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Toxicological Test Methods for Pesticides Registration - Part 15: In Vivo Mammalian Bone Marrow Polychromatic Erythrocyte Micronucleus Test

农药登记毒理学试验方法

第 15 部分：体内哺乳动物骨髓嗜多染红细胞微核试验

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Foreword

GB/T 15670 *Toxicological Test Methods for Pesticides Registration* may be divided into the following parts:

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--- Part 4: Acute Oral Toxicity Test - Miller and Taninter's Method;

--- Part 5: Acute Dermal Toxicity Test;

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Toxicological Test Methods for Pesticides Registration – Part 15: In Vivo Mammalian Bone Marrow Polychromatic Erythrocyte Micronucleus Test

1 Scope

This Part of GB/T 15670 specifies the basic principles, methods and requirements of the micronucleus test of mammalian bone marrow polychromatic erythrocyte in vivo.

This Part is applicable to the micronucleus test of mammalian bone marrow polychromatic erythrocyte in vivo for pesticides registration.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this document.

GB 14925 Laboratory Animal - Requirements of Environment and Housing Facilities

3 Terms and Definitions

For the purpose of this document, the following terms and definitions apply.

3.1 Micronucleus

In the late stage of cell mitosis, fragments of chromosomes or delayed chromosomes that cannot enter the nucleus of the daughter cells, one or several secondary nuclei formed in the cytoplasm of the daughter cells, which are consistent with the coloration of the main nucleus, and are round or oval.

3.2 Polychromatic erythrocytes; PCE

Immature erythrocytes are an intermediate stage in the process of erythrocyte maturation. At this time, because the cytoplasm still contains ribosomes, it remains basophilic for about 24h, which can be distinguished from mature erythrocytes by selective staining.

6.1.2.1 Instruments: biological microscope, constant temperature water bath, etc.

6.1.2.2 Calf serum (inactivated): After filtering the bacteria, calf serum is placed in a constant temperature water bath at 56°C for 30min for inactivation. Inactivated calf serum is usually stored in a refrigerator at 4°C. Rat and mouse serum can also be used instead.

6.1.2.3 Giemsa dye solution:

Giemsa dye: 3.8g

Methanol: 375mL

Glycerol: 125mL

Preparation: place Giemsa dye and a small amount of methanol in a mortar and grind carefully; then add methanol to 375mL; after it is completely dissolved, add 125mL of glycerol and mix well. Put it in a 37°C thermostat for 48h. Shake several times during the heat preservation period to promote the full dissolution of the dye. Take out and filter; and use it after two weeks.

6.1.2.4 Phosphate buffer solution (pH 6.8):

1/15mol/L disodium hydrogen phosphate solution: take 9.47g of Na_2HPO_4 and dissolve it in 1000mL of distilled water.

1/15mol/L potassium dihydrogen phosphate solution: take 9.07g of KH_2PO_4 and dissolve it in 1000mL of distilled water.

Mix 50 mL of disodium hydrogen phosphate solution with 50 mL of potassium dihydrogen phosphate solution; measure and adjust to pH 6.8 with a pH meter.

6.1.2.5 Giemsa application solution: take 1 portion of Giemsa dye solution and 6 portions of phosphate buffer solution (pH 6.8); mixed and prepared for current use.

6.1.2.6 Methanol (analytically pure).

6.2 Laboratory animals

6.2.1 Animal selection

Micronucleus test recommends the use of mice, but also rats. Usually use mice/rats for 7~12 weeks, mice weighing 25g-30g or rats weighing 150g-200g. The animals were randomly divided into groups, with at least 5 animals of both sexes in each group. At the beginning of the experiment, the animal weight difference shall not exceed ±20% of the average weight of the same sex.

6.2.2 Feeding environment

6.3.3.3 The negative control is a solvent control. Based on the background control data of the variation between animals and the frequency of chromosomal aberration cells, it is judged whether a negative control group is set up at each sampling time point and treated in the same way as the exposure group. If the negative control adopts a single sampling, the most suitable sampling time is the first sampling time. If there is no historical control data to prove that the used solvent is non-cytotoxic or non-mutagenic, a blank control shall be added.

6.4 Methods of exposure

6.4.1 Oral gavage or intraperitoneal injection is often used for exposure. Other reasonable routes of exposure are also acceptable. The maximum fluid volume for one gavage or injection shall not exceed 2mL/100g body weight. The use of higher volumes shall be justified. In general, isometric exposure is recommended.

6.4.2 The test can be carried out in any of the following ways for exposure:

a) Poison the animal with the test substance once; collect bone marrow samples at least twice between 24h and 48h; and the reason shall be explained before the 24h sampling;

b) Poison once a day, a total of 2 or more times (interval 24h); bone marrow may be collected once between 18h and 24h after the last exposure.

6.5 Section-making

Cervical dislocation method kills the animal, takes the sternum, then squeezes out the bone marrow fluid with small hemostatic forceps, mixes it with the fetal bovine serum at one end of the slide, and smears it according to the routine; or removes the femur and flushes the femoral bone marrow cavity with calf serum and make the cell suspension smears; the smears are dried in the air and then fixed in methanol solution for 5min-10min, and then take out for drying.

6.6 Dyeing

Stain with freshly prepared Giemsa application solution for 10min~15min; rinse immediately with pH 6.8 phosphate buffer solution; dry it; write a label; and store in a cool and dry place.

6.7 Reading

First observe under a low magnification lens; select an evenly distributed and well-dyed area; and then observe and count under an oil lens. Polychromatic erythrocytes are grey-blue; and normochromatic erythrocytes (NCE) are light orange-red. Most of the typical micronuclei are single round, with smooth and orderly edges. The chromotropism is consistent with the nucleoplasm, which is purple-red or blue-purple, and the diameter is usually 1/20~1/5 of erythrocytes. Each animal is counted for 1,000

8 Test Report

The test report shall include at least the following:

a) Test name, test organization name and contact information, report number;

b) The name and contact information of the test entrusting organization, the date of sample acceptance and the sample sealing situation;

c) The start and end date of the test, the person in charge of the test project, the technical person in charge of the test organization, and the date of issuance;

d) Test summary;

e) Name of test substance, active ingredient American Chemical Abstracts registration number (CAS number) (if known), code (if any), purity (or content), dosage form, production date (batch number), physical and chemical properties, and solvent used for preparation and preparation method;

f) The species, strain, grade, quantity, weight, gender, source (supplier name, laboratory animal quality certificate number, laboratory animal production license number), quarantine, adaptation of the laboratory animal. Breeding environment of the laboratory animal, including temperature, relative humidity, feed, single cage breeding or group breeding, laboratory animal facility use permit number;

g) Dose and group, including the principle or basis for selecting the dose, dose and group, animal grouping method and the number of animals of each gender in each group;

h) Test conditions and methods, including main instruments and equipment, route of exposure, exposure plan, operation steps, name of positive control substance, etc.;

i) Test results: summarize item by item with text description and table, poisoning performance, mean and standard deviation of polychromatic erythrocytes with micronuclei in each group, proportion of polychromatic erythrocytes in total erythrocytes, dose-response relationship, statistical analysis;

j) Test conclusion: give a conclusion on whether the test substance has mutagenic effects under the test conditions, and discuss relevant issues when necessary;

k) A description of the original record keeping.

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