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

GB 15193.11-2015

Replacing GB 15193.11-2003

**Sex-linked recessive lethal test
(English Translation)**

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Table of Contents

Foreword	
1 Scope	
2 Terms and definitions	
2.1 Lethal mutation	
2.2 Recessive mutations	
2.3 Sex-linked gene	
3 Test purposes and principles	
4 Instruments and reagents	
4.1 Instrument	
4.2 Reagents	
4.3 Preparation of Medium	
5 Test methods	
5.1 Test substance	
5.2 Experimental Animals	
5.3 Dose	
5.4 Test procedure and outcome measures	
6 Data processing and evaluation of results	
6.1 Data Processing	
6.2 Evaluation Results	
7 Test report	
8 Explanation, trial	

Foreword

This standard replaces GB 15193.11-2003 "Drosophila sex-linked recessive lethal test." this standard compared with GB 15193.11-2003, the main changes are as follows.

--- Standard name was changed to "national food safety standards Drosophila sex-linked recessive lethal test";

--- Amendments to the specific content of the "scope" of the test substance. This standard applies to the evaluation of the test substance is genotoxic;

--- Added "Terms and Definitions", "test report" and "Interpretation of Results";

--- Revised the "principle" of the part.

National Food Safety Standard Drosophila sex-linked recessive lethal test

1 Scope

This standard specifies the basic technical requirements Drosophila sex-linked recessive lethal test.

This standard applies to the evaluation of the test substance genotoxic effects.

2.1 Lethal mutation

Occurs in an altered genome, when expressed, causes the death of carriers.

2.2 Recessive mutations

A method of modifying the genome only in homozygous or hemizygous condition is expressed in.

2.3 Sex-linked gene

Gene in sex chromosome (X or Y) on the present. This refers only to the genes located on the X chromosome.

3 Test purposes and principles

Recessive gene in the sex-linked genetic cross has genetic characteristics that male flies X chromosome passed the F1 female flies, but also through the F1 generation female flies Passed F2 generation male flies. Located on the X chromosome recessive gene in the F1 generation female flies heterozygous, can not express, semi-synthetic and can type in the F2 generation male Fly manifested. Accordingly, the use of wink traits determined by genes on the X chromosome, the genetic characteristics associated with the X chromosome to make to observe the gene mutation on the X chromosome markers, so the wild-type male flies (red round eyes, normal flies) exposure, and Basc (Muler-5) female Fly (light beige rod eye on the two X chromosomes, each with an inverted position to prevent the F1 generation of the processed paternal X chromosome staining and maternal X Body swap) mating, such as male flies were subjected to the test after treatment, gene on the X chromosome occurs recessive lethal, can be regulated by the above two genetic it is manifested in the F2 generation of male flies, and membership wink marked trait to judge the results of the test. That classification according to Mendel reaction four Different phenotypes of the F2, no red round eyes of male flies in F2 generation when there are recessive lethal.

4.1 Instrument

Electric oven, incubator, stereo dissecting microscope, magnifying glass, air conditioners, bottles of anesthesia, Drosophila culture tubes, test tubes and plate rack, White porcelain

plate, sponge pad, brush, sponge plug.

Drosophila feeding utensils washed and dried after disinfection after 120 °C 2h spare.

4.2 Reagents

Ether, 75% ethanol, acetone, Tween.

4.3 Preparation of Medium

4.3.1

sugar 26g, yeast 4g, add water 150mL.

4.3.2

corn flour 34g, yeast 4g, add water 150mL.

Step 4.3.3. 4.3.1 the first medium components boiling mixed and dissolved, and then turn the ingredients into the mixing 4.3.2, boiling, most After adding propionic acid 2mL, stir, packed in Drosophila culture tubes, spare.

5.1 Test substance

Test substances should be dissolved or suspended in a suitable solvent, the solvent should be non-toxic, it does not react chemically with the test substance. The preferred solvent for the Water, water-insoluble test substance may be used vegetable oil (such as olive oil, corn oil, etc.), do not dissolve in water or oil test substance may be used carboxymethyl cellulose Su, dubbed starch suspension or paste, and then diluted with sugar water prior to sample. The test substance should be freshly prepared, our data show that its solution Except for storage or suspension stabilizer.

5.2 Experimental Animals

Drosophila melanogaster. Male flies with 3 days to 4-day-old wild-type Drosophila melanogaster (DrosophilaMelanogaster), female flies with Basc (Muler-5) line 3 days to 5 days virgin flies. Have observed 2d ~ 3d eggs or early instar larvae hatch, to check for non-virgin Flies mixed.

5.3 Dose

Determined by conventional methods Drosophila LC50 or LD50 values. Then press 1/2LC50 high dose or LD50, 1/4LD50 the dose, 1/8LD50 low dose, a separate negative (or solvent) and positive [2mmol/L methyl methane sulfonate (MMS)] in the control group. If the test Was less toxic, the test substance may be added to the medium of the maximum dose of 5% of the medium. Positive control available ethyl methane sulfonate, methyl sulfonate Methyl, N- nitroso dimethylamine.

5.4.1 Contact with the test substance

Contacting a test method for the oral administration. Freshly prepared medium when cooled to 55 °C, poured into a test substance, rapid magnetic stirring 2min, put After 4h the hunger of male flies were fed, the contact time of the test substance 3d.

5.4.2 Mating procedures and methods

To detect the test substance for which the most sensitive of the germ cells, the male flies after exposure to the test substance by 2-3-3d interval (respectively expressed fine Son, sperm cells and effector spermatocytes) and Virgin flies mating. That is, each tube with a male flies the treated sequentially with the procedure described above Two virgin flies mating, then press the F1 generation produced by female and male (1 to 1 or 1.2) were F1-F2 mate. 12d ~ 14d after observation of F2, Incubation temperature was 25 °C. Drosophila with ether after anesthetic can be grouped and F1 generation of gender separation.

Each test group should have at least 3000 samples.

6.1 Data Processing

According to the test chromosome number (ie, the female flies mating F1 generation minus the number of infertility and the number of waste pipe) and lethal positive tubes obtained mortality, press Formula (1) calculations.

Fatality rate = number of lethal tube/test chromosome number x 1000 per mille (1) Test methods suitable for the use of statistical experimental design for mortality in the control group and the test group for statistical analysis. Results should be evaluated the biological significance and statistical significance of test results at the same time be considered.

6.2 Evaluation Results

Judged as positive response relationship - test substance-induced mortality rate increased significantly, a significant difference compared with the negative control group, and a dose Sex; if no dose - response relationship, you should have at least one positive point in time of death can be repeated and statistically significant increase can also be judged as Positive.

7 Test report

7.1

Name of test, the test unit name and contact details, report number.