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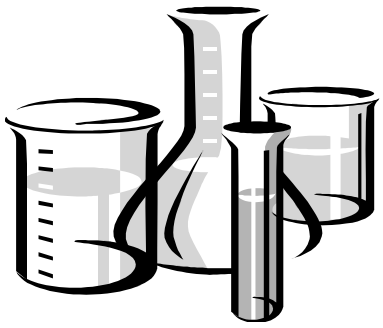
# Ecological Effects Test Guidelines

## OCSPP 850.2300: Avian Reproduction Test



**NOTICE**

This guideline is one of a series of test guidelines established by the United States Environmental Protection Agency's Office of Chemical Safety and Pollution Prevention (OCSPP) for use in testing pesticides and chemical substances to develop data for submission to the Agency under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, et seq.), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), and section 408 of the Federal Food, Drug and Cosmetic (FFDCA) (21 U.S.C. 346a). Prior to April 22, 2010, OCSPP was known as the Office of Prevention, Pesticides and Toxic Substances (OPPTS). To distinguish these guidelines from guidelines issued by other organizations, the numbering convention adopted in 1994 specifically included OPPTS as part of the guideline's number. Any test guidelines developed after April 22, 2010 will use the new acronym (OCSPP) in their title.

The OCSPP harmonized test guidelines serve as a compendium of accepted scientific methodologies and protocols that are intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA. This document provides guidance for conducting the test, and is also used by EPA, the public, and the companies that are subject to data submission requirements under TSCA, FIFRA, and/or the FFDCA. As a guidance document, these guidelines are not binding on either EPA or any outside parties, and the EPA may depart from the guidelines where circumstances warrant and without prior notice. At places in this guidance, the Agency uses the word "should." In this guidance, the use of "should" with regard to an action means that the action is recommended rather than mandatory. The procedures contained in this guideline are strongly recommended for generating the data that are the subject of the guideline, but EPA recognizes that departures may be appropriate in specific situations. You may propose alternatives to the recommendations described in these guidelines, and the Agency will assess them for appropriateness on a case-by-case basis.

For additional information about these test guidelines and to access these guidelines electronically, please go to http://www.epa.gov/ocspp and select "Test Methods & Guidelines" on the left side navigation menu. You may also access the guidelines in http://www.regulations.gov grouped by Series under Docket ID #s: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159, and EPA-HQ-OPPT-2009-0576.

## OCSPP 850.2300: Avian reproduction test.

(a) **Scope—**

(1) **Applicability**. This guideline is intended to be used to help develop data to submit to EPA under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, et seq.), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 346a).

(2) **Background**. The source material used in developing this harmonized OCSPP test guideline include the OPPT guidelines under 40 CFR 797.2130 Bobwhite Reproduction Test and 797.2150 Mallard Reproduction Test; the OPP 71-4 Avian Reproduction Test (Pesticide Assessment Guidelines Subdivision E); OECD 206, Avian Reproduction Test, and the Pesticide Reregistration Rejection Rate Analysis: Ecological Effects.

(b) **Purpose.** This guideline is designed to develop data on the reproductive effects on the northern bobwhite (*Colinus virginianus*) and mallard (*Anas platyrhynchos*) of chemical substances and mixtures ("test chemicals" or "test substances") subject to environmental effects test regulations. This guideline prescribes specific guidance for the testing of northern bobwhite and mallard, which are the Agency's preferred test species. The Agency will use these and other data to assess the chronic hazard and risks to birds that these chemicals may present through environmental exposure.

(c) **Definitions.** The definitions in the OCSPP 850.2000 guideline apply to this test guideline. In addition, the following more specific definitions apply, which refer specifically to the production and quality of eggs and subsequent development of these eggs through hatching and up to the point where young are 14 days old:

*14-day-old survivors* are birds that survive for 2 weeks following hatch.

*Cracked eggs* are eggs determined to have cracked shells when inspected with a candling lamp. Fine cracks cannot be detected without using a candling lamp and if undetected will bias data by adversely affecting measures of embryo development.

*Eggs set* refers to all eggs placed under incubation, *i.e*. total eggs produced minus cracked eggs and those selected for analysis of eggshell thickness. The number of eggs set, itself, is an artificial number, but it is essential for the statistical analysis of other development parameters.

*Eggshell thickness* refers to the thickness of the shell and the membrane of an egg at several points around the girth after the egg has been opened, washed out, and the shell and membrane dried for at least 48 hours at room temperature. Values are expressed as the average thickness of these several measured points in millimeters (mm).

*Hatchlings, normal* refers to embryos that mature, pip the shell, and liberate themselves from the eggs on day 23-25 of incubation for northern bobwhite and days 25-28 of incubation for mallards.

*Live 18-day embryos or 21-day embryos for northern bobwhite and mallards, respectively* refers to embryos that are developing normally after 18 or 21 days of incubation for northern bobwhite and mallards, respectively. This is determined by candling the eggs.

*Viable embryos (or fertile eggs)* refers to eggs in which fertilization has occurred and embryonic development has begun. This is determined by candling the eggs 11 days after incubation has begun for northern bobwhite and 14 days for mallards. It is difficult to distinguish between the absence of fertilization and early embryonic death. The distinction can be made by breaking open eggs that appear infertile and examining further. This distinction is especially important when a test substance induces early embryo mortality.

(d) **General considerations**—

(1) **Summary of the test.** Adult birds are administered the test substance continuously in their daily diet prior to the onset of breeding and continuing for an extended period after egg laying has been initiated by photostimulation. Eggs are collected, marked, stored, and subsequently incubated through hatching. Offspring are maintained on a clean diet for a period of approximately two weeks after hatching. Effects on adult birds, embryos, and hatchlings are monitored throughout the exposure period in order to assess the potential reproductive impact of the test substance. The no observable effect concentration (NOEC) for each of the monitored effects is determined and the most sensitive of these endpoints is used as the overall reproductive NOEC for the test.

(2) **General test guidance.** The general guidance in OCSPP 850.2000 applies to this guideline except as specifically noted herein.

(3) **Range-finding test.** Unless the approximate NOEC for the most sensitive reproductive endpoint is known already, a range-finding test should be conducted to help determine the concentrations to be used in the definitive test. If a range-finding test is performed, a six week dietary exposure period may provide information helpful in determining appropriate test concentrations for the definitive test.

(4) **Definitive test.** The objective of the definitive test is to determine the concentration-response relationships for avian reproductive parameters from dietary exposure to a test substance, and to determine the NOEC for reproduction. The definitive test consists of a minimum of three dietary concentrations of the test substance, plus a control. The dietary levels are confirmed by chemical analysis under test conditions. A list of reproductive response variables that are evaluated to determine a reproductive NOEC are in Table 1. A summary of test conditions is provided in Table 2 and validity elements for an acceptable definitive test in Table 3.

