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National Food Safety Standard - Test of Extended One-Generation Reproductive Toxicity

食品安全国家标准 扩展一代生殖毒性试验

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National Food Safety Standard - Test of Extended One-Generation Reproductive Toxicity

1 Scope

This Standard specifies the basic test methods and technical requirements for the extended one-generation reproductive toxicity.

This Standard is applicable to the evaluation of the reproductive and developmental toxicity of the test substance.

2 Terms and Definitions

2.1 Reproductive toxicity

Damage to male and female reproductive function or ability, and harmful effects on offspring. Reproductive toxicity may occur in female's pregnancy period, as well as in pre-pregnancy and lactation. It is manifested as the influence of exogenous chemicals on the reproductive process, such as changes in the reproductive organs and endocrine system, the influence on the sexual cycle and sexual behavior, as well as the influence on fertility and pregnancy outcome.

2.2 Developmental toxicity

The harmful effects of individuals exposed to the test substance before birth and before they develop into adults (including embryonic period, gestational period and after birth), which are manifested as structural abnormalities, growth changes, functional defects and death of the developing organism.

2.3 Nerve developmental toxicity

The abnormal changes in the structure and function of the nervous system caused by the exposure of the individual to the test substance during the development process, which may occur at any stage of the life cycle.

2.4 Developmental immunotoxicity

The immune system development is affected and dysfunction occurs, which are caused by exposure to the test substance during the early development of the individual's life (especially before and after birth), and these effects are not detected or have a short duration when the adult individual is exposed.

to be sensitive to the test substance. Generally, rodents are preferred to choose rats, and avoid strains with low reproductive rate or high incidence of developmental defects. The principles and recommendations in this Standard are based on rats. In order to correctly evaluate the influence of the test substance on the reproductive and developmental abilities of animals, animals of both sexes shall be used. The selected animals should indicate species, strain, sex, weight and age in weeks. The weight difference between the experimental animals of the same sex does not exceed ±20% of the average weight. The selected parental experimental animals must be sexually mature at the beginning of the test, and must be similar in age (day) to mating (rats are at least 13 weeks old), and the female animals shall be non-parous and non-pregnant animals.

4.2.2 The number of experimental animals

In order to obtain the basic test data with statistical requirements, correctly evaluate the toxic effect of the test substance on the reproduction and development process of the animal (including the reproduction, pregnancy and feeding process of the F0-generation animal; and the growth and development of the F1-generation animal from birth to maturity); it is necessary to ensure that at least 20 pregnant mice are obtained in each dose group and control group of the test substance. Generally, at the beginning of the test, it is recommended to prepare about 30 rats for each group of parental generation (F0-generation) rats of two sexes.

4.2.3 Preparation of animal

Before the test, the animals shall undergo at least 3d~5d of environmental adaptation and quarantine observation in the experimental animal room.

4.2.4 Animal breeding environment

The breeding conditions, drinking water, and feed of experimental animals shall comply with the relevant provisions of GB 14925, GB 5749, and GB 14924. Experimental animals are raised in single cage or separated by sex; and they may eat and drink freely. When pregnant rats are near delivery, they shall be raised separately in farrowing cages; and appropriate litter for nesting shall be placed in the cages when necessary.

4.3 Dose and grouping

Animals are randomly grouped according to their body weight; there are at least 3 test substance dose groups and 1 control group. If the test substance uses a solvent, the control group shall be given the maximum amount of the used solvent. The design of high-dose test substance group for certain test substances shall consider its impact on nutrient balance; and the dose of non-nutritive test substances shall not exceed 5% of the feed.

with carboxymethyl cellulose, starch, etc. Dissolvents or other additives to enhance the solubility of the test substance shall consider the following characteristics: whether it affects the absorption, distribution, metabolism and retention of the test substance; whether it affects the chemical properties of the test substance, so as to change its toxicity characteristics; whether it affects animal food and water consumption and affect the nutritional level of animals. In addition, in addition to water, the toxicity characteristics of solvents must be clear; and avoid the use of potentially toxic solvents (such as acetone, dimethyl sulfoxide). The test substance shall be prepared for immediate use, unless there is data showing that its solution or suspension is stable in storage.

