



"Avian Reproduction Test"

1. INTRODUCTORY INFORMATION

- Prerequisites

- Water solubility
- Vapour pressure
- Avian dietary LC50 (See Test Guideline 205)

- Guidance information

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

- Qualifying statements

- This Test Guideline cannot be used for highly volatile or unstable 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) and (2), Section 4, Literature.

2. METHOD

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

- Definitions

NOEC (No observed effect concentration) is the maximum treatment level used in a test which produces no adverse effects.

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Basal diet is the breeder ration (for adults) or starter ration (for young birds), appropriate to the species, that meets the nutritional requirements of the birds.

Eggs set are the eggs that are incubated, or all eggs produced except those with cracks and those removed for egg shell thickness measurement.

- R e f e r e n c e s u b s t a n c e s

No reference substances are recommended for this test.

- P r i n c i p l e o f t h e t e s t m e t h o d

Birds are fed a diet containing the test substance in various concentrations for a period of not less than 20 weeks. Birds are induced, by photoperiod manipulation, to lay eggs. Eggs are collected over a ten-week period, artificially incubated and hatched, and the young maintained for 14 days. Mortality of adults, egg production, cracked eggs, egg shell thickness, viability, hatchability and effects on young birds are compared with the corresponding parameters in the controls.

- C o n d i t i o n s f o r t h e v a l i d i t y o f t h e t e s t

- The mortality in the controls should not exceed 10 per cent at the end of the test.
- The average number of 14-day-old survivors per hen in the controls should be at least 14, 12 and 24 for mallard duck, bobwhite quail and Japanese quail, respectively.
- The average egg shell thickness for the control group should be at least 0.34, 0.19 and 0.19 mm for mallard duck, bobwhite quail and Japanese quail, respectively.
- If the recommended concentration scheme is followed and no effect on reproduction is detected, the results may be reported as the NOEC being greater than the highest concentration tested.

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- 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 test period.

B. DESCRIPTION OF THE TEST PROCEDURE

- Preparations

Suitable facilities for rearing birds, preferably indoors, are necessary. These include mechanisms for good ventilation, temperature, humidity and light control, as required. Artificial lighting should approximate the daylight visual spectrum, and be automatically controlled. A 15 to 30 minute transition period at dawn and dusk is recommended.

Birds are randomly distributed to test and control groups. Test and control birds should be acclimated to the facilities and basal diet for a minimum of two weeks. Incompatible birds may be re-arranged during the first week of acclimation.

A population of birds should not be used if more than three per cent of either sex die or become debilitated during the acclimation period.

Equipment

The following equipment is necessary:

- acceptable, clean pens of suitable capacity for breeding of birds and for rearing of young birds. Clean litter may be used. Brooder pens for young birds should have a temperature control device
- acceptable incubators and hatchers, preferably with automatic temperature and humidity controls and a device for turning eggs
- acceptable equipment for storing eggs at a constant temperature and humidity

- 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. The recommended species are mallard duck (*Anas platyrhynchos*), bobwhite quail (*Colinus virginianus*) and Japanese quail (*Coturnix coturnix japonica*). The recommended species are easy to rear and are widely available. If species other than those recommended are used, justification should be made in the test report. Birds can be purchased or reared in the laboratory. Birds should be examined and should appear to be free of disease and injury. All test and control birds should be from the same population of known parentage. Test mallard ducks and bobwhite quail should be similar in appearance to wild birds of the species.

TABLE 1: Recommended Conditions for Adult Birds

Species	Age at the beginning of test	Age range within a test	Minimum floor area of pen per pair*
Mallard duck	9-2 months	± 2 weeks	1 m ²
Bobwhite quail	20-24 weeks	± 1 week	0.25 m ²
Japanese quail	**	± 1/2 weeks	0.15 m ²

* If larger groups are used, the floor space should be increased in proportion.

** It is recommended that Japanese quail be proven breeders (see Table 3) before use in the test, so as to reduce variability with this species.

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Housing and feeding conditions

Adult birds should be maintained with good ventilation at $22 \pm 5^{\circ}\text{C}$ and 50 to 75 per cent relative humidity. Table 1 gives additional conditions specific to the different species.

Except for the absence of test substance in the diet, environmental conditions are the same for both the acclimation period and the test period. The use of chemicals or medication should be avoided whenever possible, but should be reported when used.

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

Environmental conditions for eggs and young birds are given in Table 2.

