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

GB/T 15670.25-2017

Replacing GB/T 15670-1995

**Toxicological test methods for pesticides registration - Part
25: Acute delayed neurotoxicity test**

农药登记毒理学试验方法 第25部分：急性迟发性神经毒性试验

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1 Scope

GB/T 15670.25-2017 is the Chinese national standard on toxicological test methods for pesticides registration - part 25:acute delayed neurotoxicity test, in the field of agriculture. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 12 July 2017 by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; Standardization Administration of China, and has been in force since 1 February 2018. Classification: ICS 65.100, CCS B17. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This Part of GB/T 15670 specifies the basic principles, methods and requirements for the acute delayed neurotoxicity test. This Part applies to the acute delayed neurotoxicity test for pesticides registration.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB 14925, Laboratory animal - Requirements of environment and housing facilities

3 Terms and definitions

The following terms and definitions are applicable to this document.

3.1 Acute delayed neurotoxicity A neurological syndrome, mainly manifested by limb weakness and spastic paralysis of upper motor neurons, that the animals gradually develop in 1 ~ 2 week(s) after neuropathic target esterase (NTE) vitality inhibition in the early stage, after being exposed to a large amount of the test substance. Neuropathological examination is manifested as distal axonal neuropathy of the spinal cord and peripheral nerves.

3.2 Organophosphorus substance Compounds containing carbon-phosphorus bonds or phosphoric acid derivatives containing organic groups, mainly including uncharged phosphate, thioorganophosphate, organic phosphoric anhydride, organic phosphoric acids, amino phosphate, thiophosphate and amino phosphorothioate, etc., some of which can cause delayed neurotoxicity. days. They shall be fed normally, without restriction to water intake. The breeding environment of test animals shall comply with the relevant provisions of GB 14925.

6.3 Dose and grouping Generally, there shall be a test substance toxicant-exposure group, a positive control group and a solvent control group. The dose for the test substance toxicant-exposure group shall be determined by a pre- test. In the pre-test, use an appropriate number of hens and dose level to determine the maximum dose achieved in the formal test. The pre-test shall cause some lethality. A variety of test methods can be used to estimate the maximum non-lethal dose of the test substances. Historical data on toxicological information from hens or other animals can aid in dose selection. In the formal test, the highest possible dose level shall be selected, and individual animals are allowed to die; however, a sufficient number of animals shall survive for biochemical (6) and histopathological examinations (6) at the 21st day. Animals can be protected from death caused by acute cholinergic effects using atropine sulfate or other prophylactic drugs known not to interfere with delayed neurotoxicity. The positive control group shall ensure that at least 6 hens (3 for biochemical tests and 3 for pathological dissection and histopathological examination) are given the positive substance for delayed neurotoxicity: tri-o-cresylphosphate (TOCP). For the solvent control group, if the solvent toxicity is known, only the solvent control group may be set; if the solvent toxicity is unknown, a blank control group shall also be added. Limited test: If the test substance is toxicant-exposed to a dose of 2 000 mg/kg body weight, and no observable toxic effect is caused, or no toxicity is estimated to occur based on the data obtained from the analysis of structurally related compounds, there is no need to carry out a test of higher dose. Quantitative testing cannot be used when higher human exposure levels indicate the need for higher dose testing.

6.4 Ways of toxicant-exposure Usually, use the oral route, including gavage, capsule swallowing or instillation in the pharyngeal isthmus, for one-time toxicant-exposure, and overnight fasting is required. The gavage volume can be given at 5 mL/kg body weight

according to the different doses of each group. In order to combat the death of animals caused by acute cholinergic effects, at 10 ~ 15 minutes before administration of the test substance, intramuscular injection of atropine sulfate known not to interfere with delayed neurotoxicity reactions can be used for protection. The dose of atropine sulfate is 10 mg/kg body weight.

6.5 Observation period and indicators

6.5.1 Clinical observation and examination Observation shall be started immediately after toxicant-exposure, once every half an hour after exposure, and once every day after 2 days, until the end of the test on the 21st day. Detailed records of all poisoning reactions, time, type, severity, and abnormal behavior of the hens shall be made. Observe the hens outside the cage, and force the hens to move in order to facilitate the observation of the slightest toxic effects. Remove the moribund hens, and sacrifice them for gross anatomy. Ataxia shall be observed and recorded according to the following manifestations: no abnormality; weak legs, slightly abnormal standing posture and gait; severely abnormal gait, falling down when walking; able to stand barely, but mostly on the tarsal; unable to stand, moving the body by flapping the wings.

6.5.2 Weight record Weigh all hens for one time before toxicant-exposure, and once every week thereafter.

6.5.3 Biochemical examination Randomly select and kill 6 hens (3 each time) from the toxicant-exposure group and the solvent control group after 24 h and 48 h of toxicant-exposure, and 3 hens from the positive control group (set at the same time) after 24 h of toxicant-exposure. Take the brain and lumbar spinal cord for the measurement of the activity of neuropathy target esterase (NTE). Observe the time of appearance of cholinergic signs, and assess the manifestations of poisoning. If it is suggested from the observation of clinical signs of poisoning that the metabolism of poisons in the body is very slow, it is best to take tissue samples, 24 h and 72 h (latest time) after toxicant-exposure each time, from 3 hens for biochemical tests. When necessary, measure the acetylcholinesterase (AChE) activity in these tissue samples. Acetylcholinesterase can spontaneously recover in vivo, thus leading to underestimation of the activity of acetylcholinesterase inhibitors (test substances).

6.5.4 Histopathological examination On the 21st day after exposure, the surviving animals shall be subjected to gross anatomical observation of brain and spinal cord, and histopathological examination of nerve tissue. These include: oblongata, pons, cerebellum and cerebral cortex, cervical segment of spinal cord, middle thoracic segment, lumbosacral junction, sciatic nerve, tibial nerve and their collaterals. Make special staining for myelin sheaths and axons.