dose or that producing some indication of cytotoxicity (e.g., partial inhibition of mitosis), or shall be the highest dose attainable.

(ii) *Route of administration.* The animals shall be exposed by inhalation for 6 hours/day for 5 consecutive days.

(C)(1) The rodent dominant lethal assay shall be conducted with MO in accordance with §798.5450 of this chapter except for the provisions in §798.5450(d)(5) (ii) and (iii).

(2) For the purposes of this section the following provisions also apply:

(i) *Dose levels.* Three dose levels shall be used. The highest dose shall produce signs of toxicity (e.g., slightly reduced fertility or body weight) or shall be the highest attainable.

(ii) *Route of administration.* Exposure shall be by inhalation for 5 days for 6 hours/day.

(D)(1) The rodent heritable translocation test shall be conducted with MO in accordance with §798.5460 of this chapter except for the provisions in §798.5460(d)(5) (ii) and (iii).

(2) For the purposes of this section the following provisions also apply:

(i) *Dose levels.* At least two dose levels shall be used. The highest dose shall result in toxic effects (which shall not reduce an incidence of fatalities which would prevent a meaningful evaluation), or shall be the highest dose attainable.

(ii) *Route of administration.* Animals shall be exposed by inhalation.

(iii) *Reporting requirements.* (A) The chromosomal aberration tests shall be completed and the final results submitted to the Agency as follows:

(1) The *in vitro* and *in vivo* (conditional) tests within 15 months of the effective date of the final Phase II test rule.

(2) The dominant lethal assay (conditional) within 24 months of the effective date of the final Phase II test rule.

(3) The heritable translocation test (conditional) within 24 months of the date of EPA's notification of the test sponsor by certified letter or FEDERAL REGISTER notice that testing shall be initiated.

(B) Progress reports shall be submitted to the Agency for the *in vitro* and *in vivo* cytogenetics assays and the dominant lethal assay at 6-month intervals, the first of which is due within 6 months of the effective date of the final Phase II rule.

(C) Progress reports shall be submitted to the Agency for the heritable translocation assay at 6-month intervals, the first of which is due within 6 months of the date of EPA's notification of the test sponsor that testing shall be initiated.

(3) *Mutagenic effects—gene mutations—*

(i) *Required testing.* (A) A *Salmonella typhimurium* mammalian microsomal reverse mutation assay (Ames assay) shall be conducted with MO.

(B) A sex-linked recessive lethal test in *Drosophila melanogaster* shall be conducted for MO if it produces a positive result in the Ames assay conducted pursuant to paragraph (c)(3)(i)(A) of this section.

(C) A gene mutation in somatic cells assay shall be conducted with MO if it produces a negative result in the Ames assay conducted pursuant to paragraph (c)(3)(i)(A) of this section.

(D) A sex-linked recessive lethal test in *Drosophila melanogaster* shall be conducted for MO if it produces a positive result in the gene mutation in somatic cells assay conducted pursuant to paragraph (c)(3)(i)(C) of this section.

(E) A mouse specific-locus test shall be conducted for MO if it produces a positive result in the sex-linked recessive-lethal test in *Drosophila melanogaster* conducted pursuant to paragraph (c)(3)(i)(B) or (D) of this section.

(ii) *Test standards—(A)(1)* The *Salmonella typhimurium* mammalian microsomal reverse mutation assay (Ames assay) shall be conducted with MO in accordance with §798.5265 of this chapter except for the provisions in

§ 798.5265 (d)(5)(ii), (d)(6)(ii) (A) and (B), and (e)(1).

(2) For the purposes of this section the following provisions also apply:

(i) *Strain specific positive controls.* Strain specific positive controls shall be included in the assay. The following controls are examples of those which may be used in the assay without metabolic activation: Strain TA 1535, sodium azide; strain TA 100, nitrofurantoin; strains TA 98 and TA 1537, 4-nitro-*o*-phenylenediamine.

(ii) *Exposure concentrations.* The test should initially be performed over a broad range of concentrations. Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of metabolic activation systems. Toxicity may be evidenced by a reduction in the number of spontaneous revertants, a clearing of the background lawn or by the degree of survival of treated cultures. Relatively insoluble compounds should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis. MO shall be tested up to 5 milligrams per plate or to the limits of solubility or toxicity. A suspected positive response not showing a clear dose-related response shall be confirmed by testing over a narrow range of concentrations.

(iii) *Test performance—Direct plate incorporation method.* The direct plate incorporation method shall be used for this test. For this test without metabolic activation, test chemical and 0.1 milliliter of a fresh bacterial culture should be added to 2.0 milliliter of overlay agar.

(B)(1) The detection of gene mutations in somatic cells in culture shall be conducted with MO in accordance with § 798.5300 of this chapter except for the provisions in § 798.5300 (d)(3)(i), (4), and (6)(i) and (e)(1).

(2) For the purposes of this section the following provisions also apply:

(i) *Types of cells used in the assay.* MO shall be tested at the HGPRT locus in the Chinese hamster ovary cell culture

test or in LK5178K mouse lymphoma cells.

(ii) *Metabolic activation.* Cells shall be exposed to MO both in the presence and absence of a metabolic activation system derived from the postmitochondrial fraction (S-9) of livers from rats pretreated with Aroclor 1254.

(iii) *Vehicle.* MO may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. The final concentration of the vehicle shall not interfere with cell viability or growth rate.

