

**OECD GUIDELINE FOR TESTING OF CHEMICALS**

**PROPOSAL FOR A NEW TEST GUIDELINE**

**Avian Reproduction Toxicity Test in the Japanese Quail or Northern Bobwhite**

**INTRODUCTION**

1. A SETAC/OECD Workshop on Avian toxicity Testing held in 1994 in Pensacola (United-States) recognised that chemical effects upon avian reproduction are of high ecological importance and that the detection of such effects should be a high priority for regulatory bodies (1). The workshop identified the need for a new avian reproduction test to be developed alongside a revised OECD Guideline 206 (2)
2. The purpose of this guideline is to describe a laboratory testing procedure that can be used to assess the effects of pesticides and other chemicals upon avian health and reproduction. Data collected from this test may be used in the assessment of risks to birds exposed to pesticides and other chemicals under field conditions.
3. The test is designed for dietary or drinking water exposure. This one-generation reproduction test monitors adult health and reproductive parameters in the Japanese quail (*Coturnix japonica*) or the Northern bobwhite (*Colinus virginianus*) over a six-week chemical exposure period (3)(4)(5)(6)(7). The test may not be appropriate for bioaccumulating substances because a steady state concentration may not be achieved sufficiently early in the test.
4. The number of 14-day old survivors per hen per day is the integrated biological endpoint of this test. However, it is also necessary to evaluate the individual steps that lead to the integrated endpoint. Such intermediate endpoints include egg production, fertility, embryonic mortality, hatchability and chick survivorship. The intermediate endpoints may enhance both the statistical power and biological impact of the test to detect effects and provide information on mechanisms of toxicity that may adversely affect overall reproductive success. In addition to the productivity parameters listed above, parental toxicity data, eggshell quality data and chick health data are also evaluated.
5. Increased statistical power to detect the effects of treatments upon birds in studies conducted under this guideline is obtained by using pairs of birds that are proven breeders. An analysis of treatment effects that corrects measurements made during the treatment period for pre-treatment differences in the birds may be performed.

## **PRINCIPLE OF THE TEST**

6. Tests performed under this guideline begin with birds that are already in egg production. The treatment period, during which birds are fed a diet containing the test substance, lasts for a period of at least six weeks. Parameters for adult toxicity and for reproduction are evaluated by making statistical comparisons between treated groups and the control group. Reproductive data are collected from all birds prior to the start of treatment to ensure that only proven breeders are used in the test and so that pre-treatment differences can be compensated for when comparing treatment and control groups. The no observed effect concentration (NOEC) and possibly the low observed effect concentration (LOEC) are determined for adult health and reproductive parameters.

## **VALIDITY CRITERIA**

7. The test substance concentration in the diet to which birds are exposed should be satisfactorily maintained and reported. Losses of 20% or less relative to initial concentrations are generally considered acceptable. Higher rates of loss must be investigated and explained.

8. When possible, all mortalities in the control group should be explained. At least sixteen breeding pairs of control birds that have produced eggs must be available at the end of the 6-week treatment period.

## **DESCRIPTION OF THE METHOD**

### **Test animals**

9. The test species is either the Japanese quail (*Coturnix japonica*) or the Northern bobwhite (*Colinus virginianus*). Birds should be approaching their first breeding season.

10. Japanese quail generally start laying eggs at or above six weeks of age. Some strains require photostimulation to initiate egg laying, while others do not. Once egg laying has begun, it will take about two to three weeks for birds to reach full egg production. Depending on the strain, eggs will be fertile when birds are about eight to ten weeks of age. If aggression is a problem, it is advisable to hold the birds on a short-day photoperiod before pairing them.

11. Northern bobwhite should be at least six months old at the onset of egg laying. Birds should be kept on a short-day photoperiod for a minimum of ten weeks prior to the anticipated onset of egg production in order to synchronize egg laying. Birds should be photostimulated approximately three weeks prior to the desired onset of egg laying. Once egg laying has begun, it will take about five weeks for birds to reach full egg production.

