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Chemical pesticide – Guideline for honeybee semi-field test

化学农药　蜜蜂影响半田间试验准则

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Foreword

This Standard was drafted in accordance with the rules given in GB/T 1.1-2009.

The main technical content of this Standard equivalently adopts EPPO Standard PP1/170(4), *Efficacy evaluation of plant protection products – Side-effects on honeybees* (English version, 2010).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. The issuer of this document shall not be held responsible for identifying any or all such patent rights.

This Standard was proposed by and shall be under the jurisdiction of the Department of Plantation of the Ministry of Agriculture.

The drafting organizations of this Standard: Institute for the Control of Agrochemicals, MOA and Hunan Province Institute of Plant Protection.

The main drafters of this Standard: Chen Ang, Yuan Shankui, Liu Yong, Jiang Hui, Li Ruizhe, Qu Honghong, Yan Qingping.

Chemical pesticide – Guideline for honeybee semi-field test

1 Scope

This Standard specifies the trial conditions, bee colony management, methods, quality control, statistical analysis, test report and other basic requirements for honeybee semi-field test for the effects of pesticides.

This Standard applies to the semi-field test of the effects of chemical pesticides on honeybee; it can work as a reference for other pesticides.

This Standard does not apply to volatile and insoluble chemical pesticides.

2 Normative References

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

GB/T 31270.10-2014, *Test guidelines on environmental safety investigation for chemical pesticides – Part 10: Honeybee acute toxicity test*

3 Terms and Definitions

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

3.1

semi-field test

A trial process in which the controllable field conditions, such as cages, tunnels and tents, are used to observe the effects of pesticides on the population and development of honeybees.

3.2

exposure

to which pesticides are applying or has been applied; the post-exposure stage is the monitoring stage, including the several investigations of colony conditions, and evaluations of honeybee mortality and activities, etc.

5 Trial method

5.1 Materials and conditions

5.1.1 Trial bees

The bee type for the trial is a representative local bee species which has a definite strain. The bee colony for the trial shall come from the same breeding strain; the original and quality shall be reliable. Bee colony having any visible biological pest or disease, shall not be used for the trial. Bee colony shall not be treated with pesticides within 4 weeks before it is used for the trial. Queen bees shall be hatched from the sister queens of the same batch which do mating flight at the same time. Only the healthy bee queens with strong fecundity, which are newly-bred before the trial, can be used for the trial. Each colony shall contain 3000 ~ 5000 worker bees and 3 full frames at least. But it can be adjusted in accordance with the objectives of the trial. Each colony shall contain all brood stages and an appropriate amount of stores of pollen/nectar for the development of brood. The colony for the trial shall be in the prime breeding period or active foraging period.

5.1.2 Test crops

Select crops which are highly attractive to bees, such as rape, cotton and so on. When the test product is a systemic seed treatment agent or granula, the realistic target crops of nectar/pollen source recommended for it shall be used.

5.1.3 Test products

Formulated products of pesticides shall be used.

5.1.4 Major equipment and facilities

5.1.4.1 Beehives

Local commonly-used beehives need to be used; or they are customized as required by the trial.

5.1.4.2 Dead-bee traps

In order to collect dead bees, dead-bee traps shall be installed at the front end of the entrance of each beehive for the trial, at least 3 d before the investigation of honeybee mortality. Dead-bee traps shall be made of nonirritant, odorless materials such as

distance between the boundary of the whole trial area and the surrounding field shall be more than 3 m. There shall be not other agricultural activities than the trial, such as fertilizer application and control of plant diseases and insect pests; it shall be ensured that the fertilizer used, pesticide products, their operating process, etc. will not exert any effect on the results of the trial.

5.2.1.2 Monitoring stage

A site with abundant nectar source (such as wild flowers) is selected as the feeding and monitoring points of the bee colony at the pre-exposure stage and post-exposure stage; the distance between the monitoring point and the test field shall be greater than 3 km to prevent honeybees from flying to the test field for the exposure stage before and after the exposure stage. Around the monitoring point, there shall be no flowering crops which attract honeybees to forage on to prevent honeybees foraging on pollen/nectar containing other pesticides, which interfere the results of the trial. When the external food is insufficient, artificial feeding can be done. The food consumption of the colony shall be recorded.

5.2.2 Test duration

The test duration is decided in accordance with the development period of test crops, the pesticide application period of control target and the objectives of the trial.

5.2.3 Management of test colony

In accordance with the investigation results of all brood state proportions of the colony before exposure, coordinate and distribute the colony between all treatments. The time of migration of the test colony can be the dusk or evening when the colony finishes the flight activities of the day, or the morning before honeybees start flight activities. When the colony is kept in the test cage, one water source free of contamination needs to be provided for honeybees (it is preferably prepared independently outside the beehive or added directly to the feed bunk inside the beehive if necessary). When the nectar/pollen of the test crops is not abundant, the colony shall be fed with an appropriate amount of sugar (honey) water and/or pollen, but in order to ensure the activeness of the foraging activities of honeybees, they shall not be fed excessively.

