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Toxicological Test Methods for Pesticides Registration - Part 21: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in Vivo

农药登记毒理学试验方法

第 21 部分：体内哺乳动物肝细胞程序外 DNA 合成（UDS）试验

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Toxicological Test Methods for Pesticides Registration - Part 21: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in Vivo

1 Scope

This Part of GB/T 15670 specifies the basic principles, methods and requirements for the unscheduled DNA synthesis (UDS) test of mammalian liver cells in vivo.

This part is applicable to unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo for the 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 document.

GB 14925 Laboratory Animal - Requirements of Environment and Housing Facilities

3 Terms and Definitions

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

3.1 Unscheduled DNA synthesis; UDS

DNA synthesis that occurs in the S-phase of the cell cycle outside of semi-reserved DNA synthesis.

3.2 Cell in repair

Cells with net nuclear grain higher than the historical negative control value of this laboratory.

3.3 Net nuclear grain; NNG

weight variation shall not exceed ±20% of the average weight of the same sex. Use the regular feed, and drinking water is not restricted. If the test substance is mixed into feed for poisoning, an appropriate feed shall be selected to ensure that the test substance is fully mixed. Animals may be raised individually or in groups of the same sex. The breeding conditions of laboratory animals shall comply with the relevant provisions of GB 14925.

6.1.2 Preparation before animal test

Before the test, the animals shall adapt to the environment of the laboratory animal room for at least 5d. The animals are randomly divided into test substance group and control group.

6.1.3 Preparation of test substance

The solid test substance shall be dissolved or suspended in an appropriate solvent/excipient, and diluted before the animal is toxicant-exposed. The liquid test substance may be directly toxicant-exposed, or diluted before being toxicant-exposed. The test substance shall be prepared fresh, unless the stability data proves that it may be stored.

6.1.4 Solvents/excipients

The solvent/excipient shall not produce toxic effects at the used dose level; and shall not react with the test substance. If a solvent/excipient that is not fully understood is used, there shall be information on its compatibility. It is recommended to consider the use of aqueous solvents/excipients first.

6.1.5 Control

Each test shall include a simultaneous positive control and a negative (solvent/excipient) control. Except for the different toxicant-exposure of the test substance, the animals in the control group shall be operated in the same way as the animals in the toxicant-exposed group.

The positive control shall expect an increase in UDS that exceeds the background value at the exposure level. The dose of the positive control shall make the effect very clear; but it does not make the reader immediately discover that it is the positive control specimen. The route of toxicant-exposure of the positive control may be different from that of the test substance. Commonly used positive control substances are shown in Table 1.

used in limit test.

6.2.5 Toxicant-exposure

The test substance is usually given by gavage. If reasonable, other routes of toxicant-exposure are also acceptable. The maximum liquid volume of one gavage does not exceed 2mL/100g body weight. If a higher volume is used, the reason shall be explained. In addition to the irritating or corrosive substances that have enhanced toxic effects at higher concentrations, the concentration shall be adjusted to reduce the volume of the test substance, and ensure that the volume is equal at all dose levels.

6.2.6 Preparation of liver cells

Liver cells are prepared 12h~16h after the animals are toxicant-exposed. If there is no obvious positive reaction, take early-stage sampling (2h~4h after toxicant-exposure). However, other sampling time may also be used based on the existing toxicokinetics data.

The liver is perfused in situ with collagenase, the liver cells are separated; and after adherence, the mammalian liver cells are cultured for a short time. The survival rate of liver cells in the negative control group shall be greater than 50%.

6.2.7 UDS determination

Newly separated mammalian liver cells are usually cultured in a ^{3}H-TdR-containing culture solution for an appropriate time, for instance, 3h~8h. At the end of the culture period, after removing the culture medium, the cells are cultured in a culture solution containing excessive unlabelled thymine to remove unincorporated radioactivity; this operation may be omitted if the culture time is longer. Then, the cells were washed, fixed, stained, and count the grains. Prepare 2~3 specimen slides for each animal.

7 Analysis and Evaluation of the Results

7.1 Analysis of the results

7.1.1 The specimen slide shall have enough cells with normal morphology for UDS evaluation. Cytotoxicity (such as nuclear pyknosis, reduced radiolabel level) shall be checked under a microscope.

7.1.2 Specimen slides shall be numbered before reading the slides. Each animal shall be read at least 100 cells from 2 specimen slides. If each animal counts less than 100 cells, the reason shall be explained. Grains are not counted for S-phase nuclei, but the proportion of S-phase cells shall be recorded.

7.1.3 Count the amount of ^{3}H-TdR incorporated in the nucleus and cytoplasm of normal

b) The name and contact information of the test entrusting organization, the date of sample acceptance and the situation of sample sealing;

c) The start and end date of the test, the person in charge of the test items, the technical person in charge of the test organization, and the date of issuance;

d) Summary of test;

e) The name of the test substance, the active ingredient American Chemical Abstracts Service accession number (CAS number) (if known), code (if any), purity (or content), dosage form, production date (batch number), appearance properties, and solvents and methods used for preparation;

f) The species, strain, grade, quantity, weight, gender, source (supplier name, laboratory animal quality certificate number, laboratory animal production license number) of laboratory animals; quarantine; adaptation conditions; and laboratory animal breeding environment, including temperature, relative humidity, feed, single cage breeding or group breeding, laboratory animal facility use permit number;

g) Dose and group, including the principle and basis for selecting the dose; the way of animal grouping; and the number of animals of each gender in each group;

h) Test conditions and methods, including main instruments and equipment; toxicant-exposure dose; route of toxicant-exposure; toxicant-exposure plan; test cycle; observation indicators, etc.; positive control and negative (solvent/excipient) control; and pre-test (if carried out) data of dose-related design; method (if carried out) to prove that the test substance reaches the systemic circulation or target organ; detailed description of the toxicant-exposure and sampling plan; method of determining toxicity; preparation and culture method of liver cells; autoradiography method; the number of prepared specimen slides and the number of counted cells; the criteria for evaluation; and the criteria for judging whether the test result is positive or negative;

i) Test results: Use the text description and table to summarize item by item of the mean values of NG, CG and NNG of each specimen slide, each animal and each group; dose-response relationship (if possible); statistical analysis (if any); and toxicity performance. Simultaneous negative (solvent/excipient) control and positive control data; background negative (solvent/excipient) control and positive control data, including range, mean and standard deviation; and the number of "cells in repair" (If determined); verify the criteria for the number of " cells in repair" (if determined); the number of S-phase cells (if determined); cell survival rate; and the rationality of the selection of statistical methods;

j) Test conclusion: Give the conclusion whether the test substance caused the increase of unscheduled DNA synthesis of mammalian liver cells in vivo under

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