

ICS 07.100
CCS C 53



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

GB 15193.8-2014

Replacing GB 15193.8-2003

**National Food Safety Standard -- Chromosome aberration test
of mouse spermatogonia or spermatocyte**

食品安全国家标准 小鼠精原细胞或精母细胞染色体畸变试验

(English Translation)

Issued on: December 24, 2014

Implemented on: May 1, 2015

Issued by: National Health and Family Planning Commission

Table of Contents

Foreword	
1 Scope	
2 Terms and definitions	
2.1 Spermatogonia	
2.2 Spermatocytes	
2.3 Structural chromosome aberrations	
2.4 Chromosome aberrations	
2.5 Chromatid aberrations	
3 Test purposes and principles	
4 Instruments and reagents	
4.1 Commonly used laboratory equipment	
4.2 Reagents	
5 Test methods	
5.1 Experimental Animals	
5.2 Test substance formulation	
5.3 Dose	
5.4 Test procedure and outcome measures	
5.5 Reading sheet	
6 Data processing and evaluation of results	
6.1 Data Processing	
6.2 Evaluation Results	
7 Test report	

Foreword

This standard replaces GB 15193.8-2003 "mouse testis chromosome aberration test." this standard compared with GB 15193.8-2003, the main changes are as follows.

- Standard name was changed to "national food safety standard mouse spermatogonia or spermatocytes chromosome aberration test";
- Modify the scope;
- Modify the terms and definitions;

- Modify the test purpose and principles;
- Modified animal requirements;
- Modify the test procedure and outcome measures;
- Increased content requires the test report.

National Food Safety Standard Mouse spermatogonia or spermatocytes chromosome aberration test

1 Scope

This standard specifies the mouse spermatogonia or spermatocytes chromosome aberration test basic test methods and technical requirements.

This standard applies to the evaluation test on mouse germ cell chromosome damage, depending on the circumstances choose spermatogonia or spermatocytes as target cells.

2.1 Spermatogonia

Male mammal seminiferous epithelium can after several mitosis and meiosis proliferation of spermatocytes produce stem cells for the original male germ cells. The same number of chromosomes somatic cells have.

2.2 Spermatocytes

Eventually differentiate into mature sperm cells through meiosis spermatogonia, divided into primary spermatocytes and secondary spermatocytes.

Secondary spermatocytes chromosome number is halved to $1n$.

2.3 Structural chromosome aberrations

In mitotic metaphase cells through a microscope it can be directly observed chromosomal structural changes. Structure can be divided into chromosomal aberrations Chromatid aberrations and distortion.

2.4 Chromosome aberrations

Structural chromosome damage, showing at the same site two chromatids are a fracture or break reconnection.

2.5 Chromatid aberrations

Structural chromosome damage, manifested as chromatid breaks or break reconnection.

2.6 The number of chromosome aberrations Chromosome number change, different from

the normal diploid karyotype, including aneuploidy and aneuploidy.

3 Test purposes and principles

Experimental animals were orally administered a test sample, the animals were killed after a certain time. Observation of testicular seminoma or spermatocyte chromosome aberration love Conditions to evaluate the mutagenicity of the test sample of male germ cells.

Before the animals were sacrificed by cell metaphase blocker treatment, remove the testes were killed after preparation after hypotonic, fixing, softening and dyeing Spermatogonia or spermatocytes chromosome specimens observed under the microscope metaphase cells analyzed spermatogonia or spermatocytes chromosome distortion.

4.1 Commonly used laboratory equipment

Anatomy of commonly used laboratory instruments, electronic scales, refrigerators, centrifuges and the like.

4.2 Reagents

4.2.1 0.1% colchicine Placed in a brown bottle, refrigerator.

4.2.2 1% sodium citrate Take 1g trisodium citrate (AR), add distilled water to 100mL.

4.2.3 60% glacial acetic acid Take 60mL glacial acetic acid (AR), add distilled water to 100mL.

4.2.4 Fixative

Methanol. acetic acid = 3, using now.

4.2.5 Phosphate buffer (pH 7.4)

Disodium hydrogen phosphate solution (1/15mol/L). disodium hydrogen phosphate (Na_2HPO_4 , analytical grade) 9.47 gm was dissolved in 1000mL of distilled water.

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

Disodium-hydrogen phosphate solution (1/15mol/L) 80mL solution of potassium dihydrogen phosphate (1/15mol/L) 20mL mixed, the pH adjusted to 7.4.

4.2.6 Giemsa dye

Weigh Giemsa dye 3.8g, was added 375mL of methanol (AR) grinding until completely dissolved before adding 125mL glycerin. Put 37 °C incubator insulation 48h shaken several times. After filtration with two weeks.

4.2.7 Giemsa Application Solution

Take 1 part of Giemsa dye was mixed with 9 parts of a phosphate buffer solution made using now.

5.1.1 Animal selection

Experimental animals should be selected in line with the relevant provisions of GB GB 14922.2 and 14922.1 of. Healthy adult male mice, 7 weeks to weeks 12 weeks, when the start of the test animal weight difference should not exceed $\pm 20\%$ of average weight. Animals should be randomly assigned to each group comprising at least 5 it can be used to analyze the animals. If the test sample has several points of time is required for each sampling time points are only used for analysis of at least 5 animal.

5.1.2 Animal Preparation

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

5.1.3 Animal Feeding

Animal breeding conditions should be consistent with GB 14925, drinking water should be consistent with GB 5749, feed should comply with the relevant provisions of GB 14924.

5.2 Test substance formulation

Test substances should be dissolved or suspended in a suitable vehicle in. The preferred solvent is water, water-insoluble test substance may be used vegetable oil (such as corn Oil, etc.), do not dissolve in water or oil test substance may be used as carboxymethyl cellulose, starch, etc. dubbed suspension or paste. The test substance should be now with the existing, Data show that, excluding its storage solution or suspension stabilizer.

5.3 Dose

5.3.1

the control group each test shall be provided with a corresponding negative (vehicle) and positive control, negative control group, except for not using the test sample, the other dealing with subject Consistent test was set.

Positive control group should be in spermatogonia and spermatocytes was observed to increase above background chromosomal aberrations. The positive control group exposed to passers-by Diameter may be different from the route of administration of the test substance, at a point in time can only sample. The positive control used cyclophosphamide (40mg/kg body Heavy, single intraperitoneal injection) or mitomycin C (1.5mg/kg body