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

GB 15193.22-2014

National food safety standard - 28 days oral toxicity test

食品安全国家标准 28天经口毒性试验

(English Translation)

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(To be issued)

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1 Scope

This standard specifies the experimental animals 28 days of basic technical requirements and test methods for oral toxicity tests.

This standard applies to the evaluation of short-term toxic effects of the test substance.

2 Terms and definitions 2.1 28 days repeated dose oral toxicity Experimental animals for 28 days after the oral health damage effect caused by contact with the test substance.

2.2 No observed adverse effect level Through animal experiments, the existing technical means and detection index was not observed any of the test substance-related toxic effects maximum dose.

2.3 Minimum observed adverse effect level Under specified conditions, the test substance causing a minimal effect dose animal tissue morphology, function, growth and development, and other harmful effects.

2.4 target organ

Organ by the test substance caused significant toxic effects of experimental animals.

2.5 Satellite Group

In toxicity studies in the design and implementation of additional group of animals, animal handling and feeding conditions and its main research similar to the test for interim or test convalescence observation and testing can also be used to observe and testing does not include other indicators and parameters in the main study.

3 test purposes and principles Determine toxic effects after 28 days of continuous oral exposure caused by the test substance, understand the test substance dose - response relationship and cytotoxicity target device Officer, determined 28 days after oral minimal observed adverse effect level (LOAEL) and not observed adverse effect level (NOAEL),

preliminary evaluation Test substance orally security, and provide longer-term and chronic toxicity test dose, observed indicators, toxicity endpoint selection for the next step in accordance with.

4 instruments and reagents

4.1 Instruments/Devices Anatomy of commonly used laboratory instruments, electronic balance, biological microscope, ophthalmoscope, biochemical analyzer, blood analyzer, blood coagulation analyzer, urine Analyzers, centrifuges, paraffin section machines.

4.2 Reagents

Formaldehyde, xylene, ethanol, hematoxylin-eosin, paraffin, blood analyzer diluent, biochemical analysis reagents, coagulation analyzer reagents, urine points Analysis of reagents.

5 Test methods

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. will Test was incorporated into feed, drinking water or gavage administration.

5.2 Experimental Animals 5.2.1 animal selection Experimental animals should be selected in line with the relevant provisions of GB GB 14922.2 and 14922.1 of. Select has been documented on the test substance sensitive Species and strains are generally preferred rodents rats, dogs preferred non-rodent (usually chosen Beagle dogs).

Recommended-week-old rats not more than 6 weeks, weighing 50g ~ 100g. Start of the test animals of each sex difference should not exceed the weight of the average weight $\pm 20\%$. each Group number of animals less than 20, male and female; if the end of the test program to do the recovery observed, should increase the number of animals (control and high dose increase Satellite groups of 10, male and female). Dogs should be used for 4 months to 6 months puppy, start of the test animal body weight of each sex was not should be more than the average weight of $\pm 20\%$, the number of animals in each group at least eight, male and female; if the end of the test program to do the recovery period observation, should be increased Number of animals (control and high dose increased satellite groups of four, male and female). Sex and number of animals in the control group should be the same group with a test substance.

5.2.2 Animal Preparation Before the test, rats in the experimental animal room should be at least 3d ~ 5d (dogs 7d ~ 14d) and adaptation to the environment quarantine observation.

5.2.3 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.

During the test animals free access to water and food, recommend a single caged rat cage can also be components of sex are reared in groups, the number of animals per cage (usually no more than

3) shall meet the minimum space required for experimental animals in order not to affect the free movement of animals and the animals were observed for signs appropriate. During each test Non-animal test should be less than 10% of mortality, dying animal blood biochemical tests should be carried out as far as possible, the gross anatomy and pathology group Histological examination of biological specimens each loss rate should be less than 10%.

5.3 dose

5.3.1 Packet

The experiment included at least three doses of the test substance group, a negative (vehicle) control group, if necessary, additional untreated control group. If the end of the trial do Recovery was observed in the control and high dose for an additional satellite group. In addition to the control group not given the test substance, the rest are treated with the test substance dose group.

