

Environmental Protection Agency

§ 799.5075

they exist on the effective date of the final rule.

[53 FR 22324, June 15, 1988; 53 FR 48645, Dec. 2, 1988, as amended at 54 FR 49760, Dec. 1, 1989; 55 FR 7324, Mar. 1, 1990; 56 FR 23232, May 21, 1991; 58 FR 34205, June 23, 1993]

§ 799.5075 Drinking water contaminants subject to testing.

(a) *Identification of test substance.* (1) 1,1,2,2-tetrachloroethane (CAS No. 79-34-5), and 1,3,5-trimethylbenzene (CAS No. 108-67-8) shall be tested as appropriate in accordance with this section.

(2) A test substance of at least 99 percent purity shall be used for Chloroethane, 1,1-dichloroethane, and 1,3,5-trimethylbenzene. A test substance of at least 98 percent purity shall be used for 1,1,2,2-tetrachloroethane.

(b) *Persons required to submit study plans, conduct tests, and submit data.* All persons who manufacture (including import and by-product manufacture) or process, or who intend to manufacture or process, the substances listed in paragraph (a) of this section after the effective date of this section to the end of the reimbursement period shall submit letters of intent to test, 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 rule-making, for the substances they manufacture subject to exclusions contained in § 790.42(a)(2), (a)(4) and (a)(5). These sections provide that processors, persons who manufacture less than 500 kg (1,100 lbs) annually, or persons who manufacture small quantities of the chemical solely for research and development as defined in § 790.42(a)(5) shall not be required to submit study plans, conduct tests and submit data, or submit exemption applications as specified in this section unless directed to do so in a subsequent notice as set forth in § 790.48(b).

(c) *Health effects testing—(1) Subacute toxicity—(i) Required testing.* (A) An oral 14-day repeated dose toxicity test shall be conducted with 1,1,2,2-tetrachloroethane, and 1,3,5-trimethylbenzene in accordance with § 798.2650 of this chapter except for the provisions in § 798.2650 (a), (b)(1), (c),

(e)(3), (e)(4)(i), (e)(5), (e)(6), (e)(7)(i), (e)(7)(iv), (e)(7)(v), (e)(8)(vii), (e)(9)(i)(A), (e)(9)(i)(B), (e)(11)(v), and (f)(2)(i). Each substance shall be tested in one mammalian species, preferably a rodent, but a non-rodent may be used. The species and strain of animals used in this test should be the same as those used in the 90-day subchronic test required in paragraph (c)(2)(i) of this section. The tests shall be performed using drinking water. However, if, due to poor stability or palatability, a drinking water test is not feasible for a given substance, that substance shall be administered either by oral gavage, in the diet, or in capsules.

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

(1) *Purpose.* To assess and evaluate the toxic characteristics of a substance, the determination of subacute toxicity should be carried out after initial information on toxicity has been obtained by acute testing. The 14-day repeated dose oral study provides information on the health hazard likely to arise from repeated short-term exposure by the oral route over a very limited period of time. It has been designed to permit the determination of the no-observed-adverse-effect level and toxic effects associated with continuous or repeated exposure to a test substance for 14 days and to evaluate reversibility, persistence, and delayed occurrence of toxic effects during a 14-day follow-up recovery period. The test is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). It will provide information on target organs and the possibility of accumulation, and can be used in selecting dose levels for subchronic studies and for establishing safety criteria for short-term human exposure.

(2) *Definitions.* Subacute oral toxicity is the manifestation of adverse effect(s) occurring as a result of the repeated daily exposure of experimental animals to a substance by the oral route for 14 days.

(3) *Principle of the test method.* The test substance is administered orally in graduated daily doses to several groups of experimental animals, one dose level

per group, for a period of 14 days. During the period of administration the animals are observed daily to detect signs of toxicity. Animals which die during the period of administration are necropsied. At the conclusion of the test, all animals, except the satellite group, are necropsied and histopathological examinations are carried out. The satellite group is necropsied after the 14-day recovery period.

(4) *Satellite group (Rodent only)*. A satellite group of 20 animals (10 animals per sex) shall be treated with the high dose level for 14 days and observed for reversibility, persistence, and delayed occurrence of toxic effects for a post-treatment recovery period of at least 14 days.

(5) *Dose levels and dose selection*. In subacute toxicity tests, it is desirable to have a dose response relationship as well as a NOAEL. Therefore, at least 3 dose levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) shall be used. Doses shall be spaced appropriately to produce test groups with a range of toxic effects. The data should be sufficient to produce a dose-response curve.

(6) *Exposure conditions*. The animals are dosed with the test substance every day for 14 days.

(7) *Observation period*. All animals shall be observed daily during the 14-day exposure period.

(8) *Observation period of satellite group*. Animals in the satellite group scheduled for follow-up observations shall be kept for at least 14 days further without treatment to detect recovery from, or persistence of, and delayed onset of toxic effects and shall be observed daily.