TABLE 2: Recommended Conditions for Eggs and Young Birds

	Temperature ($^{\circ}\text{C}$)	Relative humidity (per cent)	Turning
Mallard duck			
storage	14 - 16	60 - 85	optional
incubation	37.5	60 - 75	yes
hatching	37.5	75 - 85	no
young, first week	32 - 35	60 - 85	-
young, second week	28 - 32	60 - 85	-
Bobwhite quail			
storage	15 - 16	55 - 75	optional
incubation	37.5	50 - 65	yes
hatching	37.5	70 - 75	no
young, first week	35 - 38	50 - 75	-
young, second week	30 - 32	50 - 75	-
Japanese quail			
storage	15 - 16	55 - 75	optional
incubation	37.5	50 - 70	yes
hatching	37.5	70 - 75	no
young, first week	35 - 38	50 - 75	-
young, second week	30 - 32	50 - 75	-

The temperature and humidities given are for forced draft incubators and hatchers. In still-air, gravity-vented incubators and hatchers, temperatures should be 1.5 to 2°C higher and

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relative humidity should be increased by about 10 per cent. At high elevations, higher relative humidity is necessary. Temperatures in brooder pens should be measured at 2.5 to 4 cm above the pen floor.

- Test conditions

- *Diets containing test substances*

- A minimum of three dietary concentrations of the test substance is required for the test. The concentrations to be used should be based upon the results of a dietary LC50 test (see Test Guideline 205). The highest concentrations should approximate one half of the LC10. Lower concentrations should be geometrically spaced at fractions of the highest dose (e.g. 1/6 and 1/36 of the highest dose). The maximum recommended test concentration is 1000 ppm.

- Diets containing the required amount of the test substance are prepared by uniformly mixing the appropriate amount of the test substance with the basal diet for adultbreeding birds. Uniform distribution of the test substance in the food is the criterion for selecting the method of mixing. Typically, a carrier of low toxicity to birds is used to ensure uniform distribution. Carriers should not exceed 2 per cent by weight of the diet. If a carrier is used for test diets, the same vehicle should be added to diets of 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. Experimental justification is required for carriers for which there is no well-documented evidence of non-interference.

- No test substance, or hence no carrier, should be added to the diet of young birds.

- Performance of the test

- Birds may be kept in pens as pairs or as groups of one male and two (bobwhite quail and Japanese quail) or three (mallard duck) females. Other arrangements are not excluded if justified. Control and treated birds are kept under the same experimental conditions. For tests with pairs, at least twelve pens of birds should be used for each test concentration and for the

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control. For tests with groups, at least 8 pens of mallard ducks and twelve pens of bobwhite quail or Japanese quail should be used for each test concentration and for the control.

The test begins with exposure of test birds to diets containing the test substance. Exposure of adult birds to the test substance must be continuous throughout the test. No test substance (and hence no carrier) should be added to diets of young birds produced in the test. Clean water should be available *ad libitum*.

If the test is conducted in the preferred artificial indoor environment, birds should be held under short-day conditions (seven to eight hours light per day) for eight weeks after the test begins. During the time the dark period should not be interrupted. The photoperiod is then lengthened to 16 to 18 hours of light per day to bring the birds into breeding conditions. Egg-laying should begin two to four weeks after the photoperiod is lengthened.

If the test is conducted in an outdoor environment, the timing of the test should correspond to the natural breeding season of the species at the test location. Birds should be exposed to diets containing the test substance for at least ten weeks before egg-laying normally begins.

For either environment, the test should be continued for at least eight weeks, preferably ten weeks, after egg-laying begins.

The test substance concentration in the diet must not drop below 80 per cent of the expected concentration after the first week of the test. During the first week of the test, diets containing the highest and lowest concentrations should be analysed immediately after the initial mixing and again within four hours of replacing with freshly mixed diet, unless the stability of the test substance in the diet can be adequately demonstrated. If all analyses are within 80 per cent of expected concentrations, no further analyses are required, and the test diet should be renewed frequently enough to ensure maintaining the concentrations.

If either set of analyses indicates that concentrations of the test substance in the diet are less than 80 per cent of the expected concentration, adjustments must be made to raise initial

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concentrations or maintain the actual concentrations by more frequent renewal. Additional analyses during the second week of the test should be conducted to ensure that the adjustments have achieved the 80 per cent goal.