12. Birds used in the test should appear healthy and be free of abnormalities or injuries that may affect test results. Birds should not receive any medications beginning one week prior to the start of the pre-treatment period and continuing until the test is terminated. Age of birds and husbandry procedures should be such that successful fertilisation has taken place before the start of pre-treatment. Parental mortality during the last two weeks of acclimation should not exceed 3%. All birds used in a test should be from the same hatch.

## **Housing**

13. Temperature, ventilation and light controlled facilities are needed throughout the test. Recommended ventilation is about 8 to 15 air changes per hour. See table 1 for recommended housing conditions.

14. Artificial lighting should approximate the daylight visual spectrum and be automatically controlled. The photoperiod for adult birds, from the start of acclimation onwards, and chicks is 16 or 17 hours of light and 7 or 8 hours of darkness. Birds should be exposed to light intensity of at least 10 lux, measured at the level of the feeder. The light intensity is dictated by the amount required for daily observations. Light intensity that is too high will encourage aggressive behaviour.

15. Incubators and hatchers, preferably with automatic temperature and humidity controls and an egg-turning device, are necessary. In addition, suitable equipment is required to maintain stored eggs within the temperature and humidity ranges specified in Table 3.

16. Suitable pens of appropriate size for adults and for chicks are required. Wire pens with slanting floors and egg-catchers or other measures to prevent breakage of eggs, are recommended for adults. Pens for both adults and chicks should preferably be of stainless or galvanised steel or other inert materials. When using wire meshing for the floors, the wire mesh should be of a size sufficient to prevent foot injury but large enough to allow excreta to drop through. Measures should be taken to minimise spillage of diet, e.g. by covering the food troughs with a wire grid.

17. Adult birds should be housed in pairs (one male/one female). The parental cages should be distributed in such a way that cross-contamination and positional effects are avoided. Pen mate aggression can be a problem in both Japanese quail and Northern bobwhite. Measures must be taken to minimise injuries, stress and mortality resulting from such aggression. An example might be to separate those pairs and place them together often enough to maintain fertility of eggs. When male and female quails are housed separately, pairs should be placed together at least for half an hour per day for five days a week.

18. For rearing chicks, the use of rearing pens with thermostatically controlled warm compartments or cages, which are free of draughts and have a radiator (e.g. ceramics), are recommended. Sufficient space for feeding and drinking must be provided, especially during the first week after hatching, to reduce the problem of the weaker animals not getting access to the feed and water facilities. The optimal number of chicks per pen depends on pen size.

19. Guidance for housing conditions is provided in Table 1.

## **Feeding**

20. Diet and drinking water are provided *ad libitum*. The diet supplied should be described and should meet the specific nutrient requirements of both Japanese quail and Northern bobwhite. The caloric and water content of the diet must be reported. If the same diet is used for chicks and adults, extra calcium should be added for adult diet. Prior to and during the pre-treatment period, adult birds are fed a basal diet. During the treatment period, birds are fed basal diet mixed with the test substance at specified test concentrations.

21. Guidance for recommended nutritional values is provided in Table 2.

Table 1: Housing conditions

Age (week)	Temperature (°C)	Relative humidity (%)	Minimum floor area (cm <sup>2</sup> /bird)
1	35-38	40-80	50
2	30-35	40-80	75
3 - 4	23-27	40-80	100
> 4	16-27	40-80	625

Note: The acceptability of housing conditions of adults will be evaluated on the basis of results of reproductive performance.

Table 2: Recommended nutritional values

Nutrient	Adults	Chicks
	Recommended range (%)	Recommended range (%)
Crude protein	27 to 29	27 to 30
Crude fibre	3.5 to 5.0	3.0 to 6.0
Crude fat	2.5 to 7.0	5.5 to 7.5
Calcium	2.6 to 3.6	0.75 to 1.2
Phosphorous	0.9 to 1.1	0.6 to 1.0

## **PROCEDURE**

### **Acclimation, pre-treatment and treatment**

#### **Japanese quail**

22. An acclimation period of at least two weeks is required prior to the start of the test. If necessary, birds must be photostimulated either before or at the start of the acclimation period. Onset of egg laying can take place during the acclimation period. The test begins with the start of the pre-treatment period. The birds should be at peak egg production at the start of the pre-treatment period.

