

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

PROPOSAL FOR A NEW GUIDELINE

Avian Repellency Test

INTRODUCTION

1. This Guideline describes a method to quantify an avoidance response of birds to food containing potentially toxic substances. Avoidance responses lead to reduction of the consumption of treated food, and thus affect the adverse effects of the substance. A number of tests have been developed for quantification of avoidance, such as INRA test design (1); test designs for passerine birds by Avery and Decker (2), Avery and Nelms (3), Best and Gionfriddo (4), and Best, Stafford and Mihaich (5). Experiences with these different tests were considered in the development of this document.

2. Definitions used in this document are given in Annex 1.

INITIAL CONSIDERATIONS

3. This test is designed for the determination of a factor which quantifies the repellency, i.e. the intrinsic propensity of a substance to cause avoidance responses in a standardised study design. The information about the quantitative strength of repellency, obtained from this test, can be used as a factor in risk assessment. The results from this test are not intended for determining the hazard of the substance.

4. There are different options to quantify the degree of repellency. An easy way is to calculate a repellency factor from dietary studies such as LC50 tests (6)(7); for the calculation of the avoidance factors, see Luttkik (8), if food avoidance occurs. Since these tests are performed with different concentration levels, an evaluation of no-repellent concentration is possible in many cases. These tests, however, are designed as a short-term toxicological test associated with mortality, starvation (in case of strong repellency) and symptoms of intoxication. These test conditions are usually not appropriate for testing the strength of repellency.

5. The degree of repellency, as an inherent property of a substance, should be tested under defined conditions, preferably with a standard food with known energy content. The avoidance reaction of birds depends on the type of food and the physiological conditions of the birds, cage design and the manner of presentation of food items. Therefore the test should be performed under standardised conditions with food which is readily consumed and under physiological conditions free of stress, so that the measured response of the birds is essentially caused by the properties of the substance.

6. The determination of repellency of treated food is possible either by a "choice test" (sometimes also called "two-choice test"), in which both treated and untreated food are offered to the birds at the same time, or by a "no-choice test", in which only food treated with the test substance is offered *ad libitum* during the exposure period, whereas the control receives untreated food.

7. Choice tests better simulate the natural behaviour of the birds in the field and the desirable test conditions can be more easily achieved in a choice test than in a no-choice test, where birds may be forced to starve if the repellency of the substance is significant. A no-choice test might be considered appropriate under exceptional circumstances, e.g. when it is expected that in using the substance under practical conditions only treated food is available as food source.

PRINCIPLE OF THE TEST

8. Birds are fed on a diet containing different concentrations of the test substance. In a choice test, birds are also offered untreated food of the same type. In a no-choice test, only the control group is given untreated food. Diets are offered *ad libitum* for a 24-hour feeding period. The consumption of treated and untreated food is measured, and the repellency factor is calculated by comparing the consumptions.

VALIDITY OF THE TEST

9. During the pre-exposure acclimatisation period, mortality should not exceed 5 % of the birds. The test design is not appropriate if birds refuse untreated food during the acclimatisation period.

10. In the “no choice” test, it should be demonstrated that the food intake rate of treated and control groups is similar during the pre-test acclimatisation period when all birds have access to untreated food only.

DESCRIPTION OF THE METHOD

Test animals

11. The test should be performed with adult birds of well-established laboratory species. Species sensitive to the test substance should be chosen. The preferred species is Japanese Quail (*Coturnix coturnix japonica*). The birds are randomly assigned to the test groups and individually marked with leg-rings or wing-tags. The birds should be in good health and free from apparent malformations or diseases.

12. Each of the test groups and control groups (for no-choice test) consists of ten birds.

Housing and feeding conditions

13. The birds are housed individually, in facilities suitable for the test species. Environmental conditions should be the same during both the acclimatisation period and the test period, except that during the acclimatisation period all birds are provided with untreated food only. The recommended environmental conditions for Japanese Quails are given in Table 1. If birds of other species are used, the housing and feeding conditions should be adapted accordingly.

