

40 C.F.R. § 79.64

In vivo micronucleus assay.

- (a) *Purpose.* The micronucleus assay is an *in vivo* cytogenetic test which uses erythrocytes in the bone marrow of rodents to detect chemical damage to the chromosomes or mitotic apparatus of mammalian cells. As the erythroblast develops into an erythrocyte (red blood cell), its main nucleus is extruded and may leave a micronucleus in the cell body; a few micronuclei form under normal conditions in blood elements. This assay is based on an increase in the frequency of micronucleated erythrocytes found in bone marrow from treated animals compared to that of control animals. The visualization of micronuclei is facilitated in these cells because they lack a main nucleus.
- (b) *Definitions.* For the purposes of this section the following definitions apply:

Micronuclei mean small particles consisting of acentric fragments of chromosomes or entire chromosomes, which lag behind at anaphase of cell division. After telophase, these fragments may not be included in the nuclei of daughter cells and form single or multiple micronuclei in the cytoplasm.

Polychromatic erythrocyte (PCE) means an immature red blood cell that, because it contains RNA, can be differentiated by appropriate staining techniques from a normochromatic erythrocyte (NCE), which lacks RNA. In one to two days, a PCE matures into a NCE.

(c) Test method—(1) Principle of the test method. (i) Groups of rodents are exposed by the inhalation route for a minimum of 6 hours/day over a period of not less than 28 days to three or more concentrations of a test substance in air. Groups of animals are sacrificed at the end of the exposure period and femoral bone marrow is extracted. The bone marrow is then smeared onto glass slides, stained, and PCEs are scored for micronuclei. Researchers may need to run a trial at the highest tolerated concentration of the test atmosphere to optimize the sample collection time for micronucleated cells.

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