#### **Northern bobwhite**

23. Photostimulation should take place, prior to acclimation, about six weeks prior to the scheduled start of the pre-treatment period. This is to ensure that the treatment period will fall in the six weeks period in which egg production is least variable (approximately 8-14 weeks after photostimulation which corresponds approximately with weeks 5 to 10 of egg production).

24. The acclimation period should begin at least two weeks prior to the start of the study. The test begins with the start of the pre-treatment period. The onset of egg-laying will occur during the acclimation period.

**Japanese quail and northern bobwhite**

25. Prior to the start of treatment, birds are weighed and randomly allocated to pens and treatment groups. The number of pairs allocated to each group (for example 20) should be sufficient to ensure that there are 16 breeding pairs in the control group at the end of the treatment period. Pairs allocated to the study should have laid at least one egg during the last week prior to start of pre-treatment.

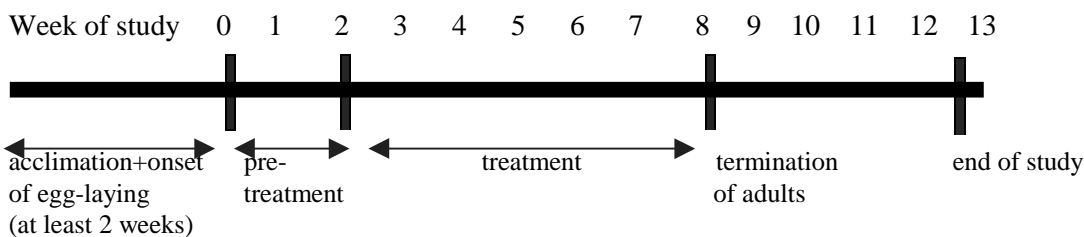
26. The pre-treatment period lasts two weeks. The purpose of the pre-treatment period is: (i) to ensure that only proven egg layers are used in the test and (ii) to compensate for pre-treatment differences when comparing treatment and control groups. Birds allocated to the study that are unlikely to meet the validity criteria of the test or are showing pen mate aggression, may still be replaced during this period. The replacement pairs must have been kept under the same conditions and the same data collected as from the pairs allocated to the study. There should preferably be no mortality among control pairs. If mortality leads to a loss of more than 15% of control pairs between start of pre-treatment and the end of the treatment period this must lead to serious reconsideration of husbandry conditions.

27. During the treatment period, the birds are exposed to the test substance for a period of six weeks. At least three groups each receive a different test concentration. The control group is fed the same diet and carrier without the test substance. Diets kept under the same environmental conditions must also be included. Generally the test substance will be mixed with the feed, although, in specific cases, it may be desirable to expose the birds via the drinking water. At the end of the treatment period all adult birds are necropsied.

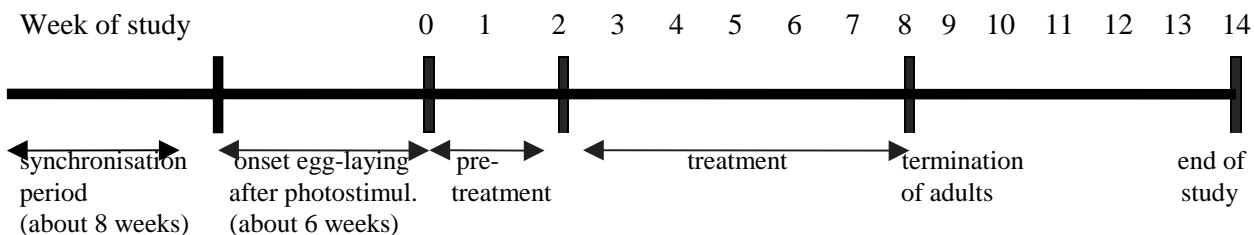
**Time line**

28. A time line is given for each species to help clarify the test design.

**Japanese Quail**



**Northern Bobwhite**



### **Dietary concentrations**

29. The concentration of the test substance in the diet is expressed as weight of the test substance per unit weight of diet with a specified water content: mg/kg of diet (or weight of test substance per weight of water when birds are exposed through drinking water). The concentration should also be expressed as mg/kg body weight per bird per day.