(e) **Test standards**—

(1) **Test substance.** The substance to be tested should be technical grade unless the test is designed to test a specific formulation, mixture, or end-use product. For pesticides, if more than one active ingredient constitutes a technical product, then the technical grade of each active ingredient should be tested separately, in addition to the combination, if

applicable. The OCSPP 850.2000 guideline lists the type of information that should be known about the test substance before testing and discusses methods for preparation of the test substance in the diet for use in testing. The Agency should be contacted prior to testing with nanomaterials.

(2) **Test duration.** The definitive test consists of three phases following acclimation to test facilities. The initial phase begins with exposure of treatment groups of adult birds to diets containing the test substance and is typically 6 to 8 weeks long. After the initial phase, the light/dark photoperiod is manipulated to bring the hens into laying condition during the second phase. This second (photostimulation) phase ends with the onset of egg-laying and is typically 2 to 4 weeks long. Unless otherwise specified, test birds should be exposed for at least 10 weeks prior to the onset of egg laying. The final phase begins with the onset of laying and lasts for at least 8 weeks, preferably 10 weeks. A withdrawal study period may be added to the test if reduced reproduction is observed and the test substance is bioaccumulative. The withdrawal period, if used, need not exceed 3 weeks.

(3) **Test organisms—**

(i) **Species.** These test protocols and standards describe tests specific to using the northern bobwhite (*Colinus virginianus* (L.)) an upland game bird, and the mallard (*Anas platyrhynchos* (L.)) for a waterfowl. Test birds should be pen-reared. The Agency will consider alternative species on a case-by-case basis.

(ii) **Source.** Birds may be reared in the laboratory or purchased from a breeder. For a satisfactory test, all control and experimental birds used in a test should be from the same source, breeding population, and strain. Purchased birds should be certified as disease-free or as bred from disease-free stocks. Rearing stock and/or test birds should be obtained only from sources that have met the requirements for "U.S. Pullorum-Typhoid Clean" classification under paragraph (j)(6) of this guideline. Birds should be obtained only from sources whose colonies have known breeding histories. Steps should be taken to prevent inbreeding. If possible, a history of rearing practices for test birds should be obtained and made available upon request. This history should include lighting practices during rearing, disease record, drug and any other medication administered, and exact age. Test birds should be phenotypically indistinguishable from wild stock. It is recommended that birds be obtained from flocks that have been outbred periodically with genetically wild stock in order to maintain a genetic composition that approximates the heterogeneity of naturally occurring birds.

(iii) **Age.** Adult test birds used are those approaching their first breeding season and are at least 16 weeks old. All test birds should be the same age within one month.

(iv) **Acclimation.** Test birds should be acclimated to test facilities and untreated basal diet for at least 2 weeks. Acclimation may be in the actual pens used in the test or in identical pens. The acclimation period may coincide with the health observation period.

(v) **Health status.** All birds should have a health observation period of at least 2 weeks prior to selection for treatment. Birds used in the test should be in apparent good health. Birds should not have been selected in any way for resistance to toxic substances. Birds are not used for testing under the following conditions.

(A) They are deformed, abnormal, sick, or injured.

(B) More than 3 percent (3%) of either sex of a population of birds becomes debilitated during the health observation period.

(C) Birds were used in a previous test, either in a control or test substance treatment group, or they are offspring of birds used in a test substance treatment group in a previous test. However, offspring of birds used as a control in a previous test are acceptable. Control offspring may be reared and used in another test as adults.

(vi) **Care and handling.** During holding, acclimation, and testing, birds should be shielded from excessive noise, activity, or other disturbance. Birds should be handled only as much as is necessary to conform to test procedures.

(vii) **Diet and feeding—**

(A) **Adult birds.** A standard commercial game bird breeder ration, or its nutritional equivalent, should be used for diet preparation. This ration or basal diet should be used for both control and treatment birds and should be constant throughout the duration of the study. Antibiotics or other medication should not be used in the diet of breeding birds. It may not be possible to obtain food that is completely free of pesticides, heavy metals, and other contaminants. However, diets should be analyzed periodically for these substances and should be selected to be as free from contaminants as possible. A nutrient analysis (quantitative list of ingredients) of the diet should be included with the test report.

(B) **Young birds.** Young birds produced during the test should be fed a commercial game bird starter ration, or its nutritional equivalent. No test substance should be added to the diets of young birds. No antibiotics or medication may be used in the diet.

(viii) **Water.** Clean water should be available *ad libitum*. Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water should be changed daily or more often. Antibiotics or other medication should not be used in the water of breeding birds. Bacitracin, or one of its forms, may be added to the drinking water of young birds, if necessary.

(4) **Administration of test substance.** The test substance is administered in the diet with *ad libitum* feeding. Any test diet remaining in feed trays should be discarded before fresh test diet is provided.

(i) **Preparation of diet treatments.**

(A) The test substance should be mixed into the diet in a manner that will ensure even distribution of the test substance throughout the diet. Diets may be mixed by commercial or mechanical food mixers. Other means are acceptable as long as they result in even distribution of the test substance throughout the diet. Screening of the basal diet before mixing is suggested to remove large particles. If possible, the test substance should be added to the diet without the use of a vehicle or diluent. If a diluent is needed, the preferred diluent is reagent water, but water should not be used for test substances known to hydrolyze readily. When a test substance is not water soluble, it may be dissolved in a reagent grade evaporative diluent (*e.g*. acetone, methylene chloride) and then mixed with the test diet. The solvent should be completely evaporated prior to feeding. Other acceptable diluents may be used, if necessary, and include table grade corn oil, propylene glycol, and gum arabic (acacia). If a diluent is used, it should comprise no more than 2% by weight of the treated diet, and an equivalent amount of diluent should be added to control diets.

(B) For many test substances, it is recommended that diets be mixed under a hood. Frequently, the test substance is added to an aliquot of the basal diet to form a premix concentrate. The premix concentrate should be stored so as to maintain the chemical concentration. For final preparation of test diets, the premix is mixed with additional basal diet to form the proper concentrations. The frequency with which final treated diets are prepared will depend upon the stability and other characteristics of the test substance. Unless otherwise specified or determined by degradation or volatility studies, it is recommended that final diets be prepared weekly, either fresh or from a concentrate. For volatile or labile test substances, test diets should be mixed frequently enough so that the concentrations are not reduced from initial concentrations by more than 20%. If the test substance is known or found to be volatile or labile to the extent that 20% or more loss occurs within 1 week, then test substance diets should be prepared (freshly or from frozen concentrate) at a frequency that will prevent more than 20% loss of test substance. The Agency should be contacted prior to testing with nanomaterials.

Sampling frequency and analysis to confirm dietary test substance concentrations and stability are conducted at a minimum as described in paragraph (e)(9)(i) of this guideline.