4.4.5 The test substance is administered by gavage, if the dissolvent is an aqueous solution, the gavage volume shall generally not exceed 10mL/kg body weight; and the maximum gavage volume shall not exceed 20mL/kg body weight. If the dissolvent is an oily liquid, the gavage volume shall not exceed 4mL/kg body weight. The intragastric volume of each group shall be the same. The gavage shall be given once a day at the same time; weighing at least twice a week; and the gavage volume shall be adjusted according to the body weight. It is recommended not to gavage the mother rats or take other treatment measures on the day of delivery.

4.5 Test method

It is recommended to select female and male rats over 10 weeks of age as the F0-generation; and start to give the test substance after at least 3~5 days of adaptation. The F0-generation female and male rats in each group shall be treated in the same way. The test substance shall be given every day for the duration of the experiment starting from 2 weeks before mating and covering the mating period, pregnancy period and F1-generation weaning. After the F1-generation is weaned, the F0-generation female and male rats can be subjected to the test of gross anatomy and reproductive indicators; but it is necessary to ensure that the F0-generation male rats are exposed to the test substance for no less than 10 weeks (at least 1 complete spermatogenesis process). F1-generation female and male offspring after weaning in each group were randomly assigned to each evaluation cohort of the group according to the test purpose; and the test substance was given until the corresponding cohort indicators were tested. See Table 1 and Table 2 for the detection time, the number of used animals and the detection content of the measurement indicators of each evaluation cohort.

5.3.3 Blood biochemical test indicators

It includes tests for blood sugar, total cholesterol, urea, creatinine, total protein, albumin and at least two enzymes that indicate liver cell damage (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transition and sorbitol dehydrogenase) and so on. If necessary, T4, TSH and sex hormone levels can be tested.

5.3.4 Urine test indicators

It includes tests for appearance, volume, osmotic pressure or specific gravity, pH, protein, urine sugar, blood cells and the like.

5.4 Sperm detection

5.4.1 Sample collection

All F0-generation and F1-generation male rats in the Cohort-1A were harvested from the epididymal tail (or vas deferens) at the end of the corresponding test to collect sperm, and the sperm motility and morphological parameters were examined. It shall be noted that the test of sperm indicators shall be performed immediately after the rat is sacrificed, and the collection process should minimize the loss of sperm.

5.4.2 Sperm detection indicators

In the evaluation of sperm morphology, each animal shall check at least 200 sperm to distinguish whether the sperm morphology is normal (the head and mid-tail are normal) or abnormal [abnormal sperm morphology includes the fusion and separation of the sperm head, and abnormity of the sperm head and (or) tail]. In addition, the percentage of sperm for sexual movement can be analyzed with the aid of instruments.

5.5 Inspection in estrus cycle

F0-generation female rats can be evaluated for the estrus cycle (vaginal cytology inspection) after starting to give the test substance; and it can continue to confirm the mating success or the end of the two-week mating period. If F0-generation female rats have some effects that are not related to reproductive toxicity (such as a significant reduction in food intake, etc.) after starting to give the test substance, then it can be considered to extend the time of giving the test substance before mating by 2 weeks (that is, give the test substance for 4 weeks before mating); so that the female rats can better adapt to the test substance; and at the same time, the corresponding F0-generation male rats are given the test substance for an extended time accordingly (but the total time of giving test substance to F0-generation male rats before they are sacrificed remains unchanged, it is still no less than 10 weeks).

The evaluation of the estrus cycle of F1-generation is mainly completed through the

5.8 Developmental immune toxicity indicator test of offspring

5.8.1 Rats in Cohort-3 of each dose group (10 males and 10 females) were tested against T cell dependent antigens [such as sheep red blood cells (SRBC) or keyhole limpet hemocyanin (KLH)] on 56±3 days after birth for main IgM antibody response (that is, T cell dependent antibody response: TDAR) test.

5.8.2 TDAR can be performed using antibody-producing cell test or specific IgM antibody test methods.

5.8.2.1 Antibody-producing cell test

Plaque forming cell (PFC) count test is to immunize SRBC by intraperitoneal injection for 4 days; mix the rat spleen cell suspension with a certain amount of SRBC; and with the participation of complement, the SRBC around the spleen cell that secretes the antibody is dissolved to form plaques visible to the naked eye and the number of hemolytic plaques can reflect the number of antibody-producing cells. PFC test can be divided into subgroups for testing on different dates, but the following conditions need to be met:

a) Determine the immunization and sacrificing time of the animals in subgroup to ensure that the peak response is reached during the test;

b) Each subgroup contains the same number of male and female animals in different groups;

c) The subgroup animals shall be the same day age at the time of death.