30. Dietary concentrations of the test substance should be chosen on the basis of toxicological data from a range-finding study or other preceding avian tests. Information may also be gained from tests with rodents or other mammals. If multiple test concentrations are to be tested, at least three different dosage groups should be tested and the highest concentration should be chosen at a level that is expected to reveal significant effects on adult health or reproductive parameters. However the highest dose administered should not cause mortality or other severe signs of parental toxicity that will preclude the evaluation of reproductive parameters. If no significant reproduction effects are expected at lower test concentrations, then the highest concentration to be tested should be the expected concentration of the chemical in the environment, with the addition of a safety factor where appropriate. If this concentration is at or lower than 1000 mg/kg diet, there is no need to test above 1000 mg/kg diet. The lowest concentration tested should be chosen so that no effects are seen on adult health or reproductive parameters. Any intermediate concentrations generally should be geometrically spaced between the highest and lowest doses.

31. If a limit test does not reveal any toxic effects when tested according to procedures described in this guideline, no additional concentrations need to be tested. The limit concentration is the expected concentration of the chemical in the environment, with the addition of a safety factor where appropriate. If this concentration is at or lower than 1000 mg/kg diet, there is no need to test above it.

32. If desired, a range finding test can be performed before initiating the main study, to aid in establishing test concentrations and to evaluate any potential avoidance effects.

### **Preparation and monitoring of the test diet**

33. To prepare the test diets, the appropriate amounts of test substance are mixed ~~directly~~ into the diet. The mixing method should be developed so as to obtain a homogeneous distribution of test substance in the diet. The use of a premix is advisable.

34. If necessary, a vehicle of negligible toxicity (e.g. food or corn oil, water, etc.) may be used to ensure a uniform distribution. Acetone may be used to dissolve a test substance, provided it is allowed to evaporate before feeding the diet to the birds. The amount of vehicle used should be as low as possible and should not exceed two per cent by weight of the diet. When used, a constant amount of vehicle should be added to each test group and the control group diet, in order to keep the caloric value and moisture content of the diet equal between dosage groups.

35. The frequency of diet preparation should be chosen so that degradation or volatilisation of the test substance in the diet does not allow the actual concentration to fall below 80% relative to initial concentration. The frequency of diet renewal should not be more than once a day and not less than once a week. All feed should be discarded from the feeders, before fresh feed is supplied to the birds. It may be desirable to keep all feed in freezing condition until use.

36. Stability analysis under typical conditions of the test, must be performed prior to the start of the test or in parallel with the range-finding study. Stability data from previous (mammalian) feeding studies may be used as guidance, if available. Stability should be verified during the main reproduction test. In

order to check stability of the test substance under test conditions, feed should be sampled and analysed from the feed hoppers at the end of the first feeding period, i.e. before diet in the feeding bowls is renewed, and again at the end of the last feeding period. A sufficient number of samples should be taken to account for variability.

37. Homogeneity of the test substance in the diet may be evaluated prior to the test or samples for homogeneity measurements may be taken at the first mix prepared for the study.

38. In order to verify test concentrations, samples of diets fed to the birds should be taken every time new diet is mixed during the treatment period to allow measurement of the actual concentration of the test substance. At least those samples taken from the beginning and towards the end of the treatment period should be analysed.

