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

GB 15193.6-2014

Replacing GB 15193.6-2003

**National Food Safety Standard -- Mammalian Bone Marrow Cell
Chromosome Aberration Test**

食品安全国家标准 哺乳动物骨髓细胞染色体畸变试验
(English Translation)

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Foreword

This standard replaces GB 15193.6-2003 "mammalian bone marrow chromosome aberration test." this standard compared with GB 15193.6-2003, the main changes are as follows.

--- Standard name was changed to "national food safety standard mammalian bone marrow chromosome aberration test";

- Modify the scope;
- Increasing the terms and definitions;
- Modified test purposes and principles;
- Revised test methods;
- Modify the data processing.

National Food Safety Standard Mammalian bone marrow chromosome aberration test

1 Scope

This standard specifies the mammalian bone marrow chromosome aberration test basic test methods and technical requirements.

This standard applies to the evaluation of the test substance to mammalian bone marrow cells genotoxicity.

2.1 Structural chromosome aberrations

It can be directly observed by a microscope to take place in the cell mitotic metaphase chromosome structural changes. Such as chromosome deletions and Fragments, chromosomes and swap within the exchange. It can be divided into structural aberrations chromosome aberrations (chromosome-type aberration) and single staining Body distortion (chromatid-type aberration).

2.2 Chromosome aberrations

Chromosome structural damage, the performance of the two chromatids of the same sites are broken or appear to change the broken reorganization.

2.3 Chromatid aberrations

Structural chromosome damage, manifested as chromatid breaks or chromatid recombination fracture injury.

2.4 The number of chromosome aberrations Chromosome number changes, unlike normal karyotype.

2.5 Endoreduplication

After the S phase of DNA replication, mitosis starts another S phase nuclei process was not performed. As a result, chromosome There 4,8,16 times chromatids.

2.6 Crack

The length of the chromosome or chromatid damage is less than the width of a chromatid, arranged in a minimum error chromatids.

2.7 Mitotic index

The number of cells and the medium-term phase of the observed ratio of the total number of cells.

3 Test purposes and principles

After administration of the test substance test animals, with metaphase blocker (eg colchicine or colchicine amine) process, inhibiting cell division when spinning Forming a hammer in order to increase the proportion of mitotic cells in the medium-term, and then drawn, producer, staining, chromosome aberration analysis.

This test can detect the whole animal test substances can cause chromosomal aberrations in bone marrow cells, the test was to evaluate the possibility of mutagenic. If the There is evidence that the test substance or its metabolites can not reach the bone marrow, are not applicable to this method.

4.1 Instrument

Commonly used laboratory equipment, constant temperature water bath ($37\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$), centrifuges, biological microscope.

4.2 Reagents

4.2.1

colchicine (0.4mg/mL). placed in a brown bottle, refrigerator.

4.2.2

potassium chloride solution (0.075mol/L).

4.2.3

fixative. 3 to 1 mix of methanol and acetic acid, temporary use when the existing service.

4.2.4

Giemsa (Giemsa) reserve bath. Take a small amount of methanol and Giemsa dye 3.8g finely ground in a mortar was gradually added methanol to 375mL, until completely dissolved, then add 125mL glycerin and mix well. Incubator at $37\text{ }^{\circ}\text{C}$ incubated 48h. During vibration insulation Shake several times, prompting sufficiently soluble dyes. Remove the filter, store at room temperature, two weeks after use.

4.2.5

Application Giemsa dye. Take 1 part by Giemsa dye stock solution with 9 parts phosphate buffer (1/15mol/L) mixture. Temporary use when formulated.

4.2.6

phosphate buffer (pH6.8).

4.2.6.1

disodium hydrogen phosphate solution (1/15mol/L). disodium hydrogen phosphate (Na_2HPO_4) 9.47g was dissolved in 1000mL of deionized water.

4.2.6.2

potassium dihydrogen phosphate solution (1/15mol/L). potassium dihydrogen phosphate (KH_2PO_4) 9.07g was dissolved in 1000mL of deionized water.

4.2.6.3

take disodium hydrogen phosphate solution (1/15mol/L) 50mL and potassium dihydrogen phosphate solution (1/15mol/L) 50mL mixed.

4.2.7

positive control. conventional cyclophosphamide, mitomycin C and the like.

5.1 Test substance

The test substance should be used in the original sample, if it can not use the original sample, the test should be treated in accordance with the principles of the test substance for proper disposal.

5.2.1 Animal selection

Common healthy young adult rats or mice, such as using the mouse to select 7 weeks to 12 weeks, when the start of the test animal body weight difference Isobutyl should not exceed 20% of the average weight. Animals should be randomly divided into groups of male and female animals each at least five. If the trial has several Sampling time points, the requirements of each sex in each group for each sampling time point for the analysis has only 5 animals.

5.2.2 Animal Preparation

Before the test, animals in the experimental animal room should be at least 3d ~ 5d acclimatization and quarantine observation.