

---

## 40 C.F.R. § 799.9530

---

### TSCA *in vitro* mammalian cell gene mutation test.

---

(a) *Scope.* This section is intended to meet the testing requirements under section 4 of TSCA. The *in vitro* mammalian cell gene mutation test can be used to detect gene mutations induced by chemical substances. Suitable cell lines include L5178Y mouse lymphoma cells, the CHO, AS52 and V79 lines of Chinese hamster cells, and TK6 human lymphoblastoid cells under paragraph (g)(1) of this section. In these cell lines the most commonly-used genetic endpoints measure mutation at thymidine kinase (TK) and hypoxanthine-guanine phosphoribosyl transferase (HPRT), and a transgene of xanthine-guanine phosphoribosyl transferase (XPRT). The TK, HPRT and XPRT mutation tests detect different spectra of genetic events. The autosomal location of TK and XPRT may allow the detection of genetic events (e.g. large deletions) not detected at the HPRT locus on X-chromosomes (For a discussion see the references in paragraphs (g)(2), (g)(3), (g)(4), (g)(5), and (g)(6) of this section).

(b) *Source.* The source material used in developing this TSCA test guideline is the OECD guideline 476 (February 1997). This source is available at the address in paragraph (g) of this section.

(c) *Definitions.* The following definitions apply to this section:

*Base pair substitution mutagens* are substances which cause substitution of one or several base pairs in the DNA.

*Forward mutation* is a gene mutation from the parental type to the mutant form which gives rise to an alteration or a loss of the enzymatic activity or the function of the encoded protein.

*Frameshift mutagens* are substances which cause the addition or deletion of single or multiple base pairs in the DNA molecule.

*Mutant frequency* is the number of mutant cells observed divided by the number of viable cells.

*Phenotypic expression time* is a period during which unaltered gene products are depleted from newly mutated cells.

*Relative suspension growth* is an increase in cell number over the expression period relative to the negative control.

*Relative total growth* is an increase in cell number over time compared to a control population of cells; calculated as the product of suspension growth relative to the negative control times cloning efficiency relative to negative control.

*Survival* is the cloning efficiency of the treated cells when plated at the end of the treatment period; survival is usually expressed in relation to the survival of the control cell population.

*Viability* is the cloning efficiency of the treated cells at the time of plating in selective conditions after the expression period.

---

(d) *Initial considerations.* (1) In the *in vitro* mammalian cell gene mutation test, cultures of established cell lines or cell strains can be used. The cells used are selected on the basis of growth ability in culture and stability of the spontaneous mutation frequency. Tests conducted *in vitro* generally require the use of an exogenous source of metabolic activation. This metabolic activation system cannot mimic entirely the mammalian *in vivo* conditions. Care should be taken to avoid conditions which would lead to results not reflecting intrinsic mutagenicity. Positive results which do not reflect intrinsic mutagenicity may arise from changes in pH, osmolality or high levels of cytotoxicity.

(2) This test is used to screen for possible mammalian mutagens and carcinogens. Many compounds that are positive in this test are mammalian carcinogens; however, there is not a perfect correlation between this test and carcinogenicity. Correlation is dependent on chemical class and there is increasing evidence that there are carcinogens that are not detected by this test because they appear to act through other, non-genotoxic mechanisms or mechanisms absent in bacterial cells.

(e) *Test method*—(1) *Principle.* (i) Cells deficient in thymidine kinase (TK) due to the mutation TK<sup>-/-</sup> are resistant to the cytotoxic effects of the pyrimidine analogue trifluorothymidine (TFT). Thymidine kinase proficient cells are sensitive to TFT, which causes the inhibition of cellular metabolism and halts further cell division. Thus mutant cells are able to proliferate in the presence of TFT, whereas normal cells, which contain thymidine kinase, are not. Similarly, cells deficient in HPRT or XPRT are selected by resistance to 6-thioguanine (TG) or 8-azaguanine (AG). The properties of the test substance should be considered carefully if a base analogue or a compound related to the selective agent is tested in any of the mammalian cell gene mutation tests. For example, any suspected selective toxicity by the test substance for mutant and non-mutant cells should be investigated. Thus, performance of the selection system/agent shall be confirmed when testing chemicals structurally related to the selective agent.

(ii) Cells in suspension or monolayer culture shall be exposed to the test substance, both with and without metabolic activation, for a suitable period of time and subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection. Cytotoxicity is usually determined by measuring the relative cloning efficiency (survival) or relative total growth of the cultures after the treatment period. The treated cultures shall be maintained in growth medium for a sufficient period of time, characteristic of each selected locus and cell type, to allow near-optimal phenotypic expression of induced mutations. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent to determine the cloning efficiency (viability). After a suitable incubation time, colonies shall be counted. The mutant frequency is derived from the number of mutant colonies in selective medium and the number of colonies in non-selective medium.

This document is only available to subscribers. Please log in or purchase access.

[Purchase Login](#)