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**Test methods for cockroach resistance to insecticides -
Bioassay methods for *Blattella germanica***

蜚蠊抗药性检测方法 德国小蠊生物测定法

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2 Normative references

This document has no normative references.

3.Terms and Definitions The following terms and definitions apply to this document.

4 Test insects

4.1 Sensitive strains Male adult insects with intact morphology, agile movement, and uniform body size were raised in a standardized laboratory environment.

4.2 The population to be tested German cockroaches were collected on-site and bred indoors to produce first-generation (F1) or second-generation cockroaches that were morphologically intact, agile, and uniform in size. F2 generation male adults.

5.Reagents Acetone (analytical grade) or other organic solvents, carbon dioxide or anhydrous ether, pesticide technical, petrolatum (analytical grade) and liquid paraffin (denier). Mixture (1.1) with a concentration of

0.84 g/mL to

0.86 g/mL.

6.Instruments and Equipment An incubator or room with controllable light, temperature, and humidity; pipettes; 500mL transparent ground glass wide-mouth bottles; micro-droplets or micro-infusion apparatus. Infusion device.

Note. The wide-mouth bottle measures 16cm in height, 8cm in bottom diameter, 5.5cm in mouth diameter, and has an internal surface area of approximately 350cm².

7 Test Conditions

7.1 Temperature. $26^{\circ}\text{C}\pm 2^{\circ}\text{C}$.

7.2 Relative humidity. $(60\pm 10)\%$.

8 Sensitive Baseline Method

8.1 Principle A toxicity regression equation was established based on the relationship between the logarithm of insecticide concentration (dosage) and the probability of insect mortality. This equation was then used to establish a toxicity regression equation for sensitive strains. The established insecticide toxicity regression line was used as the sensitive baseline. The insecticide toxicity regression line obtained from the test insect population was compared with the sensitive baseline. The resistance level of the tested population was determined by comparing the changes in the median lethal concentration (median lethal dose) $[\text{LC}_{50} (\text{LD}_{50})]$ calculated from the toxicity regression line.

8.2.1 Drug film method

8.2.1.1 Determination of Preliminary Test Concentration 8.2.1.1.1 Prepare a stock solution of a certain concentration of the tested insecticide technical material using acetone or other suitable solvent, taking into account the environment of the insect collection site. Based on the pesticide application history, the insecticide stock solution was diluted to a certain concentration using an appropriate solvent for preliminary testing.

2.5 mL of the test solution was taken. Add the medicine to a 500mL wide-mouth bottle, and immediately rotate the bottle continuously or use a bottle roller to roll the bottle to ensure that the medicine is evenly distributed below the frosted surface of the bottle neck. With an inner wall, until the liquid flow is no longer visible to the naked eye, place in a fume hood to allow the solvent to completely evaporate. Apply petroleum jelly and the liquid to the frosted glass opening of the bottle. A paraffin mixture (1.1) was used, with 10 test insects placed in each bottle. After 1 hour of contact, the test insects were transferred to a clean container for normal rearing. Mortality was recorded after 24 hours. The number of deaths. 8.2.1.1.2 Each concentration test should be repeated at least twice, with the corresponding solvent treatment serving as a control group. Adjust the test solution concentration according to the mortality rate of the test insects. The mortality rate of test insects was determined by adjusting the concentration of the pesticide solution until the lowest concentration group had a mortality rate of 0%-20%, and the highest concentration group had a mortality rate of 80%-100%. This refers to the pesticide concentration range used in the formal test.

8.2.1.2 Formal Measurement Based on the insecticide concentration range determined in the preliminary test, the insecticide stock solution was diluted into 5 fractions using appropriate solvents in a proportional or arithmetic sequence. Seven concentrations were

used as test solutions for the formal experiment. Each concentration test was repeated at least 3 times, with the corresponding solvent treatment serving as the control group. The specific procedures for preparing the drug film and treating the test insects were the same as in the preliminary test.