(ii) **Treatment concentrations.** Test concentrations of the test substance should be based on measured or calculated residues expected in the diet, unless otherwise specified. There are at least three test substance treatment groups and a control group. One test substance concentration should be equal to or greater than an actual or expected field residue exposure level. One test substance concentration should indicate a reproductive effect, (capturing the Least Observed Effect Concentration (LOEC)) or be greater than 5,000 mg/kg-diet or higher, and one

test substance concentration should be free of biological effects (capturing the no-observed effect concentration (NOEC)). The highest nonlethal concentration may be estimated from the concentration-response data generated in an avian dietary toxicity ($LC_{50}$) test or from a range-finding test. For pesticides, if the expected environmental concentration exceeds 5,000 mg/kg-diet, then the estimated environmental field concentration should be used in place of the 5,000 mg/kg-diet concentration. For pesticides, reasonable upper bound expected environmental field residue exposure level can be estimated using the Kenaga nomogram as modified by Fletcher *et al.* (see references in paragraphs (j)(1) and (j)(2) of this guideline) for short grass and the highest pesticide label rate (in lbs a.i./acre) — accounting for multiple applications that occur in a season. For example, at 1 lb a.i./acre applied twice in a season (*i.e.*, 2 lb a.i./acre) and using the Kenaga nomogram for short grass (1 lb a.i./acre—240 mg/kg) the upper bound residue level is 480 mg a.i./kg-diet. This conservatively assumes no degradation of the parent between applications.

(5) **Controls.**

(i) Every test includes a concurrent control treatment. The control birds are from the same breeding population as the test substance treatment groups and are kept under the same experimental conditions as the test substance treatment groups. The test procedures are the same for control and treated birds, except that no test substance is added to the diets of control birds. If a vehicle or diluent is used in preparation of the test diets, the same diluent is added to the diets of control birds in the highest concentration used for the test diets.

(ii) For a satisfactory test, the following values for response variables in controls should be met or at least approached at test termination. There is likely to be a problem with test procedures or conditions that should be investigated and corrected when these values are not met.

(A) Eggs laid. Normal values for both northern bobwhite and mallards are 29 to 61 eggs per hen for a 10 week egg laying period.

(B) Eggs cracked. Normal values for northern bobwhite are 0 to 7.0% of eggs laid. Normal values for mallards are 0 to 4.0% of eggs laid.

(C) Fertility (viable embryos). Normal fertility values for northern bobwhite and mallards are 80 to 100% of eggs set.

(D) Live 18-d or 21-d northern bobwhite and mallard embryos, respectively (as a percentage of viable embryos). Normal values for northern bobwhite are 97 to 100%. Normal values for mallards are 94 to 100%.

(E) Hatchability (percentage of 18-d or 21-d northern bobwhite and mallard embryos, respectively that hatch). Normal values for northern bobwhite are 85 to 100%. Normal values for mallards are 52 to 100%.

(F) Percentage of eggs set that hatch. Normal values for northern bobwhite are 71 to 95%. Normal values for mallards are 44 to 92%.

(G) 14-day-old survivors of eggs hatched. Normal values for northern bobwhite are 77 to 100%. Normal values for mallards are 94 to 100%.

(H) Eggshell thickness. Normal average values for northern bobwhite are 0.20 to 0.24 mm. Normal values for mallards are 0.316 to 0.372 mm.

(6) **Number of test organism and replicates.**

(i) The experimental unit for this test is the pen. All control and treatment birds should be randomly distributed to pens from the same population. For northern bobwhite and mallard, each of the test substance groups and the control group consist of a minimum of 16 replicate pens. Each pen contains one male and one female. The use of 20 replicate pens in the control group may yield a test with greater statistical power.

(ii) An alternative arrangement of birds may consist of multiple female birds (typically two) and one male bird in each pen. For this arrangement, each pen is considered a replicate. Productivity should be calculated on a per hen basis, with an average given for each pen. Either arrangement is acceptable if productivity reaches the definitive values given in (e)(5)(ii)(A) of this guideline. Because the behavioral interactions of birds in the two arrangements are likely to be different, testing facilities using an arrangement with which they are not familiar are advised to experiment first without test substances in order to determine the feasibility of obtaining acceptable productivity levels.

(iii) Birds should be randomly assigned to treatment and control pens. However, when birds in a pen are incompatible, they may be rearranged within a control or treatment group at any time prior to initiating treatment. Birds should be marked with leg bands.

(7) **Facilities, apparatus and supplies.** Normal laboratory equipment and supplies, and items especially listed in (e)(7)(i) through (e)(7)(vi).

(i) **Facilities.** Pens should be kept indoors in order to better control lighting, temperature, humidity, and other factors. Outdoor pens should only be used during the normal breeding season.

(ii) **Breeding pens or cages—**

(A) **Size.** The Agency recognizes that minimum cage size recommendations are evolving over time. The use of a certain cage size, as with any husbandry parameter, should result in control birds with no overt signs of stress (e.g., reproductive results are within test validity elements reported in this guideline). Northern bobwhite and mallards should be housed in breeding pens or cages of adequate size conforming to good husbandry practices (see the most recent standards of good

husbandry including, but not limited to, references provided in this guidance document).

(B) **Construction materials.** The preferred construction materials are stainless steel, galvanized sheeting, and wire mesh. For enclosed cages, floors and external walls may be wire mesh; and ceilings and common walls solid sheeting. Wire mesh for floors should be fine enough so as to not interfere with normal movement of the birds. Open-topped pens may be constructed of the same materials for the side walls and wire mesh or concrete for the floor. Concrete floors should be covered with litter such as straw, wood shavings, or sawdust. Other construction materials, except wood, are acceptable if they can be kept clean. Wood may be used as vertical framing posts for the support of wire mesh or for horizontal framing along the top of a pen. Wood should not be used for floors or lower sides of pens unless it has been coated with a nonadsorbent material such as perfluorocarbon plastic (*e.g*. Teflon), or unless the wood is replaced between tests.

(C) **Cleaning.**

*(1)* Pens should be disassembled (if feasible) and cleaned thoroughly between tests. Any used floor litter is discarded. Steam cleaning of enclosed cages is recommended. Enclosed cages may be brushed thoroughly, as an alternative method. For open-topped pens, the sides and vertical supports should be thoroughly brushed. The floor composition will dictate methods used to clean the floor. The use of detergents or bleach is acceptable, but other chemical disinfectants (such as quaternary ammonium compounds) should not be used. When necessary to control disease vectors, hot or cold sterilization techniques are recommended, as long as such techniques will not leave chemical residues on the cages. For cold sterilization, ethylene oxide is recommended.

*(2)* During the test, pens should be cleaned when necessary. However, care should be taken to keep disturbance to a minimum as birds are not to be removed from cages during cleaning.

