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Toxicological test methods for pesticides registration - Part 3: Acute oral toxicity test - Up-and-down-procedure

农药登记毒理学试验方法 第3部分：急性经口毒性试验 序贯法

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1 Scope

GB/T 15670.3-2017 is the Chinese national standard on toxicological test methods for pesticides registration - part 3: acute oral toxicity test - up-and-down-procedure, in the field of agriculture. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 12 July 2017 by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; Standardization Administration of China, and has been in force since 1 February 2018. Classification: ICS 65.100, CCS B17. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This part of GB/T 15670 specifies the basic principles, methods and requirements for the up-and-down-procedure of acute oral toxicity testing. This part applies to acute oral toxicity tests conducted for pesticide registration.

2 Normative references

The following documents are essential for the application of this document. For any dated referenced document, only the dated version applies to this document. For any undated referenced document, the latest version (including all amendments) applies to this

document.

GB 14925 Laboratory animal - Environment and housing facilities Regulations on Pesticide Registration Information (Order No. 10 of the Ministry of Agriculture of the People's Republic of China [2007])

3 Terms and definitions

The following terms and definitions apply to this document.

3.1 acute oral toxicity The health-damaging effects that occur in animals in the short term after the test substance is orally administered to experimental animals once or multiple times within 24 hours.

3.2 dose The amount of the test substance administered, which is expressed as the mass of the test substance received per unit body weight of the experimental animal (mg/kg body weight).

3.3 median lethal dose LD

50 The statistical dose of a toxicant that causes half of the total number of animals to die after the test substance is given to the experimental animals once or multiple times within 24 hours. It is expressed as the mass of the test substance received by the experimental animals per unit body weight (mg/kg body weight).

3.4 dose-response relationship The relationship between the dose and the incidence of a specific effect.

4 Purpose of the test

Acute oral toxicity test is the first step to evaluate the toxicity of the test substance. Through short-term administration of toxicant by oral, the toxicity characteristics and dose-response relationship of the test substance are preliminarily understood, which provides a basis for acute toxicity classification, label management and other toxicology test dose selection.

5 Experimental overview

The up-and-down procedure (UDP) is a step-by-step administration procedure. Animals of the same sex are tested, and one animal is administered at a time. The first animal is administered a dose that is preferably lower than the estimated LD

50.The increase or decrease in the subsequent dose of administration depends on the administration result (survival or death) of the previous animal. When the preliminary estimate of the LD50 of the test substance and the slope of the dose-response curve are not available, the computer simulation result suggests that the starting dose can be 175 mg/kg body weight, and the dose interval is calculated using the antilogarithm of 0.5

(corresponding to a default dose progression factor of 3.2). The point estimate value and 95% confidence interval of LD50 are calculated by the maximum likelihood method. NOTE. The dose progression factor is determined by the antilogarithm of the reciprocal of the slope of the dose-response curve (

3.2 is the progression factor for a slope of 2). The number of animals used in the formal test is about 6~

9.The number of animals used in the limit test is no more than

5.This method is only applicable to test substances that cause death within 1~2 days after administration, and is not applicable to test substances that are expected to cause death to happen more than 5 days (including 5 days) after administration.

6.1 Test substances

6.4.1.2 If 3 of the 5 administered animals die within 48 hours or their death is within the 14-day observation period, the administration is terminated and the formal test begins.

6.4.2 Formal test

6.4.2.1 Before the experiment, the rats were fasted overnight but had unlimited access to water.

6.4.2.2 Selection of starting dose and dose progression factor. When an estimate of the lethal dose of the test substance is not available, the default starting dose is 175 mg/kg body weight. The dose sequence set corresponding to the progression factor

3.2 of a dose-response slope of 2 is

1.75 mg/kg body weight,

5.5 mg/kg body weight,

17.5 mg/kg body weight, 55 mg/kg body weight, 175 mg/kg body weight, 555 mg/kg body weight and 2000 mg/kg body weight; or

1.75 mg/kg body weight,

5.5 mg/kg body weight,

17.5 mg/kg body weight, 55 mg/kg body weight, 175 mg/kg body weight, 555 mg/kg body weight, 1750 mg/kg body weight and 5000 mg/kg body weight. If the slope of the dose-response curve of the test substance is known to be relatively steep or relatively flat, the dose progression factor can be reduced or increased. Appendix A lists the dose levels available with a starting dose of 175 mg/kg body weight and a slope of 1~8.

6.4.2.3 At the beginning of the test, weigh the fasted animals and administer the test substance orally using a gavage needle, one animal at a time, with an interval of 48 hours

between each animal. The administration dose for the second animal depends on the result of the first animal's administration. If the animal dies or is in a dying state, the dose is adjusted down by one level according to the progression factor; if the animal survives, the dose is adjusted up by one level. When the subsequent doses show the opposite result to the starting dose, add 4 more animals to be administered the test substance sequentially and then terminate the test, or when any of the termination requirements (see 6.4.2.6) are reached, the test can be terminated. After administration, the rats shall continue to fast for 3 h~4 h. The observation period for each animal is 14 d.

6.4.2.4 If animals survive within 48 hours after administration but die later in the subsequent observation period, it indicates that the dose selection is inappropriate and consideration may be given to giving 2 more animals a dose lower than the dose that causes death, restarting the test and extending the observation period.

6.4.2.5 The test results are used to calculate the LD50 and 95% confidence interval of the test substance using the maximum likelihood method (see Appendix B).

6.4.2.6 The test can be terminated if any of the following conditions are met.

a) When three animals survive the sequential administration at the upper dose range;

6.5 Test observation and inspection

6.5.1 Clinical observation During the 24 hours after administration (especially within 30 minutes and the first 4 hours after administration) and the 14-day observation period, observe and record the poisoning reactions of each animal regularly every day. Clinical observations shall at least include.

a) Central nervous system and neuromuscular system. abnormal posture, abnormal voice, restlessness, dullness, tremor, convulsion, paralysis, movement disorder, hypersensitivity or slowness in external response, abnormal behavior such as excessive and repeated scratching around the mouth, combing, circling, even self-mutilation, walking backward, etc.;

b) Autonomic nervous system. pupil dilation or constriction, salivation and tearing;

c) Respiratory system. nasal discharge, epistaxis, flaring of the nostrils, deep and slow breathing, tachypnea, wasp waist;

d) Urogenital system. dirty perineum, discharge, vaginal or breast swelling;

e) Skin and coat. skin congestion, cyanosis; fluffy or wet coat, dirty;

f) Eyes. proptosis, conjunctival congestion, hemorrhagic discharge, corneal opacity;

g) Digestive system. diarrhea, anorexia. The time and severity of tremors, convulsions, salivation, diarrhea, lethargy and coma, the death time or the recovery time shall be recorded. Animals on the verge of death or severe pain, as well as animals that survive the