(9) *Administration of test substance*. For substances of low toxicity, it is important to ensure that when administered in the drinking water, by gavage, in the diet, or in capsules, the quantities of the test substance involved do not interfere with normal nutrition. When the test substance is administered in the diet, either a constant dietary concentration (ppm) or a constant dose level in terms of the animals' body weight shall be used; the alter-

native used shall be specified in the final test report.

(10) *Time of administration of test substance*. For a substance administered by gavage or capsule, the dose shall be given at approximately the same time each day, and adjusted on day 7 to maintain a constant dose level in terms of animal body weight.

(11) *Observation of animals*. At the end of the 14-day exposure period, all survivors, except those in the satellite group, shall be necropsied. All survivors in the satellite group shall be necropsied after a recovery period of at least 14 days.

(12) *Hematology determinations*. Certain hematology determinations shall be carried out at least two times during the test period: Just prior to initiation of dosing if adequate historical baseline data are not available (baseline data) and just prior to terminal sacrifice at the end of the test period. Hematology determinations which are appropriate to all studies are: Hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(13) *Clinical biochemical determinations*. Certain clinical biochemistry determinations on blood should be carried out at least two times: Just prior to initiation of dosing (if adequate historical baseline data are not available) and just prior to terminal sacrifice at the end of the test period. Test areas which are considered appropriate to all studies are: Electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations are: Calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with the period of fasting appropriate to the species), serum alanine aminotransferase, serum aspartate aminotransferase, gamma glutamyl transpeptidase, urea nitrogen, albumin, blood creatinine, and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include: analyses of lipids, hormones, acid/base balance,

methemoglobin, and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

(14) *Histopathology.* Histopathology of the lungs of all animals shall be performed. Special attention to examination of the lungs of rodents shall be made for evidence of infection since this provides a convenient assessment of the state of health of the animals.

(15) *Evaluation of the study results.* The findings of a subacute oral toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects and the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted subacute test should provide a satisfactory estimation of a NOAEL.

(ii) *Reporting requirements.* (A) Each subacute test shall be completed and the final report submitted to EPA within 12 months of the date specified in paragraph (d)(1) of this section, except for 1,1,2,2-tetrachloroethane. The subacute testing for 1,1,2,2-tetrachloroethane. The subacute testing for 1,1,2,2-tetrachloroethane shall be completed and the final report submitted to EPA by February 15, 1996.

(B) Except for 1,3,5-trimethylbenzene, a progress report shall be submitted to EPA for each test beginning 6 months after the date specified in paragraph (d)(1) of this section and at 6-month intervals thereafter until the final report is submitted to EPA. The progress report for 1,3,5-trimethylbenzene shall be submitted to EPA by April 10, 1995.

(2) *Subchronic toxicity*—(i) *Required testing.* (A) An oral 90-day subchronic toxicity test shall be conducted with 1,3,5-trimethylbenzene in accordance with § 798.2650 of this chapter except for the provisions in § 798.2650 (e)(3), (e)(7)(i), and (e)(11)(v). The tests shall be performed using drinking water. However, if, due to poor stability or

palatability, a drinking water test is not feasible for a given substance, that substance shall be administered either by oral gavage, in the diet, or in capsules.

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

(1) *Satellite group (Rodent only).* A satellite group of 20 animals (10 animals per sex) shall be treated with the high dose level for 90 days and observed for reversibility, persistence, and delayed occurrence of toxic effects for a post-treatment period of appropriate length, normally not less than 28 days.

(2) *Histopathology.* Histopathology of the lungs of all animals shall be performed. Special attention to examination of the lungs of rodents shall be made for evidence of infection since this provides a convenient assessment of the state of health of the animals.

(ii) *Reporting requirements.* (A) The subchronic testing for chloroethane shall be completed and the final report submitted to EPA by June 27, 1995. The subchronic testing for 1,1-dichloroethane and 1,1,2,2-tetrachloroethane shall be completed and the final report submitted to EPA by August 27, 1995. The subchronic testing for 1,3,5-trimethylbenzene shall be completed and the final report submitted to EPA by April 10, 1995.

(B) For each test, a progress report shall be submitted to EPA beginning 9 months after the date specified in paragraph (d)(1) of this section and at 6-month intervals thereafter until the final report is submitted to EPA.

(d) *Effective date.* (1) This section is effective on December 27, 1993, except for paragraphs (a)(1), (a)(2), (c)(1)(i)(A), (c)(1)(ii)(A), (c)(1)(ii)(B), (c)(2)(i)(A), and (c)(2)(ii)(A). The effective date for paragraphs (a)(2), (c)(1)(ii)(B), and (c)(2)(ii)(A) is September 29, 1995. The effective date for paragraphs (a)(1), (c)(1)(i)(A), and (c)(2)(i)(A) is February 27, 1996. The effective date for paragraph (c)(1)(ii)(A) is June 30, 1997.

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

[58 FR 59681, Nov. 10, 1993; 58 FR 1992, Jan. 13, 1994, as amended at 60 FR 56956, Nov. 13, 1995; 61 FR 7223, Feb. 27, 1996; 62 FR 35105, June 30, 1997]