(iii) **Egg storage, incubators and hatchers.** Storage and incubator equipment of sufficient size to store all eggs laid over a two week period and incubate all eggs generated during the study. All eggs should be set after candling for incubation in a commercial incubator. Storage equipment and incubators should be able to maintain stable temperature and humidity conditions. Stored and incubated eggs are turned daily. If incubators are not equipped to turn eggs automatically, they will need to be turned daily by hand. Eggs are removed to a separate incubator or hatcher on day 21 for northern bobwhite and day 24 for mallard. Forced draft incubators or hatchers should be used.

(iv) **Candling.** Candle lamps to check all eggs at set observation times for: fine cracks in egg shells; infertile eggs; and dead embryos.

(v) **Brooder pens.** After hatching, chicks or ducklings are maintained in commercial brooder pens or pens of similar construction. Pens should be constructed of galvanized metal or stainless steel. Temperature in the pens should be controlled, preferably by a thermostatically controlled device.

(vi) **Cleaning.** All materials that will come in contact with the test organisms and test substance should be cleaned before use. Cleaning procedures should be appropriate to remove known or suspected contaminants.

(8) **Environmental conditions—**

(i) **Temperature and humidity—**

(A) **Adult birds.** Temperature and humidity should be controlled during the study. The recommended temperature level for adult birds is 15 to 30 degrees Celsius ($^{o}$C) with approximately 45 to 70% relative humidity.

(B) **Eggs—**

*(1)* **Storage period.** All eggs are collected daily, marked according to the pen from which collected, and stored at 13 to 16 ºC and 55 to 80% relative humidity. Storage in plastic bags may improve uniformity of hatching. Stored eggs should be turned daily. Stored eggs are set weekly or every other week for incubation.

*(2)* **Incubation period.** During the incubation period, eggs should be maintained at 37.5 $^{o}$C ± 1 ºC and approximately 70% relative humidity.

(C) **Brooder pens.** For hatchlings, a temperature gradient in the brooder pen from approximately 35 ºC to 22 ºC will allow young birds to seek a proper temperature. Temperature requirements for young birds typically decline over this range from birth through the first several weeks of life. Humidity should be approximately 70%.

(ii) **Lighting and photoperiod—**

(A) **Adults.**

*(1)* Lights should emit a spectrum simulating that of daylight. The use of shorter wave-length "cool-white" fluorescent lights that do not emit the daylight spectrum should be avoided. Illumination intensity should be about 65 lux (for sunlight, equivalent to approximately 1.2 micromoles per square meter per second

(μmol/$m^2$/s) with a minimum of 10 lux (0.2 μmol/$m^2$/s) at the level of the birds.

*(2)* Lighting regimes (photoperiod) are critical to successful reproduction. Various photoperiod regimes have been demonstrated to give acceptable results. Any photoperiod regime that results in productivity that meets the definitive values given in paragraph Table 3 of this guideline is acceptable as long as birds are exposed to treated diets a minimum of 10 weeks prior to the onset of laying. Regardless of the methods selected, lighting should be controlled carefully, preferably by automatic timers. A 15 to 30 minute transition period between the light and dark periods is desirable. In addition, it is important during the initial phase to not interrupt the dark period unless absolutely necessary.

*(3)* A suggested photoperiod regime would consist of maintaining birds under a photoperiod for 7 or 8 hours of light during the initial phase. At the end of the initial phase, the photoperiod may be increased to 16 to 17 hours of light per day. The photoperiod may be maintained at this level for the remainder of the study, or it may be increased each week by 15 minutes per day.

(B) **Chicks and ducklings.** Lighting should be on a diurnal basis (*e.g.* 16 hours of light, 8 hours of dark, with a 15-30 minute transition at dawn and dusk, but other lighting regimes are acceptable).

(iii) **Ventilation.** Good ventilation should be maintained. Suggested ventilation rates are 10 to 15 changes per hour.

(9) **Observations—**

(i) **Measurement of test substance.** Samples of treated diets should be analyzed to confirm proper dietary concentrations of the test substance under actual test conditions. The analytical method used to determine test substance concentrations shall be validated before beginning the test, as described in OCSPP 850.2000. During the exposure period, analyses should be conducted on representative samples of test feed taken from feeders of all test concentrations at the beginning of the exposure period, midway through the test (10 to 12 weeks later), and at the end of the exposure period. If samples cannot be analyzed immediately, they should be stored appropriately (*e.g.*, frozen at a temperature of -15ºC or lower) until analysis can be performed.

(ii) **Contaminants in feed.** Diets should be analyzed periodically to identify background contaminants such as heavy metals (*e.g.*, arsenic, cadmium, lead, mercury, and selenium) and persistent pesticides, especially chlorinated insecticides. A broader pesticide screen to include other chemicals (*e.g.*, organophosphorus pesticides) may be useful.

(iii) **Basal diet composition.** A nutrient analysis of the basal diet should be included with the test report. The analysis should include percentages by weight of protein, fat, fiber, ash, calcium, and phosphorus. In addition to these analyzed components, a list of expected amounts of vitamins, minerals or other supplements should also be reported. Most commercial feed companies provide both the analysis and the list of supplements on the label.

(iv) **Environmental conditions**—

(A) **Temperature.** Temperature should be recorded at least weekly at the same time of day. For tests conducted without temperature control, temperature minimums and maximums should be recorded daily. Continuous temperature monitoring is desirable. Temperature recordings should be made at a level of 2.5 to 4 centimeters (cm) above the floor of the cage.

(B) **Humidity.** Humidity should be monitored on a constant basis in at least one representative location.

(v) **Measures of effect.** All calculations and formulae provided below assume each pen (replicate) consisted of one male bird and one female bird. If an alternative design is used (*e.g.*, one male bird and two female birds per pen), formulae will need to be adjusted accordingly; consultation with a statistician is recommended. The measurement interval for the determination of the NOECs should commence at onset of exposure of adults and finish at the end of the exposure period (typically 8-10 weeks after egg laying starts). All outcomes from eggs laid during the exposure period will be included in the statistical analysis. If a withdrawal period is used, separate summary data and statistics should be calculated based solely on data obtained from the withdrawal period. A statistician should be consulted for statistical analysis to compare data from the exposure and withdrawal periods.

(A) **Adult birds**—

*(1)* **Body weight and food consumption**. Body weights should be recorded for each adult bird at the beginning of the treatment period, at 14-day intervals until the onset of egg laying, and at termination of treatment. Taking of body weights during egg laying is discouraged because of possible adverse effects on egg production. Food consumption should be measured and recorded by pen as often as body weights are measured prior to the onset of laying and at least biweekly throughout the rest of the study.

(*2*) **Signs of toxicosis.** Observations on adult birds should be made at least once a day. or other signs of toxicity should be described and recorded by date or day of test.