### **Measurements and observations**

#### **Adult birds**

39. During the treatment period the parental birds are observed daily to detect any overt signs of toxicity or other clinical signs. The following observations are to be performed on adults during the study:

- Toxic signs and health conditions should be evaluated at least once daily, during the acclimation, stabilisation, pre-treatment and treatment periods. Observations should include mortality and clinical signs of toxicity such as lethargy, depression, wing droop, ruffled feathers, lacrimation, etc. Any injuries sustained and subsequent treatment should also be recorded.
- Food consumption (per pair) should be recorded at least weekly, during pre-treatment and treatment, as often as food is replaced in the feeders. Any apparent food spillage should be noted.
- Body weights should be determined at least at the start of pre-treatment, at the start of treatment and at the end of the treatment period. There should be no significant difference within sexes in mean body weight between test groups at the start of the pre-treatment period. If there is a significant difference between test groups, birds should be again randomised.

#### **Pathology**

40. All adults that survive to the end of the treatment period are killed as humanely as possible (e.g. by means of CO<sub>2</sub> asphyxiation). All adult birds are to undergo necropsy and gross pathology assessment. The wet weight of the liver, spleen and testes are recorded as soon as possible after death. Adult birds that die or are killed during the course of the treatment period, will be subjected to the same procedures.

41. Birds that are in severe distress will be killed *in extremis*. If one member of a pair dies or is killed *in extremis* during the treatment period, the other member of the pair is killed as well.

#### **Offspring - Eggs**

42. Prior to start of pre-treatment, egg production of all pair of birds available for the study (including potential replacements) should be recorded. These data will be used to exclude pairs that do not lay eggs, from the study.

43. During the pre-treatment period and treatment period, all eggs, with the exception of those that are cracked, broken or abnormal or used for eggshell measurements, are set, artificially incubated and allowed to hatch. All offspring are maintained on untreated diet until 14 days after hatching.

44. Eggs are collected at least once daily, numbered according to pen of origin and stored; broken eggs are numbered, recorded and then discarded. The total number of eggs should be accounted for at the end of the study.

45. The eggs are stored in a cold storage facility, for a maximum of one week, prior to setting in the incubator (see Table 3). Cool storage prevents embryo development and aids in synchronising the development of embryos of eggs laid during that week. Before placing the eggs in the incubator, they are candled to check for abnormalities and fine cracks that can be identified only by candling. After removing all cracked and abnormal eggs, the remaining eggs are equilibrated to room temperature and set in the incubator. All cracked and abnormal eggs should be recorded.

Table 3: Conditions for egg storage, incubation and hatching

	Temperature (°C)	Relative humidity (%)	Turning
Storage	13-16	55-75	optional
Incubation	37.5-37.8	50-70	yes
Hatching	37-37.5	70-75	no

Note: Optimal temperatures and humidity can vary depending on type of incubator and strain of birds. The acceptability of incubation conditions should be evaluated on the basis of performance.

46. During the pre-treatment and treatment periods one egg per pen is collected from odd numbered pens in odd numbered weeks and from even numbered pens during even numbered weeks. These eggs are used for measurements of eggshell thickness or eggshell strength. Eggshell strength is measured using a strength tester. The egg is placed on its side on the test stand so that the compression head will contact the egg at the equator. To determine eggshell thickness, each egg will be cut open around the equator and washed out; subsequently the shells are left to dry with the membrane intact for at least 48 hours at room temperature. Shell thickness is measured at a minimum of four points around the girth using a calibrated micrometer. Eggshells are measured to at least 0.01 mm and the mean value calculated per egg.

***Remark: Both eggshell strength and eggshell thickness will be recorded as part of the validation process, after which a decision will be made which parameter to choose.***

47. Incubation is best performed when eggs are set in an incubator with temperature and humidity control and an automatic turning device. From the second day of incubation onwards, eggs should be turned at least three times per day. If turned by hand, eggs should be turned an odd number of times a day.

