"Avian Dietary Toxicity Test"

1. INTRODUCTORY INFORMATION

• Prerequisites

– Water solubility
– Vapour pressure

• Guidance information

– Structural formula
– Purity of the test substance
– Methods of analysis for the quantification of the test substance in the diet
– Chemical stability in water, light and in diet
– n-Octanol/water partition coefficient
– Results of a ready biodegradability test (see Test Guideline 301)

• Qualifying statements

– This Test Guideline cannot be used for highly volatile substances.

– The test substance should possess characteristics which allow uniform mixing in the diet. A carrier of low toxicity to birds may be used to ensure uniform mixing.

• Standard documents

See references (1) through (4), Section 4, Literature.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

• Definitions

LC 50 in this Test Guideline is the median lethal concentration, i.e. that concentration of the chemical in the diet that is estimated to result in 50 per cent mortality of the birds in

response to a five day dietary exposure followed by a recovery period of three days or more.

Basal diet is a starter ration, appropriate to the species, that meets the nutritional requirements of young birds.

- Reference substances

No reference substances are recommended for this test. However, if a reference substance has been tested, the results should be given.

- Principle of the test method

Birds are fed a diet containing the test substance at a range of concentrations for a period of five days. Beginning on day 6, the birds are fed the basal diet, free of the test substance, for a minimum of three additional days. Mortalities and signs of toxicity are recorded daily.

- Conditions for the validity of the test

– The mortality in the controls should not exceed 10 per cent at the end of the test.

– There must be evidence that the concentration of the substance being tested has been satisfactorily maintained in the diet (it should be at least 80 per cent of the nominal concentration) throughout the first five days of the test period.

– The lowest treatment level should not result in compound-related mortality or other observable toxic effects.

B. DESCRIPTION OF THE TEST PROCEDURE

- Preparations

Suitable facilities for holding birds indoors are necessary. These include mechanisms for

temperature, humidity and light control as required, as well as pens of suitable capacity for rearing the birds.

Birds should be acclimated for a minimum of seven days to facilities and basal diet. They should be randomly assigned to pens, and the pens randomly assigned to concentration levels that will be used. Basal diet should be available ad libitum.

During the 72-hour period preceding testing, the health of the population should be monitored. Mortalities are recorded and the following criteria applied:

– greater than 5 per cent mortality of the population due to health or unknown causes: reject the entire group;

– less than 5 per cent mortality of the population: accept the population for testing.

• Experimental animals

Selection of species

One or more species may be used for this test. The species should be selected in accordance with the purpose for which the test is being conducted. It is desirable that the species used be selected on the basis of relevant experience in holding and testing under laboratory conditions. The birds should be in good health and free from any apparent malformations. Avianspecies recommended for testing are given in Table 1. If other species are used, the test method should be adapted to provide suitable test conditions.

The birds listed in Table 1 are easy to rear and are widely available throughout the year. Birds can be purchased or hatched from eggs. Purchased birds should be known to be free of such avian diseases as aspergillosis, Newcastle disease, pullorum, etc. or bred from stock free of such avian diseases.

All test and control birds should be from the same population of known parentage and should be within one day of age of each other, at least when using chicks from 10 to 17 days old.

Housing and feeding conditions

Environmental conditions are the same for both the holding period and the test period, except that no test substance is given in the food during the holding period. Species-specific environmental conditions are given in Table 1. The following general environmental conditions should be maintained: clean water available ad libitum; 12 to 16 hours of light per day; 5 or 10 birds per pen, except pigeons which should be housed individually; and good ventilation.

Any disturbance that may alter the behaviour of the birds should be avoided.

- Test conditions

Diets containing the test substance

A minimum of five test diets, each containing different concentrations of the test substance is required for the test. Each level should be separated by a constant factor preferably not exceeding 2.0. Definition of the concentrations to be used may require a range-finding test.

If a test at one dose level of at least 5000 ppm in the diet, using the procedures described for the study, produces no compound-related mortality or other observed toxic effects, then a full study using five dose levels may not be necessary.

Diets containing the required amount of the test substance are prepared by uniformly mixing the appropriate amount of the test substance with the prescribed basal diet for young birds. Uniform distribution of the test substance in the food is the criterion for selecting the method of mixing. If necessary, a carrier of low toxicity to birds may be used to ensure uniform distribution. Carriers should not exceed 2 per cent by weight of the diet and when used should also be added to the diets of the birds in the control. Water, corn oil or other carriers for which there is well-documented evidence that they do not interfere with the toxicity of test substances are acceptable.

TABLE 1: Recommended Bird Species and Environmental Conditions

Recommended species	Recommended conditions			
	Temperature (°C)	Relative humidity (per cent)	Age (days)	Space (cm^2/ bird)
Anas platyrhynchos (mallard duck)				
Age 0 - 7 days	32 - 35	60 - 85	10 - 17	600
8 - 14 days	28 - 32			
>14 days	22 - 28			
Colinus virginianus (bobwhite quail)				
Age 0 - 7 days	35 - 38	50 - 75	10 - 17	300
8 - 14 days	30 - 32			
>14 days	25 - 28			
Columba livia (pigeon)				
Age > 35 days	18 - 22	50 - 75	56 - 70	2500*
Coturnix coturnix japonica (Japanese quail)				
Age 0 - 7 days	35 - 38	50 - 75	10 - 17	300
8 - 14 days	30 - 32			
>14 days	25 - 28			
Phasianus colchicus (ring-necked pheasant)				
Age 0 - 7 days	32 - 35	50 - 75	10 - 17	600
8 - 14 days	28 - 32			
>14 days	22 - 28			
Alectoris rufa (red-legged partridge)				
Age 0 - 7 days	35 - 38	50 - 75	10 - 17	450
8 - 14 days	30 - 32			
>14 days	25 - 28			

* Pigeons are housed individually

• Performance of the test

Two control groups and one treatment group for each of the, at least, five dietary levels of the test substance should be used. Each group consists of 10 birds. Diets containing the test substance or control diets should be available ad libitum. The use of prophylactic medication or other chemicals should be avoided if possible, but must be reported when used.

