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

GB 15193.9-2014

Replacing GB 15193.9-2003

National Food Safety Standard -- Rodent Dominant Lethal Test

食品安全国家标准 啮齿类动物显性致死试验

(English Translation)

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Foreword

This standard replaces GB 15193.9-2003 "dominant lethal test." this standard compared with GB 15193.9-2003, the main changes are as follows.

--- Standard name was changed to "national food safety standard rodent dominant lethal test";

--- Increasing the terms and definitions;

--- Modified test purposes and principles;

--- Revised test methods;

--- Modify the data processing;

--- Increasing the test report;

--- Expanded to explain the experiment.

National Food Safety Standard Rodent dominant lethal test

1 Scope

This standard specifies the basic technical requirements and test methods for rodent dominant lethal test.

This standard applies to the evaluation of the test substance mutagenic effect.

2.1 Dominant lethal mutation

One kind of chromosome aberrations in the germ cells, structure, or change this number does not cause genetic germ cells (sperm or egg Sub) dysfunction, but the cause of death or developing embryo fertilized egg directly.

3 Test purposes and principles

Dominant lethal test is a test method for detecting test substances induced mammalian cell genotoxicity germ, which is viewed as the dominant lethal endpoints mutation. Mutagens can cause chromosomal aberrations in mammalian germ cells, that can not be combined with the opposite sex or cause a fertilized egg in the germ cells Preimplantation death, or cause early embryonic death. Generally male subjects treated rodents and mate with females, according to shun Cycle times of germ cells at different developmental stages of testing, after an appropriate time, the animals were sacrificed female uterus to check the contents to determine Implantation number, the number of live embryos and embryonic death. If the treatment group died embryos to increase or decrease the number of live embryos, and the control group was statistically Meaning a dose - response relationship between the test results can be repeated or who may be considered that the test substance is a mammalian germ cell mutagens.

Test Method 4

4.1 Test substance

4.1.1

test substance formulation. the test substance should be dissolved or suspended in a suitable solvent, the solvent should be non-toxic substances, the chemical does not occur with the test substance reaction. The preferred solvent is water, the test substance may be used liposoluble edible vegetable oils (such as olive oil, corn oil, etc.), insoluble in water or oil tested may be made using carboxymethyl cellulose, starch suspension was dubbed, not formulated as a suspension, other forms may also be formulated as a paste.

In general test substances should now use the existing, data show that except for its storage solution or suspension stabilizer.

4.1.2

give way. should gavage, or feeding method, commonly used mice fed volume of 10mL/kg body weight ~ 20mL/kg body weight, large Common rat gavage volume of 10mL/kg body weight. The method may also be used a positive control intraperitoneal injection, the injection volume of 10mL/kg of body weight -20mL/kg of body weight.

4.1.3

The administration of the test substance. Normal use once daily, continuous 5d to give way. If you think otherwise reasonably also be used, the disposable or three consecutive months of administration of the test substance.

4.2 Experimental Animals

4.2.1

Level and animal strains. Animals dominant lethal strain should choose the background value is low, the pregnancy rate and the implantation of a few more, the pre-test conception fertility Rate of more than 70% who recommended using mouse or rat. Experimental animals should meet the requirements of GB 14922.1 and GB 14922.2 and quality Certificate, healthy adult male mice (sexual maturity, 6 weeks to 8 weeks old, weighing 30g or more), or rat (sexual maturity, 8 weeks to 10 weeks Age, weight 200g or above). Mating with a female adult, it should never have been mating and reproductive history, different female mating cycle of weeks, Weight should phase approximation.

4.2.2

Number of animals. You should use the appropriate number of male animals, the number of males should be sufficient so that each mating cycle each produce 30 ~ 50 Pregnant females. Each male should be at least 15.

4.2.3

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

4.3 Dose

The experiment included at least three doses of the test substance group, high dose group animals should be able to cause some toxic symptoms, such as a slight drop in fertility, high Dose of test substance dose can be between 1/10LD50 ~ 1/3LD50. Acute toxicity test was the maximum tolerated dose administration of the test (Maximum Maximum capacity of concentration and intragastric) LD50 not find time, places 10g/kg body weight or body may intake of 100 times, or to the maximum test substance the highest dose to dose, then this dose and then set up two dose groups. General should also make positive and negative (vehicle) control group, if the same A laboratory in the last 12 months, the positive control

group Pat obtain positive results, and laboratory environmental conditions and animal strains did not change, you can not Then positive control group. The positive control was often used cyclophosphamide (cyclophosphamide), 30mg/kg body weight ~ 40mg/kg body weight, Intraperitoneal injection once a day for 5d; but also use other triethylene melamine (triethylenemelamine), methane sulfonic acid ethyl ester (ethyl methanesulfonate), methyl methanesulfonate (methylmethanesulfonate) and the like.

4.4 Test procedure and outcome measures

4.4.1

Mating. The male subjects were given the following day (once exposure method), or after the last administration of the test substance in male rats following day (multiple exposure method), male Press 1 to 1 or the female mated with 1.2 ratio after 5d, or sperm based on the vagina, check to make sure the female plug mating success, remove the other female Line feeding. After the interval 2d, then the proportion of males in the same with another batch of females mated with mice so batches for 5 to 6 batches, batch ~ 8 rats 10 batches.

4.4.2

embryos check. In male and female rats with cage date of 15 days to 17 days after the females were sacrificed, caesarean section to remove the uterus, inspect and record Recorded live embryos each female, the early death of embryos and embryos late deaths.

Embryo survival or death of identification as follows.

--- Live births. a complete shape, bright red color, natural movement, in sports after the reaction to mechanical stimulation.

--- Early death embryo. Embryo smaller body, the shape is not complete, the placenta is small or inconspicuous. The earliest embryonic death in utero Film uplift a small tumor. As has been completely absorbed, leaving a dark brown ridge point was only in the endometrium.

--- Late embryonic death. forming, dark color, no natural motion, no motor response after mechanical stimulation.

5.1.1 Statistical Analysis

Check the results of dominant lethal test in the test group were calculated in units of the following metrics for each mating cycle. Press the test group and control the indicators group of animals were used appropriate statistical analysis methods, such as chi2 test, ANOVA or rank sum test, in order to assess by Mutagenicity test substance. Pregnancy rates available chi2 test, the average number of implantation, the average number of available embryos death ANOVA or rank sum test France, embryonic mortality by rank sum test anyway analysis or ANOVA with post-string conversion.