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

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**Test guidelines on environmental safety assessment for
chemical pesticides - Part 11: Silkworm acute toxicity test**

化学农药环境安全评价试验准则 第11部分：家蚕急性毒性试验

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1 Scope

This document describes the test method for acute toxicity to silkworms in the environmental safety assessment for chemical pesticides.

This document is applicable to acute toxicity tests on silkworms conducted for the registration of chemical pesticides.

2 Normative References

GB/T 6682

NY/T 2882.5

NY/T 4195.4

3 Terms and Definitions

The following terms and definitions are applicable to this document.

3.1 Median lethal concentration

In toxicity test by the spraying method, the concentration of the test substance in mulberry leaves causing 50% mortality in the test organisms, which is expressed as LC50.

NOTE. the unit is mg test substance/kg mulberry leaves or mg a.i./kg mulberry leaves.

4.1 Toxicity Test by Spraying Method

Use spray-treated mulberry leaves to feed second-instar silkworms. Observe the poisoning symptoms and mortality of the silkworms under test after 24 h, 48 h, 72 h and 96 h. After the test, the data are statistically analyzed to determine the median lethal concentration (LC50) and its 95% confidence interval.

4.2 Toxicity Test by Fumigation Method

Tests simulating indoor application conditions for hygiene drugs shall be conducted in a fumigation test device or fumigation chamber that meets the test requirements. The fumigation test device or fumigation chamber shall be designed with relevant parameters in accordance with the recommended dosage, while meeting the test requirements. The test substance is quantitatively combusted (or electrically heated) in the test device or fumigation chamber. From the start of fumigation, observe and record the toxic reaction symptoms of silkworms in the fumigation test device at 0.5 h, 2 h, 4 h, 6 h and 8 h. After 8 h, remove the silkworms from the test device, and under normal silkworm rearing conditions, continue to observe for mortality at 24 h and 48 h.

5 Test Conditions

The test temperature is 25 °C ± 2 °C, the relative humidity is 70% ~ 85%, and the photoperiod (light-dark ratio) shall be 16 h. 8 h.

6.1 Reagent

6.1.1 Unless otherwise specified, all reagents are recommended to be of analytical grade, and water shall be Grade III water as specified in GB/T 6682.

6.1.2 Acetone (C₃H₆O, CAS No.. 67-64-1).

6.1.3 Dimethylformamide (C₃H₇NO, CAS No.. 68-12-2).

6.1.4 Dimethyl sulfoxide (C₂H₆OS, CAS No.. 67-68-5).

6.1.5 Agar (CAS No.. 9002-18-0).

6.2 Test Organisms

Representative silkworm (*Bombyx mori*) varieties, such as Jingsong x Haoyue shall be

selected and cultured in accordance with the requirements of NY/T 4195.4. Second-instar silkworms are used as test silkworms.

6.3 Mulberry Leaves for Testing

Collect fresh, clean and uncontaminated young leaves from the top of the mulberry tree (*Morus alba*) for the test. Unused mulberry leaves can be wrapped in plastic wrap around the petioles, placed in a sealed bag, and refrigerated for up to one week.

6.4 Petri Dishes

Preferably, use petri dishes with a diameter of 20 cm.

7 Instruments and Equipment

7.1 Sprayer (shall evenly spray and allow for quantitative calculation).

7.2 Artificial climate chamber.

7.3 Constant temperature and humidity incubator.

7.4 Electronic balance (with a division value of 0.0001 g or higher). 7.5 Fumigation box.

7.6 Temperature and humidity recorder, etc.

8 Test Substances

Active compound (parent material) or formulation of pesticide. Before the test, it is advisable to understand the solubility of the test substance in water and organic solvents, its stability in water and photolytic stability, the recommended field application rate, and the number of applications, etc.

9.1.1 Preliminary test

In accordance with the conditions of the formal test, set up treatment groups with large intervals to determine the concentration range of the formal test. The preliminary test may not have replicates; each treatment shall contain 20 second-instar silkworms.

9.1.2.1 Test design

Based on the results of the preliminary test or known toxicity data, at a certain proportional interval (the geometric difference shall not exceed 2.2), set up 5 ~ 7 concentration groups, and a blank control. If a solvent is used to aid the dissolution of the test substance, a solvent control shall also be set up. Each treatment group and control group shall have 3 replicates, with 20 second-instar silkworms per replicate.

9.1.2.2 Preparation of test substance solution

Use water to prepare test solutions of the test substance to different concentrations. For substances that are poorly soluble in water, a small amount of organic solvents (for example, acetone) with low toxicity to silkworms, emulsifiers or dispersants may be used to aid the dissolution; their amount shall not exceed 1% (volume fraction), and the amount shall be consistent across all treatment groups.

9.1.2.3 Exposure

9.1.2.3.1 Select mulberry leaves of similar size and mass, preferably retaining a 1 cm ~ 2 cm petiole, with each leaf weighing 2.0 g ~ 3.0 g. Use a sprayer to spray the test solution onto the underside of the mulberry leaves, ensuring uniform exposure. Weigh the mass of the mulberry leaves before and immediately after spraying to determine the accurate amount of test substance applied to each leaf. After each treatment, use a solvent of low toxicity to silkworms to clean the inner wall of the sprayer to prevent water droplets from forming and causing uneven spraying. After the mulberry leaves have dried, place 3 leaves in each petri dish as one replicate.

During the test, take measures to keep the leaves fresh, for example, inserting the petioles into 1% agar medium.

9.1.2.3.2 Select healthy, uniformly sized second-instar silkworms and randomly transfer them onto the mulberry leaves in the petri dishes. During the test, the silkworms are continuously fed the exposed mulberry leaves. If the leaves withered, the exposed mulberry leaves shall be replaced in time.

9.1.2.4 Observation and recording

At 24 h, 48 h, 72 h and 96 h after exposure, observe and record the symptoms of silkworm poisoning and the number of deaths. If the difference in mortality rate between the treatment group and the control group at 72 h and 96 h is greater than 10%, replace them with unexposed fresh mulberry leaves and extend the observation period, until the mortality rate increases by less than 10% within 24 h.

9.1.3 Limit test

The limit concentration is 5,000 mg of test substance/kg mulberry leaves or 70 times the predicted exposure concentration (PEC) calculated in accordance with NY/T 2882.5 (take the higher value). At least three replicates shall be set up for the treatment and control groups, with 20 second-instar silkworms per replicate. If the mortality rate in each replicate at the limit concentration is not greater than 10%, then, a formal test is not required.