



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 15193.5-2014

**National food safety standard - Mammalian Erythrocyte
Micronucleus Test**

食品安全国家标准 哺乳动物红细胞微核试验

(English Translation)

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1 Scope

This Standard specifies the basic test methods and technical requirements for mammalian erythrocyte micronucleus test.

This Standard is applicable to the evaluation of the genotoxicity of test substances.

2.1 Micronucleus

During the cell mitosis anaphase, when the chromosomes regularly enter the daughter cells to form the cell nucleus, the entire chromatids or acentric fragments or rings of chromosomes that remain in the cytoplasm. In the telophase, one or several regular subnuclei are formed separately and are contained in the cytoplasm of the cell.

2.2 Centromere

During the phase of cell division, the region where chromosomes connect to spindle fibers allows the orderly movement of daughter chromosomes to the poles of daughter cells.

2.3 Normochromatic erythrocytes

Mature erythrocytes that lack ribosomes and can be distinguished from immature polychromatic erythrocytes by selective ribosome dyes.

2.4 Polychromatic erythrocytes

Immature erythrocytes are in the intermediate stage of development and still contain ribosomes, so they can be distinguished from mature normochromatic erythrocytes with

selective ribosome dyes.

2.5 Total erythrocytes

The sum of normochromatic erythrocytes and polychromatic erythrocytes.

5.1 Test substances

5.1.1

Preparation of test substances

The test substances shall be dissolved or suspended in a suitable solvent, where water is the preferred solvent; for water-insoluble test substances, vegetable oils (such as olive oil, corn oil, etc.) can be used; for water- insoluble or oil-insoluble test substances, carboxymethyl cellulose, starch, etc. can also be used to prepare suspensions or pastes. Test substances shall be prepared before use, unless there is data indicating that their solutions or suspensions are storage stable.

5.1.2

Administration route

Oral gavage shall be used. Positive control substances can also be injected intraperitoneally. The gavage volume shall generally not exceed 10 mL/kg body weight. If it is an aqueous solution, the maximum gavage volume can reach

20 mL/kg body weight; if it is an oily liquid, the gavage volume shall not exceed 4 mL/kg body weight; the gavage volume of each group shall be the same.

5.2 Experimental animals

5.2.1

Animal species and strain selection

When using bone marrow, it is recommended to use mice or rats. When using peripheral blood, it is recommended to use mice. If it has been proven that the spleen of a certain strain of animal does not clear polychromatic erythrocytes with micronuclei, or is sufficiently sensitive to detect chemicals that cause structural or numerical aberrations of chromosomes, such animals can be used. Usually, mice aged 7 ~ 12 weeks and weighing 25 g ~ 35 g or rats weighing

200 g ~ 300 g are used. At the beginning of the test, the difference in animal weight shall not exceed $\pm 20\%$ of the average weight of each gender. Use at least 5 animals of each gender in each group.

5.2.2

Animal preparation

Before the test, the animals shall undergo environmental acclimatization and quarantine

observation for at least 3 ~ 5 days in the experimental animal room.

5.2.3 Animal feeding: Experimental animal feeding conditions, drinking water, and feed

shall comply with national standards and relevant regulations (GB 14925, GB 5749, GB 14924.1, GB 14924.2, GB 14924.3). The animals in each test group shall be housed in separate cages according to gender, with 5 animals in each cage. During the experiment, the experimental animals shall be fed basic feed and have free access to water.

5.3 Dosage

The test substances shall be set up in three dose groups. In principle, the maximum dose group is the dose at which animals show severe poisoning and/or individual animals die, which can generally be $1/2$ LD₅₀. The low dose groups shall not show toxicity, and

$1/4$ LD₅₀ and $1/8$ LD₅₀ shall be taken as the medium and low doses, respectively. When the animal does not die at the maximum dose (maximum use concentration and maximum gavage capacity) of the test substances in the acute toxicity test and the LD₅₀ cannot be calculated, the high dose groups shall be in the following order:

- a) 10 g/kg body weight;
- b) 100 times the possible human intake;
- c) Design the maximum gavage dose, and then set up medium and low dose groups.

Set up a solvent control group. The positive control can be given at cyclophosphamide 40 mg/kg body weight orally or intraperitoneally (oral is preferred).

5.4.1 Administration of test substances

Oral gavage. According to the cell cycle and the characteristics of the action of different substances, a preliminary test can be done first to determine the sampling time. The 30-hour test substance administration method is commonly used. That is, the test substance is administered twice with an interval of 24 hours, and the bone marrow sample is collected 6 hours after the second administration of the test substance. The experiment can also have the following two sampling methods:

- a) Administer the test substance to the animal once. Collect bone marrow samples at least twice at appropriate intervals, starting no earlier than 24 hours after administration and ending no later than 48 hours after administration. The reason for sampling earlier than 24 hours after administration shall be stated.

Collect peripheral blood samples at least twice at appropriate intervals, starting no earlier than 36 hours after administration and ending no later than 72 hours after administration. If

a positive result is found at one sampling time, no further sampling is required.

b) Administer once a day for 2 or more times (24 h intervals). Bone marrow can be collected once between 18 h and 24 h after the last administration, and peripheral blood can be sampled once between 36 h and 48 h after the last administration.

If the rate of micronucleated cells in peripheral blood normochromatic erythrocytes is used as the experimental observation endpoint, the animal shall be administered for more than 4 weeks.

5.4.2.1 Bone marrow sample: After killing, take the sternum or femur; use hemostatic

forceps to squeeze out the bone marrow fluid and mix it with the calf serum at one end of the slide; make a conventional smear. Alternatively, use calf serum to rinse the femoral bone marrow cavity to make a cell suspension smear; after the smear is naturally dried, put it in methanol to fix it for 5 min ~ 10 min. Store it after fixation on but there is no dose-response relationship, the test shall be repeated. Repeatable results can be considered positive.

7 Test report

7.1

Test name, test unit name and contact information, and report number.

7.2 Name and contact information of the test entrusting unit, and sample acceptance

date.

7.3 Test start and end dates, test item leader, test unit technical leader, and issue date.

7.4 Test summary.

7.5 Test substances: name, batch number, dosage form, status (including sensory, properties, packaging integrity, and labeling), quantity, pretreatment method, and relevant information of the positive control.

7.6 Experimental animals: species, strain, grade, quantity, age, weight, sex, source

(supplier name, experimental animal production license number), animal quarantine, adaptation, breeding environment (temperature, relative humidity, experimental animal facility use license number), feed source (supplier name, experimental animal feed production license number).