5.3.2 Design of dose High doses of the principle 5.3.2.1 should make some animals appear more obvious toxicity, but does not cause death; low dose should not have any concept of Observed toxic effects (equivalent NOAEL), and higher than the actual human exposure levels; dose ranging between, there may be mild toxic effect it should be to arrive at LOAEL. Group spacing generally declining doses 2 to 4 times with appropriate, such total dose of test substance is too large span, the dose can be added provided group. The reference design test dose acute toxicity LD50 dose and the actual human intake of conduct.

5.3.2.2 LD50 test substance can be determined to LD50 10% to 25% as 28 days oral toxicity test of the highest dose group, the LD50 Select the percentage of the main reference LD50 dose - response curve slope. Then this dose group consists of several doses, at least the lowest dose group Three times the expected human intake.

5.3.2.3 not seek LD50 test substance, the test dose should be possible to cover the expected intake of 100 times the human dose, without affecting animals Under the premise of feeding and nutritional balance should try to improve dose high dose group. Intended for human intake of larger test substance, the high-dose group also to give the maximum amount by design.

5.4 Test procedure and outcome measures

5.4.1 The test is administered 5.4.1.1 according to the characteristics of the test substance and test purposes, select the test was incorporated into feed, water or orally administered.

If the test was its driving Was palatability, should be given orally. The test substance should be administered continuously for 28 days.

5.4.1.2 test was orally administered to the test substance is dissolved or suspended in a suitable vehicle, the preferred solvent is water, water-insoluble test substance You can use vegetable oils (such as olive oil, corn oil, etc.), do not dissolve in water or oil can also make use of the test substance carboxymethyl cellulose, starch, etc. dubbed suspended Liquid or paste and the like. The test substance should now use the existing, data show that except for its storage solution or suspension stabilizer. Should be daily during the same period Gavage once weighed twice weekly gavage volume weight adjustment. Gavage volume of generally not more than 10mL/kg body weight, such as a water-soluble when liquid, the maximum volume of rats fed up to 20mL/kg of body weight, dogs 15mL/kg body weight; the case of an oily liquid, the volume should not exceed gavage 4mL/kg body weight; each group was given a consistent volume.

5.4.1.3 incorporated into feed or drinking water test substance administered to the test substance and feed (or water) and mixed well to ensure the stability of the test substance formulation Qualitative and uniformity, without affecting the animal feeding, nutritional balance and water intake to the principle of the test substance incorporated into feed ratio is generally less than the mass fraction 5%, while if more than 5% (maximum not more than 10%), the control diet was adjusted nutrient levels (when the test substance no heat or nutrients, and when you add a proportion of more than 5% in the control group diet should be filled methyl cellulose, incorporated in an amount equivalent to the high dose), and it was tested nutritional group Hormone levels consistent, while additional untreated control group; also depends on the subject matter of heat or nutrient status of the test substance dose adjustment feed Nutrient levels, making it consistent with the control group diet nutrient levels. Test substance dosage units per kilogram of body weight is ingested the test substance Mg (or g) number, namely mg/kg body weight (or g/kg body weight), when the test was incorporated into feed dosage unit can be expressed as mg/kg (or g /kg) of feed, the incorporation of water is expressed as mg/mL of water. When the test was incorporated into the feed, the need to test substance dose (mg/kg body weight) by each animal Food intake, body weight of 100g converted into animal feed test concentration (mg/kg feed), usually 28 days oral toxicity test according to the daily food intake of rats Weight 10% conversion.

5.4.2 General Clinical Observation Observation period 28d, assuming that the recovery observed after administration of the test animals should stop was to continue to observe 14d, to observe the test substances toxic Reversibility, persistence and delayed effects. Usually observed at least once daily during the clinical manifestations of animal testing and record animals poisoning Signs, and the extent and duration of death. Observation includes hair, skin, eyes, mucous membranes, secretions, excretions, respiratory system, nervous System, autonomic activity (eg. lacrimation, piloerection reaction, pupil size, unusual respiratory) and behavior (eg. gait, posture, response to treatment, there is no tonic or