Table 1: Recommended environmental conditions and diet for Japanese Quails

Bird species	Temperature (°C)	Relative humidity (%)	Daily feed ration	Recommended food	Daylight period
<i>Coturnix coturnix japonica</i> (Japanese Quail)	21 ± 3	50-75	<i>ad libitum</i>	Standard diet or seed (e.g. wheat or hemp)	Light period: 8 hrs; Dark period: 16 hrs.

14. The test should be performed using a standard diet, appropriate for the species used in the test, in order to avoid the problem of differences in the attractiveness of the food.

15. For testing the repellency of a substance that is mainly used for seed treatment, the test may be performed with natural food items such as seeds. If seeds to be tested are rather unattractive to birds (e.g. rape seeds), the birds should be adequately accustomed to the seeds prior to exposure to guarantee an adequate intake.

Duration

16. The duration of the test is at least eleven days. This includes at least seven days of acclimatisation, one day during which the birds are fed the test diet, (or both the test and the untreated control diets) and at least three days during which only untreated control normal diet (standard diet or seed) is offered.

Acclimatisation period

17. Prior to exposure to treated food, an acclimatisation period of at least one week is necessary to accustom the birds to the test facilities. If the test is performed with a standard diet, birds are offered a standard diet *ad libitum* during acclimatisation period.

18. In case the test is performed with seeds, birds have to be accustomed to this food item. This can be achieved by first mixing standard diet and test seed in a ratio of 1:1, and then after three days before application (day -3), offering seeds only. In case the seed is not accepted by the test species, this design is not appropriate.

19. Daily consumption of food is measured during the last three days of the acclimatisation period.

20. A pre-test starvation period is not recommended as the food intake rate of the control on day -1 has to be comparable to the food intake rate on the exposure day.

Conditions of exposure and dose levels

21. Diets containing the required amount of the test substance are prepared by mixing the appropriate amount of test substance with the food/seed to be used for the test. The mixing method should assure homogeneous distribution of the test substance in the diet.

22. The food is offered daily in dishes or bowls. In order to avoid site preferences the treated food and the untreated food should be presented to each bird either at the same site in the cage or at different sites offering the same environment. Treated and untreated food will be randomly assigned to the two dishes/bowls.

23. Treated and untreated food should be offered *ad libitum*.

24. A range of test concentrations should be chosen to allow the quantification of repellency at relevant predicted environmental concentrations. Normally at least three concentrations are used.

Observations

25. The following observations should be made during the test:

- Signs of intoxication and other abnormal behaviour: observations in intervals of 2 hours (or hourly in the case of symptoms) over a period of 8 hours on the day of exposure (day 0), and once a day for the following three days.
- Body weights should be determined in the morning of the following days: at the beginning of the acclimatisation period (day -7), on days -4, -1 of the acclimatisation period, on the day of treatment (day +1), and at the end of the test (day +3).
- Daily food consumption should be measured over 24-hour periods: every day after the fourth day of the acclimatisation period (day -3) until the end of the test (day +3).
- Gross necropsy of birds that die during the test.

DATA AND REPORTING

Treatment of Result

26. Primary endpoint of the study is the repellency factor (RF). RF is calculated by comparing Food Intake Rates (FIR) for treated and untreated food. FIR refers to the daily food consumption per body weight (mg/kg). Body weights determined in the morning of a particular day are used to calculate the FIR for that day. For each bird, the FIR should be calculated from day -3 until the end of the test. Based on the FIR of all the birds, mean FIR is calculated for each treatment group.

27. In a “choice test”, RF is calculated for each bird by comparing the consumption of treated food to the complete food consumption as follows:

$$RF = \frac{\text{FIR for treated food}}{\text{total FIR for treated and untreated food} \times 1/2}$$

28. In the case of a no-choice test, the repellency factor is defined as the average food intake per test group in relation to average food intake of control group during the exposure period.

$$RF = \text{FIR for treatment group} / \text{FIR for control group}$$

29. The RF should be calculated for each concentration. It may be possible to obtain the NOEC (no effect concentration) for repellency, which means the highest concentration where the RF of the treated group is not statistically different from the control group.

Test report

30. The test report must include the following information:

Test substance:

- physical nature, purity and, where relevant, physico-chemical properties (including isomerisation);
- identification data, including CAS number;
- active ingredient and concentration;
- average amount of active ingredient per food particle (i.e., seeds);
- carrier material (in case of baits and granules).

