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

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Replacing GB/T 15670-1995

**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:

- Part 1: General Principles;
- Part 2: Acute Oral Toxicity Test - Horn's Method;
- Part 3: Acute Oral Toxicity Test - up-and-down-Procedure;
- Part 4: Acute Oral Toxicity Test - Miller and Taninter's Method;
- Part 5: Acute Dermal Toxicity Test;
- Part 6: Acute Inhalation Toxicity Test;
- Part 7: Dermal Irritation/Corrosion Test;
- Part 8: Acute Eye Irritation/Corrosion Test;
- Part 9: Skin Sensitisation Test;
- Part 10: Short-Term Repeated Dose 28-day Oral Toxicity Study;
- Part 11: Short-Term Repeated Dose 28-day Dermal Toxicity Study;
- Part 12: Short-Term Repeated Dose 28-day Inhalation Toxicity Study;
- Part 13: Sub-chronic Toxicity Study;

1 Scope

GB/T 15670.15-2017 is the Chinese national standard on toxicological test methods for pesticides registration - part 15: in vivo mammalian bone marrow polychromatic erythrocyte micronucleus test, in the field of agriculture. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 12 July 2017 by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; Standardization Administration of China, and has been in force since 1 February 2018. Classification: ICS 65.100, CCS B17. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

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 Na₂HPO₄ and dissolve it in 1000mL of distilled water. 1/15mol/L potassium dihydrogen phosphate solution: take 9.07g of KH₂PO₄ 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;