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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

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Table of Contents

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 15670.16-2017 is the Chinese national standard on toxicological test methods for pesticides registration - part 16: in vivo mammalian bone marrow cell chromosome aberration 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/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

6.1 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 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 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 2n±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