(iv) *Test performance.* Cells shall be exposed to MO both with and without exogenous activation. Exposure shall be for 4 hours unless a different exposure time is justified by the investigator.

(C)(1) The sex-linked recessive lethal test in *Drosophila melanogaster* shall be conducted with MO in accordance with § 798.5275 of this chapter except for the provisions in paragraph (d)(5)(iii).

(2) For the purposes of this section the following provisions also apply: *Route of administration.* Exposure shall be by exposure to MO vapors.

(D)(1) The mouse visible specific locus test shall be conducted with MO in accordance with § 798.5200 of this chapter except for the provisions in § 798.5200(d) (5) (ii) and (iii).

(2) For the purposes of this section the following provisions also apply:

(i) *Dose levels.* A minimum of 2 dose levels shall be tested. Exposure shall be for 6 hours a day. Duration of exposure shall be dependent upon accumulated total dose desired for each group.

(ii) *Route of administration.* Animals shall be exposed to MO by inhalation.

(iii) *Reporting requirements—*(A) The gene mutation tests shall be completed and final results submitted to the Agency as follows:

(1) The *Salmonella typhimurium* mammalian microsomal reverse mutation assay and the gene mutation in somatic cells assay (conditional) within 12 months of the effective date of the final Phase II test rule.

(2) The sex-linked recessive-lethal test in *Drosophila melanogaster* (conditional) within 25 months of the effective date of the final Phase II test rule.

(3) The mouse specific-locus test (conditional) within 48 months of the date of EPA's notification of the test sponsor by certified letter or FEDERAL REGISTER notice that testing shall be initiated.

(B) Progress reports shall be submitted to the Agency for the *Salmonella phimurium* mammalian reverse mutation microsomal assay, gene mutation in mammalian cells in culture assays, and *Drosophila* sex-linked recessive lethal test at 6-month intervals, the first of which is due within 6 months of the effective date of the final Phase II rule.

(C) Progress reports shall be submitted to the Agency for the mouse specific locus assay at 6-month intervals, the first of which is due within 6 months of the date of EPA's notification of the test sponsor that testing shall be initiated.

(4) *Oncogenicity*—(i) *Required testing.* An oncogenicity bioassay shall be conducted by inhalation for MO if MO gives positive results in any one or more of the following tests:

(A) *In vitro* cytogenetics test, conducted pursuant to paragraph (2)(i)(A) of this section.

(B) *In vivo* cytogenetics test, conducted pursuant to paragraph (2)(i)(B) of this section.

(C) Gene mutation in somatic cells assay, conducted pursuant to paragraph (c)(3)(i)(C) of this section.

(D) *Drosophila melanogaster* sex-linked recessive-lethal test, conducted pursuant to paragraph (c)(3)(i)(B) or (D) of this section.

(ii) *Test standard.* (A) (1) An oncogenicity bioassay shall be conducted by inhalation with MO in accordance with §798.3300 of this chapter except for the provisions in §798.3300(b)(1)(i), (4), and (6).

(2) For the purposes of this section the following provisions also apply:

(i) *Species and strain.* MO shall be tested in both rats and mice. Commonly used laboratory strains should be employed. The tester should provide justification/reasoning for their selection.

(ii) *Exposure conditions.* Animals shall be exposed to MO for at least 6 hours per day on a 5-day per week basis over a period of at least 24 months for rats and 18 months for mice.

(iii) *Administration of the test substance.* Animals shall be exposed to MO by the inhalation route.

(B) [Reserved]

(iii) *Reporting requirements.* (A) The oncogenicity tests shall be completed and final results submitted to the Agency 53 months after the date of EPA's notification of the test sponsor by certified letter or FEDERAL REGISTER notice that testing shall be initiated.

(B) Progress reports shall be submitted to the Agency at 6-month intervals, the first of which is due within 6 months after the date of EPA's notification of the test sponsor that testing shall be initiated.

(d) *Effective date.* The effective date of this final Phase II rule for mesityl oxide is July 6, 1987.

[50 FR 51866, Dec. 20, 1985; 51 FR 1793, Jan. 15, 1986, as amended at 52 FR 19093, May 20, 1987; 58 FR 34205, June 23, 1993]

#### § 799.2700 Methyl ethyl ketoxime.

(a) *Identification of test substance.* (1) Methyl ethyl ketoxime (MEKO, CAS No. 96-29-7) shall be tested in accordance with this section.

(2) MEKO of at least 99 percent purity shall be used as the test substance.

(b) *Persons required to submit study plans, conduct tests, and submit data.* All persons who manufacture (including import) or process or intend to manufacture or process MEKO, including persons who manufacture or process or intend to manufacture or process MEKO as a byproduct, or who import or intend to import products which contain MEKO, after the date specified in paragraph (e) of this section to the end of the reimbursement period, shall submit letters of intent to conduct testing, submit study plans, conduct tests and submit data, or submit exemption applications, as specified in this section, subpart A of this part, and parts 790 and 792 of this chapter for single-phase rulemaking. Persons who manufacture, import, or process MEKO only as an impurity are not subject to these requirements.

(c) *Health effects testing*—(1) *Pharmacokinetics testing*—(i) *Required testing.* Pharmacokinetics testing shall be conducted with MEKO in accordance with paragraph (c)(1)(ii) of this section.