
40 C.F.R. § 799.9539

TSCA mammalian erythrocyte micronucleus test.

(a) *Scope.* This section is intended to meet the testing requirements under section 4 of TSCA.

(1) The mammalian erythrocyte micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals, usually rodents.

(2) The purpose of the micronucleus test is to identify substances that cause cytogenetic damage which results in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes.

(3) When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded; any micronucleus that has been formed may remain behind in the otherwise anucleated cytoplasm. Visualization of micronuclei is facilitated in these cells because they lack a main nucleus. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals is an indication of induced chromosome damage.

(b) *Source.* The source material used in developing this TSCA test guideline is the OECD guideline 474 (February 1997). This source is available at the address in paragraph (g) of this section.

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

Centromere (kinetochore) is a region of a chromosome with which spindle fibers are associated during cell division, allowing orderly movement of daughter chromosomes to the poles of the daughter cells.

Micronuclei are small nuclei, separate from and additional to the main nuclei of cells, produced during telophase of mitosis (meiosis) by lagging chromosome fragments or whole chromosomes.

Normochromatic erythrocyte is a mature erythrocyte that lacks ribosomes and can be distinguished from immature, polychromatic erythrocytes by stains selective for ribosomes.

Polychromatic erythrocyte is an immature erythrocyte, in an intermediate stage of development, that still contains ribosomes and therefore can be distinguished from mature, normochromatic erythrocytes by stains selective for ribosomes.

(d) *Initial considerations.* (1) The bone marrow of rodents is routinely used in this test since polychromatic erythrocytes are produced in that tissue. The measurement of micronucleated immature (polychromatic) erythrocytes in peripheral blood is equally acceptable in any species in which the inability of the spleen to remove micronucleated erythrocytes has been demonstrated, or which has shown an adequate sensitivity to detect agents that cause structural or numerical chromosome aberrations. Micronuclei can be distinguished by a number of criteria. These include identification of the presence or absence of a kinetochore or centromeric DNA in the micronuclei. The frequency of micronucleated immature

(polychromatic) erythrocytes is the principal endpoint. The number of mature (normochromatic) erythrocytes in the peripheral blood that contain micronuclei among a given number of mature erythrocytes can also be used as the endpoint of the assay when animals are treated continuously for 4 weeks or more. This mammalian *in vivo* micronucleus test is especially relevant to assessing mutagenic hazard in that it allows consideration of factors of *in vivo* metabolism, pharmacokinetics and DNA-repair processes although these may vary among species, among tissues and among genetic endpoints. An *in vivo* assay is also useful for further investigation of a mutagenic effect detected by an *in vitro* system.

(2) If there is evidence that the test substance, or a reactive metabolite, will not reach the target tissue, it is not appropriate to use this test.

(e) *Test method*—(1) *Principle*. Animals are exposed to the test substance by an appropriate route. If bone marrow is used, the animals are sacrificed at appropriate times after treatment, the bone marrow extracted, and preparations made and stained (test techniques described in the references under paragraphs (g)(1), (g)(2), and (g)(3) of this section may be used). When peripheral blood is used, the blood is collected at appropriate times after treatment and smear preparations are made and stained (the test techniques described in the references under paragraphs (g)(3), (g)(4), (g)(5), and (g)(6) of this section may be used). For studies with peripheral blood, as little time as possible should elapse between the last exposure and cell harvest. Preparations are analyzed for the presence of micronuclei.

(2) *Description*—(i) *Preparations*—(A) *Selection of animal species*. Mice or rats are recommended if bone marrow is used, although any appropriate mammalian species may be used. When peripheral blood is used, mice are recommended. However, any appropriate mammalian species may be used provided it is a species in which the spleen does not remove micronucleated erythrocytes or a species which has shown an adequate sensitivity to detect agents that cause structural or numerical chromosome aberrations. Commonly used laboratory strains of young healthy animals should be employed. At the commencement of the study, the weight variation of animals should be minimal and not exceed $\pm 20\%$ of the mean weight of each sex.

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