The minimum duration of the test is eight days: five days on the test diet followed by a minimum of three days on normal diet. If mortalities occur on days 7 or 8, or if signs of toxicity remain on day 8 and are not clearly in remission, the test should be continued until two successive days pass without a mortality and it is assured that the birds will recover or until 21 days after the beginning of the test, whichever comes first.

Observations

The following observations at a minimum should be made during the test:

– signs of intoxication and other abnormal behaviour: twice on day 1, daily thereafter

– mortality: twice on day 1, daily thereafter

– body weights: day 0, 5, 8, and end of test (if extended beyond 8 days)

– food consumption: days 0-5, 5-8, and 8-end of test (if extended)

3. DATA AND REPORTING

• Treatment of results

The median lethal concentration (LC 50) can be determined by probit analysis, other appropriate statistical methods, or graphically. Examples of suitable methods are included in Section 4, Literature, references (7), (8), and (9). Where the data permit, the 95 per cent confidence limits are determined by a suitable method, and a test for statistical heterogeneity is performed to ascertain the validity of the data.

When the data obtained are inadequate for use of probit methods in calculating the LC50 (due to most of the results being either no deaths or total mortality) and where a spacing ratio between concentrations of 2.0 or less has been employed, the highest concentration causing no mortality and the lowest concentration causing 100 per cent mortality should be used, together whith any partial mortality data, to determine the LC50. Examples of this approach are included in references (9), (10), and (11).

When mortality at the level of 5000 ppm, the highest recommended treatment level, is less than 50 per cent and the LC50 cannot be calculated, the LC50 should be reported as greater than 5000 ppm and the no-effect level reported as well.

• Interpretation of results

If it is observed that the stability or homogeneity of the test substance in the diet cannot be maintained, care should be taken in the interpretation of the results and note made that these may not be reproducible.

• Test report

The test report should include the following information:

Test substance: chemical identification data

Test animals: scientific name of species, strain, age of birds at the beginning of the test (in days); if species other than those recommended are used, justification must be made

Test conditions:

– housing conditions (including type, size and material of pen, pen temperatures, approximate test room humidity, photoperiod and lighting intensity)

– description of the basal diet, including source, composition, manufacturer's nutrient analysis (protein, carbohydrate, fat, calcium, phosphorus, etc.) and any supplements and carriers used)

– test diets: method of preparation, number of concentrations used, nominal and (where determined) measured dietary concentration of 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

– acclimation procedures and methods of randomly assigning birds to test pens

– for each concentration and control number of cages and number of birds per cage

– frequency, duration and methods of observation

– names of toxicants (if any) used as reference substances and method of preparation of test diets

Results:

– number of deaths at each treatment level and in the control groups

– average body weights for live birds in each pen at the beginning of the test, the end of the exposure period, and the end of the test; individual weights of all birds that die during the test

– description of all signs of intoxication (e.g. convulsions, lethargy) and other abnormal behaviour (e.g. unusual interactions with other birds), including day of onset, duration, severity (including death), and numbers affected in the different dietary concentrations and controls each day of the test period

– estimated food consumption, by weigh-back method, per pen for the exposure period and for the post exposure period

– results of range-finding test (if conducted)

– calculated LC50 value, 95 per cent confidence limits, slope of the concentration-response curve, the results of a goodness-of-fit test (e.g. chi-square test), highest concentration causing no mortality and lowest causing 100 per cent mortality.

The statistical methods used should be described or the references given.

– anything unusual about the test, any deviation from the above procedures, and any other relevant information

4. LITERATURE

1. U.S. EPA: Registration of Pesticides in the United States - Proposed Guidelines, *Federal Register 43*, No. 132 (July 10, 1978).

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3. Pesticides Safety Precautions Scheme, Working Document D5: Evaluating the Acute Oral and Short-Term Cumulative Oral Toxicity of Pesticides to Birds, Tolworth Laboratory, Ministry of Agriculture, Fisheries and Food, U.K. (1979).

4. Protocols for Sub-acute Toxicity Test (LC50-8 days) in Quail and Mallards, Central Institute for Nutrition and Food Research, TNO, The Netherlands.

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8. J.J. Litchfield and F. Wilcoxon, *J. Pharmacol. Exper. Ther. 96*, 99-113 (1949).

9. C.E. Stephan, in *Aquatic Toxicology and Hazard Evaluation* (edited by F.L. Mayer and J.L. Hamelink), ASTM STP 634, pp. 65-84, American Society for Testing and Materials (1977).

10. W.R. Thompson, *Bacteriological Review 11*, 115-145 (1974).

11. C.S. Weil, *Biometrics 8*, 249-263 (1952).