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Toxicological test methods for pesticides registration - Part 10: Short-term repeated dose 28-day oral toxicity study

农药登记毒理学试验方法 第 10 部分：短期重复经口染毒（28 天）毒性试验

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Toxicological test methods for pesticides registration - Part 10: Short-term repeated dose 28-day oral toxicity study

1 Scope

This Part of GB/T 15670 specifies the basic principles, methods, requirements for short-term repeated dose 28-day oral toxicity study.

This Part applies to short-term repeated dose 28-day oral toxicity study, for pesticide registration.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB 14925 Laboratory animal - Requirements of environment and housing facilities

3 Terms and definitions

The following terms and definitions apply to this document.

3.1

Short-term repeated dose oral toxicity

Health-damaging effects, which are caused by repeated oral administration of the test substance for a period shorter than 10% of the life span of the experimental animal.

3.2

No observed adverse effect level; NOAEL

Under the specified test conditions, using existing technical means and detection indicators, the highest dose or concentration of the test substance, at which harmful effects related to poisoning cannot be observed.

3.3

Lowest observed adverse effect level; LOAEL

Under the specified test conditions, using existing technical means and detection indicators, the lowest dose or concentration of the test substance, at which harmful effects related to poisoning are observed.

3.4

Target organ

Organs, in which the test substance causes significant toxic effects in the body.

4 Test purpose

Through short-term repeated dose 28-day oral toxicity study, determine the toxic effects, which are caused by repeated oral exposure to the test substance in a short period of time; preliminarily understand the toxicological characteristics, dose-response relationship and target organs of the test substance; obtain the non-observed adverse effect dose level (NOAEL) and the lowest observed adverse effect dose level (LOAEL), so as to provide a basis for the selection of sub-chronic and chronic toxicity test doses and observation indicators.

5 Test overview

The experimental animals are repeatedly orally exposed to the poison for 28 days, in a short period of time. Observe the toxic reactions of the animals. Weigh the body weight and calculate food intake, regularly. Measure the hematological indicators, blood biochemical indicators, histopathological examination indicators, etc., to evaluate the short-term repeated intake toxicity of the test substance, thereby initially determining the dose and target organs of the harmful effects, which are caused by the test substance in animals.

6 Test methods

6.1 Preparation of test substance

When the test substance is mixed into feed or drinking water, in order to ensure the uniformity and stability of the preparation of the test substance, it shall measure the stability, content, uniformity. The concentration fluctuation in the feed or drinking water shall be within ±15% of the theoretical concentration; it will not affect the nutritional quality and water balance of the feed. The amount added into the feed shall not exceed 5% of the feed concentration. When the test substance is added to feed or water to affect the palatability of animals, the test substance shall be administered orally. When injecting poison into the stomach, the test substance shall be dissolved or suspended in

6.3.3 Limit test

If it is expected that the dose of 1000 mg/kg body weight is unlikely to produce any toxic effects, OR it is predicted that it will not produce toxicity based on the structure, there is no need to set three doses.

6.4 Routes of exposure

The test substance can be mixed into feed or drinking water, OR administered by gavage. Animals shall be exposed to the poison for 7 days per week. If the gavage method is used, the gavage method shall be administered once a day at the same time and place; the amount of gavage shall be adjusted regularly, according to the body weight (the body weight shall be weighed twice a week for adjustment), to maintain the dose of the exposure constant. The intragastric volume generally does not exceed 10 mL/kg body weight. The maximum intragastric volume of aqueous solution can reach 20 mL/kg body weight. The intragastric volume of each group is consistent. The exposure time is 28 days. The animals in the additional group are observed for at least 14 days during the recovery period.

6.5 Test observation and inspection

6.5.1 Clinical observation

During the test, the general clinical manifestations of the animals are observed at least once a day. The signs, extent, duration of poisoning and death of the animals are recorded. Observations include at least the following: changes in skin, hair, eyes, mucous membranes, respiratory system, circulatory system, nervous system, limb activities, behavior, etc. Dead animals shall be dissected promptly; dying animals shall be disposed of promptly.

6.5.2 Body weight, food and water intake records

Record body weight and food intake every week. If the test substance is administered through drinking water, it shall record the weekly water intake.

6.5.3 Hematology tests

At the end of the experiment, fasting blood samples are collected before the animals are sacrificed (or when they were sacrificed), for determination of hematological indicators. Measurement indicators include hemoglobin concentration, red blood cell count, hematocrit, total white blood cell count and classification, platelet count, coagulation function (prothrombin time and activated partial thromboplastin time). If it affects the blood system, it shall add the reticulocyte and bone marrow smear cytology tests.

6.5.4 Blood biochemical tests

At the end of the experiment, before the animals are sacrificed, blood is collected on an

empty stomach, to measure blood biochemical indicators. The measurement indicators shall at least include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea nitrogen (BUN), creatinine (Cr), blood glucose (GLU), Albumin (ALB), total protein (TP), total cholesterol (TCH), potassium, sodium; if necessary, indicators such as γ-glutamyl transpeptidase (GGT), ornithine decarboxylase (ODC), triglycerides, bile acid, cholinesterase, hormones, calcium, phosphorus.

6.5.5 Urine test

Generally, it is not necessary. When relevant toxic effects are suspected or observed, urine needs to be collected for urine indicator measurement, including appearance, specific gravity, pH, urine protein, glucose, blood cells.

6.5.6 Pathological examination

6.5.6.1 Gross anatomy examination

At the end of the test, a general examination of all animals shall be conducted, including body surface, cranial cavity, chest cavity, abdominal cavity and their organs. Dissect brain, pituitary gland, heart/aorta, thyroid and parathyroid glands, esophagus, lungs/trachea, thymus, stomach, duodenum, small intestine, large intestine, liver, kidneys, spleen, pancreas, adrenal glands, ovaries, testicles, epididymis, representative lymph nodes, spinal cord (cervical, thoracic and lumbar segments), uterus, prostate, bladder, sciatic nerve, sternum for fixation. If necessary (if there are lesions on gross observation), dissect the breasts, muscles, femurs, lacrimal glands, salivary glands, skin, eyeballs, for fixation. Also weigh the brain, heart, thymus, adrenal gland, liver, kidney, spleen, testis, ovary, to calculate the organ coefficient.

6.5.6.2 Histopathological examination

The principles of histopathological examination are:

a) Gross anatomy examination of all fixed and preserved organs and tissues in the high-dose group and control group for histopathological examination;

b) After discovering the lesions in the high-dose group, perform histopathological examination on the corresponding organs and tissues in the medium- and low-dose groups;

c) Histopathological examination shall be conducted, on other diseased organs and tissues visible to the naked eye, during gross anatomical examination;

d) If there are abnormal changes in the poisoning treatment group, histopathological examination shall be conducted, on the corresponding organs and tissues of the animals in the additional group;

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