5.8.2.2 Specific IgM antibody test

5 days after intraperitoneal injection of SRBC or KLH, the titers of specific IgM antibodies in the serum were determined by enzyme-linked immunosorbent assay (ELISA).

5.9 Continuing evaluation of potential reproductive toxicity

If the test results of F1-generation rats in Group-1A suggest that the test substance may have reproductive toxicity, the male and female rats (13 weeks old) in the Cohort-1B of each dose group can be certified by pathological examination after sacrifice, and can also continue to give the test substance at least until the 90 days after birth (but no more than 120 days), and then perform female-male mating (avoid the mating of female and male rats in the same litter) to obtain F2-generation animals to evaluate the potential reproductive toxicity effects; the treatment method is the same as that of F0-generation animals. When the F2-generation pups reach the 4 days after birth, if obtained test data can explain the problem, the test can be ended; and there is no need to continue feeding until weaning or longer.

suspicious, the histological examination of the animals in the Cohort-1B is required for verification.

6.3.2.2 Cohort-2

After completing the neurobehavioral test (75 days after birth, but no more than 90 days), all rats in the high-dose group and the control group in the Cohort-2A were harvested the brain and other tissues for neuropathological examination. On the 21 days (or 22 days) after birth, all rats in the high-dose group and the control group in the Cohort-2B were harvested brain and other tissues for histopathological examination. When the organs are found to have significant pathological changes related to the test substance, all animals in the middle and low dose groups need to be further checked to determine the NOAEL.

For rats in the Cohort-2A and Cohort-2B, the brain olfactory bulb, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, and midbrain (tunicate, tegmental, and cerebral peduncle), brain stem and cerebellum can be comprehensively examined through the multi-section of the brain. In addition, on the basis of the above examinations, the rats in Cohort-2A can increase eye (retina and optic nerve) and peripheral nerve specimens, muscle and spinal cord examinations.

7 Data Processing and Result Evaluation

7.1 Data processing

Summarize all the data and results in the form of a table. The data can be counted in a table. The table shall show the number of experimental animals in each group, the number of male animals that mate, the number of female animals that conceive, various toxic reactions and their occurrence percentage. Reproductive and physiological development indicator data shall be counted in litters. The results shall be statistically analyzed by appropriate methods. The measurement data shall be analyzed by variance, and the mean comparison between multiple test groups and the control group shall be performed. The classification data shall use Fisher's exact distribution test, chi-square test, and rank sum test; and the grade data shall use Ridit analysis, rank sum test, etc.

7.2 Evaluation of results

Compare the observation indicator and pathological examination results of the test group animals and the control group animals to see whether there are significant differences and dose-response relationship; and assess whether the test substance has reproductive and developmental toxicity; and then further determine the NOAEL and LOAEL of its reproductive and developmental toxicity. At the same time, it is also possible to further estimate the characteristics of reproductive and developmental

urine analysis;

f) Phenotypic analysis results of spleen cells (T cells, B cells, NK cells);

g) The number of F0-generation and F1-generation female animals in normal or abnormal estrus cycles;

h) mating time (the number of days from the start of mating to the successful mating);

i) Toxic or other effects on reproduction, including the number and percentage of animals that have completed mating, pregnancy, childbirth and lactation; the number and percentage of male animals that have successfully mated; and the number and percentage of female animals with symptoms of dystocia/prolonged delivery or difficulty delivery;

j) The duration of pregnancy and childbirth (if recordable);

k) Number of implants, litter size and sex ratio of pups;

l) The number of miscarriages after implantation, the number and percentage of live births and still births;

m) Pup litter weight;

n) Observe the number of obviously abnormal pups with naked eyes;

o) Physiological development index results of pups;

p) The data of F1-generation rats sexual maturity indicators;

q) The body weight, absolute and relative organ weight data of F0-generation and F1-generation rats at the time of slaughter;

r) Gross anatomy and histopathological examination results;

s) The results of sperm motility and morphology analysis of male epididymal tail of F0-generation and F1-generation male animals.

8.8.2 Neurodevelopmental toxicity test results:

a) The analysis results of the auditory startle test;

b) Nervous system function observation combination indicator, and explanation of the result scoring operation;

c) The results of the test and analysis of neurobehavioral development indicators;

d) Neuropathological gross anatomy and histopathological examination results.

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