8.2.2 Infusion Method

8.2.2.1 Determination of Preliminary Test Concentration 8.2.2.1.1 Prepare a stock solution of a certain concentration of the tested insecticide technical material using acetone or other suitable solvent, taking into account the environment of the insect collection site. Based on the pesticide application history, the insecticide stock solution was diluted to a certain concentration using an appropriate solvent for preliminary testing. Test insects were then tested using carbon dioxide or... Anesthetize with ether. Using a micro-drip device, apply 1 μL of the test drug solution to the ventral plate between the middle and hind coxas of the male adult insect. For each treatment... Ten test worms were administered via intravenous drip. The treated worms were then transferred to a clean container for normal rearing. The number of deaths was recorded after 24 hours. 8.2.2.1.2 Each concentration test should be repeated at least twice, with a drop of the corresponding solvent serving as a control group. Adjust the test solution concentration according to the mortality rate of the test insects. The mortality rate of test insects was determined by adjusting the concentration of the pesticide solution until the lowest concentration group had a mortality rate of 0%-20%, and the highest concentration group had a mortality rate of 80%-100%. This refers to the pesticide concentration range used in the formal test.

8.2.2.2 Formal Measurement Based on the insecticide concentration range determined in the preliminary test, the insecticide stock solution was diluted in a series of 5 to 7 fractions using appropriate solvents in a proportional or arithmetic sequence. A specific concentration was used as the test solution for the formal experiment. Each concentration test was repeated at least 3 times, with the corresponding solvent dripped as a control group. The specific procedures for treating the test insects were the same as those for the preliminary test.

8.3 Determination of Death If the test insect is not moved or cannot crawl normally when gently touched with a sharp instrument such as a dissecting needle, it is considered dead.

8.4 Data Processing Probit analysis was used to correlate the logarithmic values of different test drug concentrations (dosages) with the corresponding probability of mortality. The values were used to derive the toxicity regression equation, calculate the median lethal concentration (median lethal dose) [LC50 (LD50)] and 95% confidence interval.

Note. When using the droplet method for bioassays, the dosage of the test agent is determined based on the concentration of the test solution and the amount of agent received by the test insect, and the LD50 is calculated accordingly; when using the film-forming method, the LD50 is calculated... Calculate LC50.

8.5 Presentation of Results Results are expressed as resistance multiples, where resistance multiple = LC50 (LD50) value of the tested population/LC50 (LD50) value of the susceptible strain. Common insecticides are used to... The LC50/LD50 of the sensitive strain German cockroach is shown in Table A.1 of Appendix A.

8.6 Result Determination The 95% confidence limits of the LC50 (LD50) of the sensitive strain and the tested population do not overlap, and the resistance multiple is greater than or equal to 5 times that of the tested population.

9 Diagnostic Dosimetry

9.1 Principle Based on the toxicity regression line of the insecticide to the sensitive strain, a dosage that can distinguish between sensitive individuals and resistant individuals or resistant heterozygotes is determined. The diagnostic dose is generally determined by using 2 times (or several times) the 99% lethal concentration (99% lethal dose) [LC99 (LD99)] of the sensitive strain. This agent... A certain number of individuals in the test population are treated with a specific amount of pesticide, and the mortality rate is used to determine whether the test population has developed pesticide resistance.

9.2 Operating Procedures

9.2.1 Drug film method Prepare a stock solution of the tested insecticide at a certain concentration using acetone or other suitable solvent, and then dilute the stock solution to a diagnostic concentration using the appropriate solvent. The required concentration for dosage (LC99 of commonly used insecticides against the sensitive strain of German cockroach is shown in Table A.1) is determined by adding

2.5 mL of the pesticide solution to 500 mL of [amount missing]. Immediately after placing the bottle in the wide-mouthed container, rotate it continuously or use a bottle roller to ensure the liquid is evenly distributed across the entire inner wall below the frosted surface of the bottle neck, until... The liquid flow is not visible to the naked eye; place it in a fume hood to allow the solvent to completely evaporate. Apply a mixture of petroleum jelly and liquid paraffin to the frosted glass opening of the bottle. (1.1) Ten test insects were placed in each bottle. After 1 hour of contact, the test insects were transferred to a clean container for normal rearing. The number of deaths was recorded after 24 hours. The experiment continued until... Repeat the experiment at least 10 times, with the corresponding solvent used as a control group.

9.2.2 Infusion Method Prepare a stock solution of the tested insecticide at a certain concentration using acetone or other suitable solvent, and then dilute the insecticide stock solution with the appropriate solvent. The concentration required for diagnostic testing (LD99 of commonly used insecticides for susceptible strains of German cockroaches is shown in Table A.1). Test insects are exposed to carbon dioxide or ether. Intoxication, use a micro-drip device to drip 1 μ L of the drug solution onto the ventral plate between the middle