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

GB/T 31270.9-2025

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**Test guidelines on environmental safety assessment for
chemical pesticides - Part 9: Avian short-term dietary toxicity
test**

化学农药环境安全评价试验准则 第9部分：鸟类短期饲喂毒性试验

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Foreword

GB/T 31270 Test Guidelines on Environmental Safety Assessment for Chemical Pesticides can be divided into the following 21 parts:

- Part 1: Transformation in Soils;
- Part 2: Hydrolysis;
- Part 3: Photo-transformation;
- Part 4: Adsorption/Desorption in Soils;
- Part 5: Leaching in Soils;
- Part 6: Volatility;
- Part 7: Bioconcentration Test;
- Part 8: Degradation in Water-Sediment Systems;
- Part 9: Avian Acute Toxicity Test;
- Part 10: Honeybee Acute Toxicity Test;
- Part 11: Silkworm Acute Toxicity Test;
- Part 12: Fish Acute Toxicity Test;
- Part 13: Daphnia Sp. Acute Immobilization Test;
- Part 14: Alga Growth Inhibition Test;
- Part 15: Earthworm Acute Toxicity Test;

- Part 16: Soil Microorganism Toxicity Test;
- Part 17: Trichogramma Acute Toxicity Test;
- Part 18: Amphibian Acute Toxicity Test;
- Part 19: Effects on Non-Target Plants;
- Part 20: Livestock Short-Term Dietary Toxicity Test;
- Part 21: Macro-Crustacean Toxicity Test.

This Part belongs to Part 9 of GB/T 31270.

Test Guidelines on Environmental Safety Assessment for Chemical Pesticides - Part 9:
Avian Acute Toxicity Test

1 Scope

This Part of GB/T 31270 specifies the basic requirements for materials, conditions, operation, quality control, data processing, test reports, etc. of avian acute oral toxicity test and acute feeding toxicity test.

This Part is applicable to the avian acute oral toxicity test and acute feeding toxicity test registered for chemical pesticides. Other types of pesticides can be used as reference.

This Part is not applicable to the chemical pesticides that are volatile and difficult to dissolve.

2 Terms and Definitions

For the purposes of this document, the following terms and definitions apply.

2.1 Median lethal dose

In the acute oral toxicity test, the dose of the test substance which caused 50% of the test organisms to die; expressed by LD50.

NOTE: unit is mg a.i./kg body weight.

2.2 Median lethal concentration

In the acute feeding toxicity test, the concentration of the test substance which caused 50% of the test organisms to die; expressed by LC50.

NOTE: unit is mg a.i./kg feedstuff.

2.3 Test substance

Substance to be tested in the test.

granular formulations, the capsule injection method can be used for contamination.

3.3 Acute feeding toxicity

The acute feeding toxicity refers to using a sprayer to spray the drug solutions with different concentrations on the food; mix as spray till mix evenly. Feed the test birds for 5d by feedstuff containing different-concentration test substances; feed the test birds for 3d with feedstuff not containing the test substances from the 6th day; record the poisoning and death of the birds every day; and calculate the 8d LC50 value and 95% confidence limit.

4.1.1 Test organism

Depending on the purpose of the test, one or more species are available for selection.

The test species can breed on their own; or the standardized breeding materials can be purchased. The selected test birds shall be in good health and have no obvious deformity; the mortality of the test species in the first 7d after introduction into the laboratory shall be < 5%; and they are considered in good health conditions if their growth state conforms to the growth law of these species. The recommended species can refer to Appendix A. The test birds shall pass animal quarantine to ensure that there are no diseases. The test birds shall come from the same female population and hatches on the same day.

4.1.2 Test substance

The test substance shall use pure pesticides, technical material or formulation. For those hard to dissolve into water, they can use a small amount of organic solvent with little toxicity to birds to assist in dissolving. The amount of organic solvent shall not exceed 0.1mL (g)/L.

4.1.3 Main apparatus

The main apparatus is as follows:

- Bird cage for test;
- Electronic balance;
- Pipette;
- Syringe, etc.

Set at least 5 concentration groups at a certain interval against the concentration range determined by the pre-test. 10 birds in each group, half male and half female. Set the blank control group; while the solvent control group shall also be set for those that need solvent to assist in dissolving. No repetition shall be set for the control group and each concentration group. The geometric difference in concentration level between concentration groups shall not exceed 2 times. Observe and record the poisoning symptoms and death of the test birds every 24h. After the completion of the test, the data shall be subjected to

mathematical statistics to calculate median lethal concentration LC50 value and 95% confidence limit.

4.2.2.3 Limit test

According to the classification criteria of pesticide toxicity to birds, the upper limit is set to 5000 mg a.i. /kg body weight; i.e. if the bird does not die when the test substance reaches 5000 mg a.i. /kg body weight; then the test does not have to be carried out. At this point, it can be determined that the oral toxicity of the test substance to birds is low toxicity.

4.3.1 Selection of statistical analysis methods

The Karber's Method, linear interpolation method and probability unit graphic method can be used to calculate the median lethal dose LD50 and median lethal concentration LC50 of the avian oral toxicity and feeding toxicity at each observation time; the calculation and analysis can also be conducted through data statistic software.

4.3.2 Karber's method

Use Karber's method to calculate the LD50 value and 95% confidence limit of the avian oral toxicity on the 7d; and the LC50 value and 95% confidence limit of the feeding toxicity on the 8d.

The calculation of LD50 (LC50) can refer to Formula (1): Where:

X_m - logarithm of the highest concentration;

i - logarithm of adjacent concentration ratio;

ΣP - the sum of the mortality rates in each group (expressed in decimal fraction).

The calculation of 95% confidence limit can refer to Formula (2):