



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

GB 15193.3-2014

**National food safety standard - Acute toxicity test
(English Translation)**

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Foreword

This standard replaces GB 15193.3-2003 "Acute toxicity test".

Compared with GB 15193.3-2003, the main changes of this standard are as follows.

- CHANGE the standard name into "National food safety standard - Acute toxicity test";
- MODIFY the Scope;
- ADD test report and test interpretation;
- MODIFY the experimental animals and test methods;
- DELETE the maximum tolerated dose method; ADD the limit test and the up-down procedure;
- MODIFY the Appendix.

National food safety standard - Acute toxicity test

1 Scope

This standard specifies the basic test methods and technical requirements, for acute oral toxicity test.

This standard applies to the evaluation of the acute oral toxicity of the test substance.

2.1 Acute oral toxicity

The toxic effects on animals, in a short period of time, after one or more oral administration of the test substance to experimental animals within 24 h.

2.2 The half lethal dose (LD50)

The dose of the test substance, that can cause animal mortality to be 50%, after oral administration of the test substance once or multiple times within 24 h. It is the calculated value, which is obtained through statistics.

3 Test purpose and principle

The acute oral toxicity test is the most basic test, for detecting and evaluating the toxic effects of the test substance, that is, after the test substance is administered orally once or multiple times within 24 hours, the toxic reactions produced by animals, including poisoning sign and death, are observed in a short period of time. It is usually expressed as LD50.

4.1.1 Preparation of test substances

It shall dissolve or suspend the test substance in a suitable solvent. The preferred solvent is water.

4.1.2.1 Approach

Oral gavage.

4.1.2.2 Fasting before the test

Animals need to fast before the test. Generally, rats need to fast overnight (generally about 16 h); mice need to fast for 4 h ~ 6 h; BUT they can drink water freely. After administration of the test substance, rats need to continue fasting for 3 h ~ 4 h; mice need to continue fasting for 1 h ~ 2 h. If the test substance is administered in batches multiple times, the animals can be given a certain amount of feed, according to the length of the exposure interval.

4.1.2.5 Observation period

General observation period is 14 d, which may be extended to 28 d if necessary. It shall be observed for at least 7 d, in special emergency situations.

4.2.1 Animal selection

The selection of experimental animals shall comply with national standards and relevant regulations (GB 14923, GB 14922.1, GB 14922.2). Choose healthy adult rats (180 g ~ 220 g) and/or mice (18 g ~ 22 g) of two genders, OR choose other experimental animals. Females shall be unmated and unpregnant. The difference in body weight, between animals of the same gender, shall not exceed $\pm 20\%$ of the average body weight.

4.2.2 Animal preparation

Before the test, the experimental animals shall undergo at least 3 d ~ 5 d environmental adaptation and quarantine observation, in the test environment.

4.3.1.1 Pre-test

According to the nature of the test substance and the known data, the following methods are used. Generally, it uses the dose of 100 mg/kg body weight, 1000 mg/kg body weight, 10000 mg/kg body weight, each of which uses 2 ~ 3 animals for pretest. According to the death situation within 24 h, estimate the possible range of LD50. Determine the dose group of the formal test.

4.3.1.2.1 Number of animals

Generally, there are 10 animals in each group, half male and half female.

4.3.2.1 Scope of application

This method is applicable to the test substance, whose data shows little or no toxicity. When the animal is administered with a certain dose of the test substance, there is still no death.

4.3.2.2 Number of animals

It generally selects 20 animals, half male and half female.

4.3.3.1 Scope of application

This method is mainly applicable to the test substance, which has high purity, high toxicity, low intake, AND the animal will die, within 1 d ~ 2 d after administration of the test substance. For the test substance, which causes the animal to die at 5 d and above, after administration, it is not applicable. The test can be carried out, according to the dose sequence selected by the tester OR under the guidance of a special software package; the test results can be counted.

4.3.4.1 Pre-test

Unless otherwise required, the dose, at which all animals die or more than 90% of the animals die, AND the dose, at which no animals die or less than 10% of the animals die, shall be obtained in the pre-test, as the highest dose and the lowest dose in the formal test, respectively.

4.3.6.1 Principle

When two or more test substances exist at the same time, three different combined action modes of antagonism, addition or synergy may occur. These three different actions can be determined, according to certain formulas and judgement criteria.

4.3.6.3.1 Each test substance in the mixture is mixed in an equal toxic ratio, so the

calculated LD₅₀ is multiplied by the ratio of each test substance, to obtain the dose of each test substance.

4.4.1 Clinical observation

Observations include skin, hair, eyes, mucous membranes, respiratory system, genitourinary system, digestive system, nervous system, etc. Special attention shall be paid to the presence or absence of tremor, convulsions, salivation, diarrhea, sluggishness, lethargy, coma (see Appendix F).