(*3*) **Gross pathology.** Gross pathological examinations should be conducted on all birds that die during the test period, and for all

survivors at the end of the test. At a minimum, the examination should include the GI tract, liver, kidneys, heart, reproductive organs, and spleen. The subcutaneous fat and muscles should also be examined for evidence of deterioration. It is preferred that a sufficient number of samples of two or more tissues (*e.g*. muscle, fat) be analyzed for test substance residues unless it can be demonstrated that the elimination rate is less than 24 hours.

(B) **Eggs**—

*(1)* **Eggs laid.** All eggs laid should be collected daily, counted and marked according to the pen from which collected, and stored. Storage in plastic bags may improve uniformity of hatching. Stored eggs should be turned daily. Eggs should be removed daily, marked, and stored until there is a sufficient quantity for incubation.

*(2)* **Cracked eggs, egg shell thickness, and eggs set.**

*(a)* At weekly or biweekly intervals, eggs should be removed from storage and be candled to detect eggshell cracks. All eggs should be candled at day 0 for cracks and all cracked eggs are counted and discarded. Except for eggs with cracked shells and those eggs removed for eggshell thickness measurements, all eggs should be set after candling for incubation and the number of eggs set recorded.

*(b)* Once every 2 weeks all eggs newly laid that day should be removed and measured for eggshell thickness. Eggs should be opened at the girth (the widest portion), the contents washed out (or used or saved for egg residue analysis), and the shell air-dried for at least 48 hours. The thickness of the shell plus the dried membrane should be measured at a minimum of 3 points around the girth using a micrometer calibrated at least to 0.01 millimeter (mm) units.

*(3)* **Fertility and early death of embryos.** Eggs should be candled again on day 11 for northern bobwhite or day 14 for mallards of incubation to determine fertility and early death of embryos.

*(4)* **Embryo survival.** A final candling should be done on day 18 for northern bobwhite or day 21 for mallards to measure embryo survival. Eggs should be removed to a separate incubator or hatcher on day 21 for northern bobwhite or day 24 for mallard.

(C) **Chicks and ducklings—**

*(1)* **Number of hatchlings.** Count the number of embryos that pip shell, and embryos that liberate themselves. Hatching will normally be complete by the end of day 24 for both species. By day 24 or 27 of incubation, the hatched bobwhite chicks and ducklings, respectively, should be removed from the hatcher or incubator. Chicks or ducklings should be either housed according to the appropriate parental pen group or individually marked (such as by leg bands) as to parental group and housed together.

*(2)* **Signs of toxicosis or abnormalities.** Chicks or ducklings should be observed daily from hatching until they are 14 days old. Mortality, signs of toxicity, and other clinical abnormalities should be recorded at least cumulatively through day 5 and recorded by age from days 5 through 14.

*(3)* **Body weight of hatchlings.** Each chick or duckling is weighed individually upon hatching. An average hatchling weight for each pen is calculated.

*(4)* **Body weight of 14-d-old survivors.** Each chick or duckling is weighed individually on day 14. An average hatchling weight for each pen is calculated.

(f) **Treatment of results—**

(1) **Response variable calculation.** For all equations in paragraph (f)(1) of this guideline, the index $j$ = 1 to the total number of pens per treatment group (typically 16).

(i) **Adult body weight gain.** The change in adult body weight (males and females are tracked separately) between test initiation and test termination, $\Delta bw_j$, for pen $j$ is the measure used in this test guideline to evaluate the inhibitory effects of the test substance on adult growth. The change in adult body weight (male or female), assuming one bird of each sex per pen, is calculated using Equation 1. Additionally, the change in adult body weight during the course of the test prior to the onset of laying (day 0 to 14, 14 to 28, 28 to 42, *etc*.) is calculated and plotted to assess effects on the pattern of growth (*e.g*., *t2* = day 14 and *t1* = day 0; *t2* = day 28 and *t1* = day 14).

$$\Delta bw_j = bw_{jt2} - bw_{jt1} \qquad \textbf{Equation 1}$$

where:

$bw_{jt}$ = male (or female) body weight in pen $j$ at time $t$, where *t1* is test initiation; and *t2* is test termination.

(ii) **Average hatchling weight.** The response measure for hatchling weight is the average hatchling weight per pen which is calculated using Equation 2.

$$\overline{HATWT}_j = \sum_{k=1}^{NH_j} HATWT_{kj} \Big/ NH_j \qquad \textbf{Equation 2}$$

where:

$k$ = index number of a hatchling in pen $j$ from 1 to $NH_j$;

$NH_j$ = total number of hatchlings in pen $j$; and

$HATWT_{kj}$ = body weight of hatchling $k$ in pen $j$.

(iii) **Average 14-d survivor weight.** The response measure for 14-d old survivor weight is the average survivor weight per pen which is calculated using Equation 3.

$$\overline{SURVWT}_j = \sum_{k=1}^{HS_j} SURVWT_{kj} \Big/ HS_j \qquad \textbf{Equation 3}$$

where:

$k$ = index number of a 14-d old hatchling in pen $j$ from 1 to $HS_j$;

$HS_j$ = total number of 14-d old surviving hatchlings in pen $j$; and

$SURVWT_{kj}$ = body weight of 14-d old surviving hatchling $k$ in pen $j$.

(iv) **Average egg shell thickness.** The response variable for egg shell thickness is the average thickness per pen which is calculated using Equation 4.

$$\overline{THICK}_j = \sum_{k=1}^{m_j} THICK_{kj} \Big/ m_j \qquad \textbf{Equation 4}$$

where:

$k$ = index number of egg shell thickness measurement in pen $j$ from 1 to $m_j$;

$m_j$ = total number of eggs with shell thickness measured in pen $j$; and

$THICK_{kj}$ = average shell thickness of egg *k* in pen *j*., measured as described in paragraph (e)(9)(v)(B)*(2)(b)* of this guideline.

(v) **Total food consumption per adult per pen.** Total food consumption per adult bird between test initiation and test termination, $TFOOD_j$, for pen *j* is the measure used in this test guideline to evaluate adversion or inhibitory effects of the test substance on consumption of food by adults. The total food consumption per adult bird per pen is calculated using Equation 5. Additionally, the weekly (or biweekly after the onset of laying) food consumption rate per adult (*e.g.*, *t1*, *t2*, *etc.*) during the course of the test is calculated and plotted to assess effects on the pattern of food consumption.

$$TFOOD_j = \sum_{t=1}^{term} \left( \frac{FOOD_{jt}}{m_{jt}} \right) \qquad \textbf{Equation 5}$$

where:

*t* = index of weekly and biweekly measurements of food consumption, with *t*=1 being week 1 of the study and *t=term* being the test or exposure termination week;

$FOOD_{jt}$ = total food consumption in pen *j* at time *t*;

$m_{jt}$ = number of adult birds in pen *j* at time *t*;

(vi) **Proportion of uncracked eggs.** The proportion of uncracked eggs is calculated using Equation 6.

$$UE_j = \left( EL_j - EC_j \right) \Big/ EL_j \qquad \textbf{Equation 6}$$

where:

$EL_j$ = total number of eggs laid in pen *j*; and

$EC_j$ = number of eggs cracked in pen *j*.

