

### III Avian Reproduction Test

#### Objective

The objective of this test is to demonstrate the toxicity of the test substance for the reproduction of birds by administering the test substance on adult birds and by observing the effects on the mortality of the adult birds, the number of eggs laid, the number of eggs with cracked shells, thickness of egg shells, the rate of embryonic growth, the proportion of hatched eggs and young birds.

#### 1. Definition

The following definitions of the terms should be defined in this test method:

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

#### 2. Physico-chemical characteristics of the test substance

In order to conduct the test, a reliable method of analysis is needed to measure water solubility and vapor pressure of the test substance and to determine the quantity of the test substance within the feed. It is also necessary to collect as much information as possible on structural formulas, purity, stability against water and light, and stability within the feed of the test substance, which are closely related to testing techniques.

#### 3. Preliminary tests

In order to grasp the rough toxicity of the test substance beforehand, an avian dietary toxicity test is conducted according to the OECD Test Guideline 205.

#### 4. Test organisms

One or more species may be used for this test. The recommended species is Japanese quail (*Coturnix coturnix japonica*), but other species such as mallard duck (*Anas platyrhynchos*) and bobwhite quail (*Colinus virginianus*) can also be used. 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 when they are brought in 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.

## **5. Test method**

### **5-1. Test facilities and equipment**

#### **(1) Test facilities**

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 (it is desirable to gradually light up or black out over 15 to 30 minutes).

#### **(2) 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

### **5-2. Acclimatization**

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.

### **5-3. Implementation of tests**

#### **5-3-1. Test conditions**

##### **(1) Environmental 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. (any significant disturbances to the environment that influence the behaviour of the birds should be avoided as much as possible.)

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

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 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.

Table 1: Recommended Conditions for Adult Birds

Species	Age at the beginning of the test	Age range within a test	Minimum floor area of pen per pair*
Japanese quail	**	±1/2 weeks	0.15m <sup>2</sup>
Mallard duck	9-12 months	±2 weeks	1m <sup>2</sup>
Bobwhite quail	20-24 weeks	±1 week	0.25m <sup>2</sup>

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

\*\* It is recommended that Japanese quail be proven breeder before use in the test, so as to reduce variability with this species.

Table 2: Recommended Conditions for Eggs and Young Birds

	Temperature (°C)	Relative humidity (per cent)	Turning
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	-
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	-

## (2) Administration of 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 an avian dietary toxicity test. 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 adult breeding 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.

Table 3: Normal\* Values for Reproduction Parameters

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

Eggshell thickness, mm	0.19-0.23	0.35-0.39	0.19-0.24
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\* 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.

### **(3) Test operations**

Birds may be kept in pens as pairs or as groups of one male and two (Japanese quail and bobwhite 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 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

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 16 for Japanese quails, day 23 for mallard ducks and day 21 for bobwhite quail. Hatching should be completed by day 17 to 18 for Japanese quail, day 25 to 27 for mallard ducks, and day 23 to 24 for bobwhite 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.

### **5-3-2. Observations**

The following observations should be made during the test:

- 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.

#### **5-4. Validity of the test**

The following conditions must be satisfied to validate the test:

- 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 24, 14 and 12 for Japanese quail, mallard duck and bobwhite quail, respectively.
- The average egg shell thickness for the control group should be at least 0.19, 0.34 and 0.19 mm for Japanese quail, mallard duck and bobwhite quail, respectively.
- The test substance concentrations in the diet should be adequately maintained (at least 80 per cent of the expected concentrations).

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.

#### **6. Treatment of results**

Test groups should be individually compared to the control group using generally acceptable statistical method, such as analysis of variance.

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.