When the colony is kept at the monitoring point, feeding management can be done by following the experiences of the local bee keepers, but during the whole trial process, the following operation shall not be done for the test colony:

a) using pesticides toxic to queen bees or worker bees to control plant diseases and insect pests of honeybees;

b) switching frames between different colonies or other behaviors, which will affect the structure and quantity of colonies.

monitoring point, at least continue to observe for 3 weeks, to ensure the evaluation cycle after the end of exposure includes one development period of worker bee broods at least.

5.2.5 Pesticide trial

5.2.5.1 Pesticide application method

The application shall be carried out using the application method recommended for the test plans or test products, according to the local good agricultural practices (GAP). If the test products need to be used after preparation, they shall be used immediately after preparation.

5.2.5.2 Pesticide application appliances

Select frequently-used appliances in production; record all the information of the appliances used, including type, description, model number and operating conditions (working pressure and orifice diameter). Before each use, calibrate the pesticide application appliances and calculate their application rate to decide a method for uniform application for a plot. If the seeding equipment is used, the equipment shall be calibrated to seed uniformly, quantitatively.

5.2.5.3 Time and frequency of pesticide application

The time and frequency of pesticide application shall be in accordance with the recommended application method for the characteristics of the test pesticides.

5.2.5.3.1 Time of pesticide application

The pesticide application shall be carried out during the daytime when honeybees are foraging most actively; or it is adjusted as appropriate in accordance with different objectives of the trial. When the effects of long period residual effect pesticides on honeybees are evaluated, the time of application shall be at a certain time interval before the exposure of honeybees, to prevent the effects of pesticides caused by direct exposure to honeybees. If the effects of risk mitigation measures are evaluated, they shall be applied before the active flight activities of honeybees. In addition to this, consideration shall be given to the local general agricultural practices. For the direct atomization treatment method, try to avoid rainfall before the treatments become dry: normally, there shall be no rainfall within 2 h after application; the air speed in cages during the application shall be less than 2 m/s.

5.2.5.3.2 Frequency of pesticide application

Normally, apply once during the flowering period, but full consideration shall be given to the maximum dose, residue, acute effects and different objectives of trial research and the recommended pesticide application method, before the frequency of

investigation area shall be fully removed.

5.2.6.1.2 Investigation content

When worker bees are incapable of removing the dead bees inside the beehives because of a massive mortality of honeybees, it also needs to investigate the dead bees at the bottom of all test beehives. Under the dry field conditions, it also needs to record the number of dead bees on the ground (by counting the dead bees on the screen laid on the ground). In accordance with the differences in test objectives and crops, part or whole of the ground area can be investigated.

5.2.6.2 Honeybee flight conditions

The flight condition investigation needs to be carried out when honeybees are moved to cages until the end of the exposure. During the investigation, count the honeybee number foraging on flowers and flying across the area, within a certain area (e.g. 1 m^2) within a certain time (at least 15 s) or on a certain number of flowers (e.g. 15 flowers). Select at least 3 observation points randomly for each cage (avoiding the area before the beehive entrance).

The flight investigation shall be carried out under normal conditions after the flowers of the crops in cages bloom, at the time when honeybees fly the most actively; evaluation shall be carried out once at the same time each day, but on key test points, e.g. the first day of the exposure, the frequency of flight investigation shall be increased, e.g. 1 h, 2 h, 4 h, 6 h and other periods of time after the exposure.

5.2.6.3 Honeybee activities

At the exposure stage of the cage trial, investigate the activities of honeybees on the crops and around the beehives in the process of assessing the honeybee mortality and flight conditions. Compared with the blank controls, at least the following activities shall be observed and recorded:

a) toxic symptoms, such as convulsion, tremble and unbalanced movement;

b) gathering at the entrance of the beehives;

c) aggressivity;

d) hanging bee beard;

e) inertia;

f) flying in high density without falling on the crops;

g) other abnormal behaviors.

6 Quality control

The criteria for quality control include:

a) the mortality of the reference product treatment group and the mortality of the blank control group have significant differences in statistics. If the mortality of the blank control group is excessively high or the mortality of the reference product treatment group is excessively low, the trial shall be carried out once again;

b) the mortality of honeybees of the reference product treatment group shall be increased significantly after the pesticide application.

7 Statistical analysis

The evaluation of test products shall be given by comparing the data of the test product treatment group and of the blank control and the reference product treatment (the data before and after the application). The data needing to be compared includes the following parts:

a) mortality: the mortality of honeybees in the dead-bee traps and the mortality of honeybees falling on the screen on the ground (in case of a dry field);

b) flight intensity: the number of honeybees foraging on a unit area of crops or a unit quantity of flowers, within a unit time;

c) colony condition: the colony population, and the proportions of egg, larva, pupa and food store accounted for the frame.

The original data shall meet the requirements for the trial and a proper method shall be used to do statistical analysis. In theory, first ensure the end-point data is suitable for the statistical analysis, e.g. in the analysis of mortality and flight intensity, the difference significant level of all test data (including normal distribution test and homogeneity test of variances) shall be 0.05. Normally, the two-tailed test shall be carried out for the data before the pesticide application; the one-tailed upper limit test shall be done when the mortality data is subjected to the statistical analysis after the application. However, the flight intensity data shall be subjected to the single-tailed lower limit test. Or, in accordance with the test objectives and the design requirements, select an appropriate statistical method.

8 Test report

The test results shall be fully reflected in the report. The test report shall at least include

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