(vii) **Proportion of normal eggs.** The proportion of normal eggs is calculated using Equation 7.

$$NE_j = \left( EL_j - EC_j - EA_j \right) \Big/ EL_j \qquad \textbf{Equation 7}$$

where:

$EL_j$ = total number of eggs laid in pen *j*;

$EC_j$ = number of eggs cracked in pen *j*; and

$EA_j$ = number of irregular or abnormal eggs in pen $j$.

(viii) **Other proportions.** Eight additional proportions are calculated as new variables for each pen: proportion of eggs set per eggs laid ($ES_j/EL_j$); proportion of viable embryos per eggs laid ($VE_j/EL_j$); proportion of live 18-d-old northern bobwhite or 21-d-old mallard embryos per viable embryos ($LE_j/VE_j$); proportion of normal hatchlings per eggs set ($NH_j/ES_j$); proportion of hatchlings per live 18-d-old northern bobwhite or 21-d-old mallard embryos ($NH_j/LE_j$); proportion of hatchlings per eggs laid ($NH_j/EL_j$); proportion of 14-d-old survivors per eggs set ($HS_j/ES_j$); proportion of 14-d-old survivors per hatchlings ($HS_j/NH_j$).

(2) **Descriptive statistics—**

(i) **Environmental conditions.**

(A) Calculate descriptive statistics (mean, standard deviation, minimum, maximum) for temperature, relative humidity, and light intensity during the three exposure phases (initial, photostimulation and laying) for adults, and for chicks or ducklings in the brooder pens.

(B) Calculate descriptive statistics (mean, standard deviation, minimum, maximum) for temperature, and relative humidity during egg storage and incubation.

(ii) **Dietary test substance concentrations.** Calculate descriptive statistics (mean, standard deviation, coefficient of variation, minimum, maximum) by treatment level of the test substance concentration in the diet.

(iii) **Basal diet.** Calculate descriptive statistics (mean, standard deviation) of the percentages by weight of protein, fat, fiber, ash, calcium, and phosphorus.

(iv) **Reproductive response variables.** For each treatment group including the control, calculate and plot summary statistics (mean, median, minimum, maximum, first quartile, and third quartile) for each reproductive response variable in Table 1. Additionally, calculate the standard deviation, coefficient of variation, standard error of mean, and 95% confidence interval of mean for each treatment group including the controls.

(3) **Percent inhibition—**

(i) **Inhibitory effects.** Except for the two response variables, number of cracked eggs and number of irregular and abnormal eggs, all other response variables are expected to exhibit increasing inhibition or reduction in the measured response with increasing test substance concentration in the diet. For all response variables in Table 1 the percent inhibition (%I) as compared to the control at each test substance concentration is calculated using Equation 8.

$$\%I = \frac{(C - X)(100)}{C}$$ **Equation 8**

where:

$C$ = the control mean treatment response value (*e.g.* number of eggs laid); and

$X$ = the test substance treatment mean response value (*e.g.* number of eggs laid). Stimulation or a greater response in the test substance treatment than the control is reported as negative %I.

(ii) **Stimulatory effects.** For the response variables number of cracked eggs and number of irregular and abnormal eggs, the interest is in the increase or stimulation of these events with increasing test substance concentrations rather than in their reduction or inhibition. The percent stimulation or increase is also calculated using Equation 8 except stimulation is reported as negative values of %I. Negative %I values indicate an increased or stimulatory effect over the control response. If working with negative numbers is confusing, the analyst may find multiplying the %I value by -1 reduces confusion when discussing the increase in cracked eggs and irregular or abnormal eggs with increased test substance concentration.

(4) **NOEC.** A NOEC and LOEC are determined for each of the reproductive response variables in Table 1 using appropriate statistical methods. All methods used for statistical analysis should be described completely. Experimental units (replicates) are the individual pens within each treatment level. The overall study NOEC and LOEC are the lowest values (most sensitive) of all response variables considered.

**Table 1.—Reproductive Response Variables To Evaluate**

| |
|---|
| **Measured response variables** |
| Number of eggs laid per pen ($EL_j$) |
| Number of irregular or abnormal eggs per pen ($EA_j$) |
| Number of cracked eggs per pen ($EC_j$) |
| Number of eggs set per pen. ($ES_j$) |
| Number of viable embryos per pen ($VE_j$) |
| Number of live embryos (18-day-old northern bobwhite or 21-day-old mallard embryos) per pen ($LE_j$) |
| Number of normal hatchlings per pen ($NH_j$) |
| Number of 14 day-old survivors per pen ($HS_j$) |
| **Calculated response variables** |
| Proportion of uncracked eggs per pen ($EL_j - EC_j$)/($EL_j$) |
| Proportion of eggs set of eggs laid per pen ($ES_j$/$EL_j$) |
| Proportion viable embryos of eggs set per pen ($VE_j$/$ES_j$) |
| Proportion of live embryos of viable embryos per pen ($LE_j$/$VE_j$) |
| Proportion of normal hatchlings of eggs laid per pen ($NH_j$/$EL_j$) |
| Proportion of normal hatchlings of eggs set per pen ($NH_j$/$ES_j$) |
| Proportion of normal hatchlings of live embryos per pen ($NH_j$/$LE_j$) |
| Proportion of 14 day-old survivors of eggs set per pen ($HS_j$/$ES_j$) |
| Proportion of 14 day-old survivors of normal hatchlings per pen ($HS_j$/$NH_j$) |
| Average egg shell thickness per pen ($THICK_j$) |
| Average hatchling body weight per pen ($HATWT_j$) |
| Average 14 day-old survivor body weight per pen ($SURVWT_j$) |
| Adult male body weight gain per pen ($\Delta bw_j$) |
| Adult female body weight gain per pen ($\Delta bw_j$) |
| Total food consumption per adult bird per pen ($FOOD_j$) |

**(g) Tabular summary of test conditions**. Table 2 lists the important conditions that should prevail during this test. Meeting these test conditions will greatly increase the likelihood that the completed test will be acceptable or valid.

