
40 C.F.R. § 799.1700

Fluoroalkenes.

(a) *Identification of test substances.* (1) Vinyl fluoride (VF; CAS No. 75–02–5), vinylidene fluoride (VDF; CAS No. 75–38–7), tetrafluoroethene (TFE; CAS No. 116–14–3), and hexafluoropropene (HFP; CAS No. 116–15–4) shall be tested in accordance with this section.

(2) VF, VDF, TFE, and HFP of at least 99 percent purity shall be used as the test substances.

(b) *Persons required to submit study plans, conduct tests and submit data.* All persons who manufacture VF, VDF, TFE, or HFP, other than as an impurity, from July 22, 1987 to the end of the reimbursement period shall submit letters of intent to conduct testing or exemption applications, submit study plans, conduct tests in accordance with the TSCA Good Laboratory Practice Standards (40 CFR part 792), and submit data as specified in this section, subpart A of this part, and part 790 of this chapter for single-phase rulemaking, for the substances they manufacture.

(c) *Health effects testing—(1) Mutagenic effects—Gene mutation—(i) Required testing.* (A) (1) A detection of gene mutations in somatic cells in culture assay shall be conducted with TFE and HFP in accordance with § 798.5300 of this chapter except for the provisions in paragraphs (c), (d)(3)(i), (4), (5) and (6) and (e).

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

(i) *Reference substances.* No reference substance is required.

(ii) *Test method—Type of cells used in the assay.* Mutation induction at the HPRT locus shall be measured in Chinese hamster ovary (CHO) cells. Cells shall be checked for Mycoplasma contamination and may also be checked for karyotype stability.

(iii) *Test method—Metabolic activation.* Cells shall be exposed to the test substance only in the presence of a metabolic activation system for TFE, and in both the presence and absence of a metabolic activation system for HFP. The metabolic activation system shall be derived from the post-mitochondrial fraction (S–9) of livers from rats pretreated with Aroclor 1254.

(iv) *Test method—Control groups.* Positive and negative controls shall be included in each experiment. In assays with metabolic activation, the positive control substance shall be known to require such activation. Nitrogen shall serve as the negative control and diluting gas.

(v) *Test method—Test chemicals.* The test should be designed to have a predetermined sensitivity and power. The number of cells, cultures, and concentrations of test substance used should reflect these defined parameters. The number of cells per culture is based on the expected background mutant frequency; a general guide is to use a number which is 10 times the inverse of this frequency. Several concentrations (usually at least four) of the test substance shall be used. These shall yield a concentration-related toxic effect. The highest concentration shall produce a low level of survival (approximately 10 percent), and the survival in the lowest concentration shall

approximate that of the negative control. Cytotoxicity shall be determined after treatment with the test substance both in the presence and in the absence of the metabolic activation system.

(vi) *Test performance.* Cells in treatment medium with and without metabolic activation shall be exposed to varying concentrations of test gas-air mixtures by flushing treatment flasks (or chambers) with 10 volumes of test gas-air mixture at a rate of 500 mL/min or that rate which will allow complete flushing within 1 minute. In the case of a test chamber volume of 1.67 L, a flow rate of 10 L/min is appropriate. Each flask shall be closed with a cap with a rubber septum. Headspace samples shall be taken at the beginning and end of the exposure period and analyzed to determine the amount of test gas in each flask. Flasks shall be incubated on a rocker panel at 37 °C for 5 hours for tests with metabolic activation. For the non-activated portion of the test, the incubation time shall be 18 to 19 hours at 37 °C. At the end of the exposure period, cells treated with metabolic activation shall be washed and incubated in culture medium for 21 to 26 hours prior to subculturing the viability and expression of mutant phenotype. Cells treated without metabolic activation shall be washed and subcultured immediately to determine viability and to allow for expression of mutant phenotype. Appropriate subculture schedules (generally twice during the expression period) shall be used. At the end of the expression period, which shall be sufficient to allow near optimal phenotypic expression of induced mutants (generally 7 days for this cell system), cells shall be grown in medium with and without selective agent for determination of numbers of mutants and cloning efficiency, respectively. This last growth period is generally 7 days at 37 °C. Results of this test shall be confirmed in an independent experiment.

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