GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

ICS 65.100

B 17

GB 15670.16-2017

Partially replacing GB/T 15670-1995

Toxicological Test Methods for Pesticides Registration - Part 16: In Vivo Mammalian Bone Marrow Cell Chromosome Aberration Test

农药登记毒理学试验方法

第 16 部分：体内哺乳动物骨髓细胞染色体畸变试验

Issued on: July 12, 2017 **Implemented on: February 01, 2018**

Issued by: General Administration of Quality Supervision, Inspection and Quarantine;

Standardization Administration of PRC.

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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 16: In Vivo Mammalian Bone Marrow Cell Chromosome Aberration Test

1 Scope

This Part of GB/T15670 specifies the basic principles, methods and requirements of the in vivo mammalian bone marrow cell chromosome aberration test.

This Part is applicable to the in vivo mammalian bone marrow cell chromosome aberration test for pesticide 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 Chromatid-type aberration

Chromosomal structural damage, which is manifested by damage of chromatids break or chromatids break and recombination.

3.2 Chromosome-type aberration

Chromosome structure damage, which is manifested by break or break recombination changes at the same site of two chromatids.

3.3 Endoreduplication

The process, after the S phase of DNA replication, the nucleus does not enter the

6 Test Methods

6.1 Test substance and instrument reagent

6.1.1 Test substance

Distilled water is preferred as the solvent. If the test substance is insoluble in water, it can be prepared into an emulsion or suspension with edible oil, edible starch, and 0.5% carboxymethyl cellulose sodium. The test substance shall be prepared fresh before gavage, unless some data show that it is stable when stored in solution (or emulsion, suspension). If it is not a common solvent, there shall be reference materials and explain its ingredients and the reason for the selection of solvent.

6.1.2 Instruments and reagents

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

6.1.2.2 0.1% colchicine: place in the brown bottle, and store in the refrigerator at 4°C.

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 9 portions of phosphate buffer solution (pH 6.8); mixed and prepared for current use.

6.3.2 Limit test

If a single exposure (or two exposures on the same day) dose is above 2000mg/kg body weight, there is still no observable toxic effect, and no genotoxicity is expected based on the data of structurally related compounds; then 3 doses are not required for test. If the exposure time is within 14d, the dose is set to 2000 mg/kg body weight; if the exposure time is longer than 14d, the dose is set to 1000 mg/kg body weight. If the human's possible (expected) exposure is too large, the limit test shall be carried out at a dose level of 5000 mg/kg body weight or a higher dose shall be selected.

6.3.3 Setting of the control group

6.3.3.1 For each test, each gender shall set up corresponding positive and negative controls (solvent/vehicle). Except that the test substance is not used for exposure, the animals in the control group shall be operated in the same way as the animals in the exposure group.

6.3.3.2 The positive control group shall be expected to detect an increase in chromosome structure aberrations in vivo that exceeds the background value to confirm the sensitivity of the test system. The route of exposure of the positive control may be different from the route of exposure of the test substance, and sampling is only performed at a single time point. It is optimal to use a positive control related to the chemical structure of the test substance. Refer to Appendix A for commonly-used positive controls.

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 aberrations, it is judged whether a negative control group is set 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 Gavage is often used for exposure, and the positive control group may also be injected intraperitoneally. It is recommended that the test substance be exposed to the poison once. The test substance with a large sample amount may also be exposed to the poison in several times, such as the interval of no more than 6h in the same day, and the exposure is carried out twice. The recommended time for the first sample collection is 12h~18h after the last exposure (1.5 times the normal cell cycle); and the recommended time for the second sample collection is 24h after the first sampling time.

6.4.2 Other methods of exposure shall explain the reasons. If the exposure is more than one day, one sampling can be taken 12h~18h after the last exposure (1.5 times the normal cell cycle).

of section-making often leads to changes in the proportion of metaphase with chromosome loss, the number of chromosomes contained in the counted cells shall be controlled at 2*n*±2.

6.7.3 The observation items include:

a) Changes in the number of chromosomes: aneuploidy, polyploidy, endoduplication;

b) Changes in the structure of chromosomes: breaks, tiny bodies, loops with centromeres, loops without centromeres, monomer exchanges, double tiny bodies, non-specific type changes (such as crushing, slender centromeres, adhesion), etc.

7 Test Results and Evaluation

7.1 Test results

Show the data of each animal in a table. The test unit is each animal. For each animal, the following items shall be evaluated, such as the number of cells counted, the number of aberrations in each cell and the percentage of cells with chromosomal structural aberrations. The number and frequency of different types of chromosome structural aberrations in the exposure group and the control group shall be listed. The gaps shall be counted and reported separately, but generally not included in the total aberration frequency. If there is no evidence of gender difference, the data of male and female animals may be combined for statistical analysis. Statistical analysis may be tested by χ^2, or use statistical methods such as Fisher test, etc.

7.2 Evaluation of test results

7.2.1 The standard for judging a positive result include: when comparing exposure group with the negative control group, and the increase of chromosome aberration rate has statistically significance and there is significant dose-response relationship, then it may be confirmed as a positive result. If the statistical difference is significant, but there is no dose-response relationship, the test shall be repeated; and if the difference may be repeated, it may be regarded as positive. The evaluation shall be analysed from two aspects of biological significance and statistical significance.

7.2.2 The test substance whose result does not meet the above criteria may be considered as a negative result in this test system.

7.2.3 The increase in polyploidy indicates that the test substance may induce chromosomal numerical aberration. Increased endoreduplication indicates that the test substance may inhibit cell cycle progression.

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