Regardless of the stability of the test substance in the diets, food in the pens should be renewed on at least a weekly basis. If a substance is stable only to the extent that diets would need to be renewed daily, the test may be inappropriate.

Once laying begins, eggs should be collected daily and marked according to pen. Eggs should be stored and set weekly or every other week for incubation (see Table 2 for conditions). Prior to incubation, all eggs should be candled to detect cracks. Cracked eggs should not be incubated. Eggs set for incubation should be candled again after six to eleven days to determine viability.

At least two eggs from each pen, designated in advance (e.g. the third and tenth eggs, or all eggs collected on days 5, 20, and 35 of laying), should be measured for eggshell thickness. Cracked eggs should not be measured, but their numbers should be recorded. Eggs should be opened, washed out, dried with the membrane intact and measured at three to four points around the girth.

Eggs should be transferred from incubation conditions to hatching conditions on day 23 for mallard ducks, day 21 for bobwhite quail and day 16 for Japanese quails. Hatching should be completed by day 25 to 27 for mallard ducks, day 23 to 24 for bobwhite quail, and day 17 to 18 for Japanese quail.

Hatchlings should be housed in groups according to pen of origin or individually marked and housed together. Hatchlings should be maintained on appropriate diets (free of test substance) for 14 days. Temperature and humidity for young birds are given in Table 2. Preferably, lighting should be on a diurnal basis (e.g. 14 hours of a light, 10 hours of dark) with a 15 to 30 minute transition at dawn and dusk; other lighting regimes are acceptable.

Observations

The following observations should be made during the test:

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- mortality and signs of toxicity: daily
- body weights of adults: at start of exposure period, prior to onset of egg laying, and at termination of study
- body weights of young: at 14 days of age
- food consumption of adults: one or two-week intervals throughout the study
- food consumption of young: first and second week after hatching
- gross pathological examination: all adult birds.

Residue analysis of selected tissues may be useful particularly for test substances with a value of log P(n-octanol/water) higher than 3.0.

3. DATA AND REPORTING

• Treatment of results

Test groups should be individually compared to the control group by a statistical procedure designated in the study plan. Any generally acceptable statistical method, such as analysis of variance or other applicable methods given in reference (8) may be used. Analysis should include the parameters given in Table 3 and also, if possible, the percentage of hens laying eggs, the body weight of adult birds and the body weight of 14-day old survivors.

• 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 beginning of test (in weeks or months), source of birds, any pre-treatment, etc.

Test conditions:

- housing conditions: type, size, and material of pen, pen temperatures, approximate test room humidity, photoperiod, ventilation, and any changes during the test

"Avian Reproduction Test"*TABLE 3: Normal* Values for Reproduction Parameters*

Parameter	Mallard duck	Bobwhite quail	Japanese quail
Egg production - number of eggs laid per hen (ten weeks)	28 - 38	28 - 38	40 - 65
Percentage of cracked eggs	0.6 - 6	0.6 - 2	-
Viability (per cent viable embryos of eggs set)	85 - 98	75 - 90	80 - 92
Hatchability (per cent hatching of eggs set)	50 - 90	50 - 90	65 - 80
Percentage of hatchlings that survive to 14 days	94 - 99	75 - 90	93
Number of 14-day old survivors per hen	16 - 30	14 - 25	28 - 38
Eggshell thickness, mm	0.35 - 0.39	0.19 - 0.24	0.19 - 0.23

* These values are typical, but not necessarily representative for all facilities. If control birds do not meet or closely approach these values, the test procedures and conditions should be investigated for potential problems.

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- 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 method of randomly assigning birds to test pens and any rearrangements due to incompatibility
- number of birds per pen and number of replicate pens per concentration level and for controls
- methods of identification of eggs and birds
- conditions of egg storage, incubation, and hatching, including temperature, humidity, and turning frequency
- names of toxicant (if any) used as reference substances and method of preparation of test concentration,

Results:

- frequency, duration, and description of signs of toxicity, along with severity, numbers affected and any remissions
- food consumption and body weights for adults and young birds
- details of gross pathological examinations
- results of residue analysis (if performed)
- egg production, eggs set, viability, hatchability (including normal hatchlings), survival of young, and eggshell thickness (in summary by concentration level and for each pen by week for the test period, preferably in tabular form)

- methods of statistical analysis and interpretation of results
- NOEC and any statistically significant effect levels
- anything unusual about the test and other relevant information which might have influenced the results

4. L I T E R A T U R E

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