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- microbiological status of the birds, when known;
- number, age and sex of animals;
- acclimatisation procedures and methods of randomly assigning birds to test pens;
- source, (including type, size and material of pen, bowl/dish) diet, housing conditions, diet, etc.

Test conditions:

- rationale for initial dose level selection, dose progression factor and for follow up dose levels
- details of test substance formulation including method of preparation, number of concentrations used, nominal and (where determined) measured dietary concentration of the test substance at each level, assay method used to determine actual concentrations, frequency of mixing and renewal, carrier (if used), storage conditions, method of application;
- description of standard diet, including source, composition, manufacturer's nutrient analysis (including energy content in units of dry matter), and in case seed is used in the test, description of the type of seed (including source) and any supplements and carriers used;
- details of quality (including diet type/source, water source).

Results:

- body weight/body weight changes;
- individual weights of animals at day: -7,-4,-1,+1,+3;
- daily food intake from day -3 until day +3;
- repellency factors;
- time of onset of signs of toxicity and whether these were reversible for each animal;
- necropsy findings for each animal that died during the test;
- statistical treatment of results.

REFERENCES

- (1) INRA (1990) Methodologie d'acceptation de nourriture ou de semences traitées par une substance repulsive, par des oiseaux en conditions de captivité. G. Grolleau, 1/06/1990, Laboratoire de phytopharmacie unite d'eco-etho-toxicologie, Versailles Cedex.
- (2) Avery, M.L. and Decker, D.G. (1991): Repellency of fungicidal rice seed treatment to red-winged blackbirds. *J. Wildl. Mange.* 55(2), 327-334.
- (3) Avery; M.L. and Nelms, C.O (1993): Food Avoidance by red-winged blackbirds conditions with Pyrazin odor. *The Auk* 107, 544-549.

- (4) Best,L.B. and Gionfriddo;J.P.(1994): House Sparrow Preferential Consumption of Carriers Used for Pesticide Granules. *Environmental Toxicology and Chemistry* 13 (6): 919-925.
- (5) Best;L.B.; Stafford,T.M and Mihaich;E.M. (1996): House Sparrow Preferential Consumption of Pesticide Granules with Different Surface Coatings. *Environmental Toxicology and Chemistry* 15(10), 1763-1996.
- (6) OECD (1984). OECD Guideline for Testing Chemicals 205. Avian Dietary Toxicity Test
- (7) EPA (1995). Ecological Effects Test Guidelines. OPPTS 850.2200. Avian Dietary Toxicity Test.
- (8) Luttk, R (1998): Assessing Repellency in a Modified Avian LC50 Procedure Removes the Need for Additional Tests. *Ecotoxicology and Environmental Safety* 40; 201-205

ANNEX 1 DEFINITIONS

Avoidance refers to the reaction of birds to reduce food ingestion due to the repellent properties of a substance. The degree of avoidance depends on the repellent properties of the substance, the type of the treated or contaminated food, and the physical conditions of the exposed birds. Usually the birds have to taste at least some of the treated food, before avoidance begins.

Repellency is defined as an inherent property of a test substance, which is determined under standard test conditions as the reduction of ingestion of treated food by birds in comparison to the ingestion of untreated food.

Repellency Factor (RF) is a quantitative value to estimate the strength of repellency. In a choice test the consumption of treated food should be related to the complete food consumption of the test period. If no repellency exists the relation between consumed treated food and the complete food consumption equals to 0.5 (i.e half of the daily food consumed is treated the other half untreated). The repellency factor (RF) is defined as the deviation from this theoretical value and reads:

$$RF = \text{Food consumption of treated food} / \frac{1}{2} \text{ Food consumption (untreated + treated food)}$$

This factor can range between 0 and 1. RF=0 means complete avoidance of that food sort, whilst RF=1 means no repellency. A repellency factor can be transformed into a percentage of food reduction (FR); eg. RF = 0.2 means 80% reduction of food consumption.

$$\text{Food reduction in \%: FR} = 100 \% - (RF \times 100\%)$$

Standard diet refers to a standard food for adult birds, appropriate to be readily consumed by the species, that meets the nutritional requirements, used during the pre-treatment period and as control diet.