**Table 2.—Summary of Test Conditions for Avian Reproduction Test**

| | |
|---|---|
| Test duration | Test birds should be exposed for at least 10 weeks prior to the onset of egg laying and for at least 8 weeks, preferably 10 weeks, following the onset of laying |
| Temperature | 15 to 30ºC for adults; A gradient between approximately 22ºC and 35ºC for hatchlings |
| Light quality | Lights should emit a spectrum simulating that of daylight |
| Light intensity | 10 - 65 lux (0.2 to 1.2 µmol/$m^2$/s) |
| Photoperiod | Variable (see text) |
| Humidity | Approximately 45 to 70% |
| Pen size | See the most recent standards of good husbandry including references provided in this guidance document. |
| Number of pens per concentration level | 16 pens per test concentration and control are preferred. |
| Test species | Northern bobwhite and mallard (additional species may tested as an option) |
| Age of test organisms | 16 weeks or slightly older at study initiation |
| Number of birds per concentration level | Paired design (one male and one female) is preferred |
| Number of concentration levels | Minimum of three, plus a control group |
| Administration of test substance | Through diet |
| Measures of Effect (Measurement Endpoints) | NOEC for each reproductive parameter (see Table 1) and feed consumption |

(h) **Test validity elements.** This test would be considered to be unacceptable or invalid if one or more of the conditions in Table 3 occurred. This list should not be misconstrued as limiting the reason(s) that a test could be found unacceptable or invalid. However, except for the conditions listed in Table 3 and in OCSPP 850.2000, it is unlikely that a study will be rejected when there are slight variations from guideline environmental conditions and study design unless the control organisms are significantly affected, the precision of the test is reduced, the power of a test to detect differences is reduced, and/or significant biases are introduced in defining the magnitude of effect on measurement endpoints as compared to guideline conditions. Before departing significantly from this guideline, the investigator should contact the Agency to discuss the reason for the departure and the effect the change(s) will have on test acceptability. In the test report, all departures from the guideline should be identified, reasons for these changes given, and any resulting effects on test endpoints noted and discussed.

**Table 3.—Test Validity Elements for Avian Reproduction Test**

| |
|---|
| 1. Birds were not randomly assigned to treatment and control pens. |
| 2. More than 10% of the control birds died or became moribund during the test. |
| 3. The average number of eggs laid per hen in the control group was less than 29 for northern bobwhite or mallard. |
| 4. The number of viable embryos in the control group was less than 80% of the eggs set for northern bobwhite or mallard. |
| 5. The number of 18-d-old northern bobwhite and 21-d-old mallard embryos of eggs set in the control group was less than 97% for northern bobwhite or less than 94% for mallard, respectively. |
| 6. The number of normal hatchlings in the control group was less than 85% of the viable embryos for northern bobwhite or less than 52% of the viable embryos for mallard. |
| 7. The number of normal hatchlings in the control group was less than 71% of the eggs set for northern bobwhite or less than 44% of the eggs set for mallard. |
| 8. The number of 14 day old survivors in the control group was less than 77% of the normal hatchlings for northern bobwhite or less than 94% of the normal hatchlings for mallard. |
| 9. The average eggshell thickness in the control group is less than 0.20 mm for northern bobwhite or 0.316 mm for mallards. |
| 10. There are greater than 13% cracked eggs in the control group. |

(i) **Reporting—**

(1) **Background information.** Background information to be supplied in the report consists at a minimum of those background information items listed in paragraph (j)(1) of the OCSPP 850.2000 guideline.

(2) **Guideline deviations.** Provide a statement of the guideline or protocol followed. Include a description of any deviations from the test guideline or any occurrences which may have influenced the results of the test.

(3) **Test substance.**

(i) Identification of the test substance: common name, IUPAC and CAS names, CAS number, structural formula, source, lot or batch number, chemical state or form of the test substance, and its purity (*i.e.* for pesticides, the identity and concentration of active ingredient(s)), radiolabeling if any, location of label(s), and radiopurity.

(ii) Storage conditions of the test chemical or test substance and stability of the test chemical or test substance under storage conditions if stored prior to use.

(iii) Methods of preparation of the test substance and the treatment concentrations used in the range-finding and definitive tests.

(iv) If a vehicle (solvent) is used to prepare the test diet provide: the name and source of the vehicle, the nominal concentration(s) of the test substance in the vehicle in stock solutions, and the vehicle concentration(s) used in the test substance diet and control diet.

(v) Storage conditions and stability of the test substance concentration in the treated diets throughout the duration of the study.

(4) **Test organisms.**

(i) Scientific and common name of the species test.

(ii) Age of birds (in months) at test initiation.

(iii) History of the birds used in the test: source, name of supplier, batch or lot number.

(iv) Description of housing for stock birds during pre-test observation and acclimation: type, size, material of pens, and loading (number of birds per pen).

(v) Description and documentation of any acclimation performed.

(vi) Description of pre-test observation period: date, duration, feeding regime, and environmental conditions (temperature, humidity, photoperiod, light intensity).

(vii) Results of health observations during pre-test observation period: occurrence and rate of mortality, occurrence, type and rate of sickness and injuries.

(5) **Test system and conditions.** Provide a description of the test system and conditions used in the definitive test, and any preliminary range-finding test.

(i) Date of test initiation, duration of test, and for adults the duration of initiation, photostimulation, and egg-laying phases and withdrawal phase if applicable.

(ii) Description of housing for adult birds used in range-finding and definitive tests: type, size, and material of pen.

(iii) Detailed description of the basal diet: source, composition, diluents (if used), supplements (if used), and a nutrient analysis of the basal diet.

(iv) Exposure regime to test diet and watering during exposure phases and withdrawal phase if appropriate.

(v) Frequency and method of determining food consumption per pen and any repellancy or food palatability issues.

(vi) Description of any medication given during the test: type, frequency, and an explanation of how it was administered and justification of why it was given.

(vii) The number of test substance and control treatments and the nominal test substance dietary concentrations (in mg a.i./kg-diet for pesticides).

(viii) Statement about the selection basis or source for the highest dietary test substance concentration such as the highest expected environmental concentration based on the highest pesticide label rate (in lbs a.i./acre), accounting for multiple applications that occur in a season, or actual highest measured test substance residue levels.

(ix) Number of replicate test pens used per test substance concentration and control treatments and number of birds of each sex per pen at test initiation.

(x) Description of arrangement of pens to prevent cross-contamination.

(xi) Methods of assigning birds to test pens, including method of randomization.

(xii) Method of marking all adult and hatchling birds and eggs.

(xiii) Methods and frequency of environmental monitoring performed during the initiation, photostimulation, and laying phases for air temperature, humidity, and light intensity.

(xiv) Test substance dietary residue sampling methods and frequency to document homogeneity and stability of the test substance in the diet throughout the study duration.

(xv) Egg collection interval from pens.

(xvi) Description of storage housing for eggs: type, size, placement in bags if applicable.

(xvii) Interval for removing eggs for egg shell thickness determinations and the number of eggs used per pen for egg shell thickness

(xviii) Duration of an egg in storage and frequency of setting eggs.

(xix) Frequency of turning eggs in storage and during incubation.

(xx) Times (days) eggs were candled and the purpose for which they were candled.

(xxi) Time (days) when eggs were transferred to a hatcher.

(xxii) Time (days) when hatched eggs were removed and counted.

(xxiii) Methods and frequency of environmental monitoring performed during the storage and incubation of eggs: air temperature and humidity.

(xxiv) For the definitive test, a description of all analytical procedures and additionally the accuracy of the method, method detection limit, and limit of quantification.

(xxv) Frequency, duration, and types of observations conducted on adult birds during initial, photostimulation, and laying phases and withdrawal phase if appropriate, and on eggs, and hatchlings.

(6) **Results.**

(i) Environmental monitoring data results (air temperature, humidity and light intensity) for adults in the initial, photostimulation, and laying phases and the withdrawal phase if appropriate; for hatchlings; and for chicks or ducklings in tabular form (provide raw data for measurements not made on a continuous basis), and descriptive statistics (mean, standard deviation, minimum, maximum).

(ii) Egg storage and incubation temperature, and relative humidity results (provide raw data for measurements not made on a continuous basis), and descriptive statistics (mean, standard deviation, minimum, and maximum).

(iii) Mean, standard error, minimum and maximum test substance concentrations (mg a.i./kg-diet for pesticides) by observation time and treatment level and an evaluation of the results in terms of documenting the stability of the test substance concentration in the diet throughout the duration of the study.

(iv) Tabulation of number of eggs laid, number of irregular or abnormal eggs, number of cracked eggs, number of eggs set, number of viable embryos, number of live embryos, number of normal hatchlings, and number of 14-day old survivors by treatment, observation week, and pen. Descriptive statistics (mean, standard deviation, standard error, 95% confidence interval, median, first and third quartiles, minimum, maximum) and plot of these effects (mean, median, first and third quartiles, minimum, maximum) by treatment level. Percent inhibition calculations as compared to control. Provide sufficient raw data for performance of an independent statistical analysis.

(v) A tabulation of percentage of eggs set of eggs laid, viable embryos of eggs set, live 18-day old embryos, 14-day old survivors of eggs laid, and 14-day old survivors of hatchlings. Descriptive statistics (mean, standard deviation, standard error, 95% confidence interval, median, first and third quartiles, minimum, maximum), plot of these effects (mean, median, first and third, minimum, maximum) by treatment level, and a tabulation of the %I. Provide sufficient raw data for performance of an independent statistical analysis.

(vi) A tabulation of egg shell thickness by treatment and pen and calculated average egg shell thickness per pen. Descriptive statistics (mean, standard deviation, standard error, 95% confidence limits, median, first and third quartiles, minimum, maximum) of average egg shell thickness, plot of the mean, median, first and third quartiles, minimum and maximum average egg shell-thickness by

treatment level, and a tabulation of the %I. Provide sufficient raw data for performance of an independent statistical analysis.

(vii) Description of any signs of intoxication or any other abnormal behavior in adult birds, including time of onset, duration, severity (including death), and numbers affected (including accidental deaths or injuries), and any remissions.

(viii) List of the number of the birds for which necropsies were performed and details of the necropsies.

(ix) For young birds, description of any signs of toxicosis or any other abnormal behavior, including time of onset, duration, severity (including death), and numbers affected (including accidental deaths or injuries), and any remissions during first and second week after hatching.

(x) Tabulation of body weights of adult male and female birds by pen and observation time (provide raw data) and the body weight gain for each sex by pen between test initiation and termination. Descriptive statistics of body weight gain for each sex (mean, standard deviation, standard error, 95% confidence limits, median, first and third quartiles, minimum, and maximum) by treatment level, plot of mean, median, first and third quartiles, minimum and maximum, and a tabulation of the %I. Tabulation and plot of the mean change in adult body weight by observation interval and treatment level during the course of the test prior to the onset of laying (day 0 to 14, 14 to 28, 28 to 42, *etc*.).

(xi) Tabulation of body weights of surviving young at 14 days of age by pen, and observation time (provide raw data), descriptive statistics (mean, standard deviation, minimum, and maximum), plot of mean, median, first and third quartiles, minimum and maximum, and a tabulation of the %I.

(xii) For adult birds and chicks or ducklings, the food consumption data results and the calculated food consumption per bird by observation time and pen in tabular form. Descriptive statistics (mean, standard error) and plots of food consumption per bird by treatment level and observation time to evaluate patterns in food consumption throughout the duration of the study. Descriptive statistics (mean, standard deviation, median, first and third quartiles, minimum, and maximum) and plots of these for total food consumption per bird by treatment level.

(xiii) Description of statistical method(s) used for NOEC and LOEC determination, including software package, and the basis for the choice of method.

(j) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Fletcher, J.S., J.E. Nellesson and T.G. Pfleeger. 1994. Literature review and evaluation of the EPA food-chain (Kenaga) nomogram, an instrument for estimating

pesticide residues on plants. Environmental Toxicology and Chemistry 13(9): 1383-1391.

(2) Hawkins, P., Morton, D.B., Cameron, D., Cuthill, I., Francis, R., Freire, R., Gosler, A., Healy, S., Hudson, A., Inglis, I., Jones, A., Kirkwood, J., Lawton, M., Monaghan, P., Sherwin, C., and Townsend, P., 2001. Laboratory birds: refinements in husbandry and procedures. Laboratory Animals. 35(1**)**: 1-163. October, 2001

(3) Hoerger, F. and E.E. Kenaga. 1972. Pesticide residues on plants: correlation of representative data as a basis for estimation of their magnitude in the environment. In F. Coulston and F. Corte, eds. Environmental Quality and Safety: Chemistry, Toxicology and Technology, Volume 1. Georg Theime Publishers, Stuttgart, Germany, pp 9-28.

(4) National Research Council of the National Academies. 2010. Guide for the Care and Use of Laboratory Animals. The National Academies Press, Washington, D.C.

(5) Organization for Economic Co-operation and Development, 1984. TG-206, Avian Reproduction Test, adopted April 1984.

(6) U.S. Environmental Protection Agency, 1982. Pesticide Assessment Guidelines Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Office of Pesticide and Toxic Substances, Washington, D.C. EPA-540/9-82-024, October 1982.

(7) U.S. Environmental Protection Agency, 1994. Pesticide Reregistration Rejection Rate Analysis: Ecological Effects. Office of Prevention, Pesticide and Toxic Substances, Washington, D.C. EPA 738-R-94-035, December, 1994.

(8) U.S. Department of Agriculture, 1979. National Poultry Improvement Plan, Report No. 2., in Directory of Participants Handling Waterfowl, Exhibition Poultry, and Game Birds. USDA, Science and Education Administration, Beltsville, MD 20705.