48. Fertility and embryo viability are checked by removing the eggs from the incubator and candling them (see table 4). This is done after approximately 8 days for Japanese quail or 11 days for Northern bobwhite. All eggs that appear to contain live embryos are placed back into the incubator. Those eggs that do not appear to contain a live embryo, are opened up and examined under a dissecting microscope, in order to distinguish between infertility and early embryonic death. Fertility, infertility, viability and

embryonic death are recorded. Approximately 2 or 4 days before hatching for Japanese quail and Northern bobwhite, respectively, the eggs are candled again for viability of embryos; all live embryos are transferred to a hatcher and recorded as late viable embryos. Those embryos that are dead are discarded and recorded as late embryonic dead. During candling, eggs should not be allowed to cool to room temperature, since this may delay embryonic development. After hatching, chicks should be dry before they are taken out of the hatcher. Those chicks that have not hatched within approximately 24 hours of the majority of chicks hatching, should be considered unhatched. No assistance should be given to chicks during hatching.

**Table 4:** Approximate time-points during incubation (after n days of incubation)

Species	Candling for fertility and viability (days)	Transfer to hatcher and candling (days)	Hatching (days)
Japanese Quail	8	15-16	17-18
Northern Bobwhite	11	20-21	24-25

**Remark:** *There is still some debate within the working group whether or not a distinction should be made between infertility and early embryonic death by opening up the eggs at first candling. Some argue that this information is essential for risk assessment, because it is the only endpoint that might address male fertility problems; others argue that the information on recording embryonic mortality has no value for risk assessment. All appear to agree that discerning embryonic mortality from infertility can only be done by opening up the eggs. The second candling is generally considered less important from a data collection point of view, but may be useful to avoid placing dead embryos in the hatcher. It is proposed to perform candling as described in paragraph 45 during the validation and to evaluate the usefulness and practicality of the process after the validation.*

**Chicks**

49. After hatching, chicks are identified and weighed individually or by pen of origin. They may be housed together, in groups of approximately equal number, by week and preferably by treatment group. Chicks are held for 14 days and observed daily for clinical signs. After 14 days the chicks are weighed again and killed as humanely as possible. Chicks which are in severe distress will be killed *in extremis*.

50. Typical control values for reproductive parameters in Japanese quail and Northern bobwhite are provided in Annex 2. If control values do not meet these values, this must lead to serious reconsideration of the test procedure and husbandry conditions.

**DATA COLLECTION AND TREATMENT OF RESULTS**

**Data collection**

51. The following data are collected during the study:

**Adults :**

- Mortality per bird
- Food consumption per pair, at least per week
- Body weights per bird per sex
- Clinical observations per bird
- Pathological findings per bird
- Organ weights (liver, spleen and testes) per bird

#### **Reproductive parameters (per parental pen per week)**

- Eggs laid
- Eggs cracked; eggs broken
- Egg abnormalities
- Eggs set
- Eggshell thickness or eggshell strength
- Eggs fertile
- Embryos viable
- Normal hatchlings
- Abnormal hatchlings
- Clinical signs of toxicity, abnormalities and mortality
- 14-day old surviving chicks
- Chick body weight at hatching and 14 days after hatching

52. If a pair is removed during the study due to death, injury, etc., data from all preceding weeks are reported. If data are used from a pen in which a mortality occurred or a bird was injured, this should be justified.

#### **Treatment of results**

53. Numerical data should be presented in tabular form, separating clearly the pre-treatment and treatment periods and the weeks in which the data were collected.

54. Measurements of endpoints made on adult birds and reproductive parameters will be evaluated by comparing values obtained from birds in treated groups with values obtained from control birds. Available methods for statistical analysis are listed and explained in ref (9) (the cited report by Duncan MacLeod report will be updated).

55. In addition for reproductive parameters pre-treatment differences may be compensated for when comparing treatment and control groups during or at the end of the treatment period. Methods that compare individual performance pre and post dose using a covariate analysis are provided in (10).

56. It must be noted that under this guideline, where exposure to the test substance starts after egg-laying has begun, an effect may occur in the course of the six-week period after one or more gamete cycles have taken place. If, in evaluating the results of this study, there appears to be delayed toxic responses, then averaging the results from all the treatment weeks may lead to a substantial reduction in the power of the test to detect effects. In such cases the results should be evaluated in one or two week increments.

57. A "no observed effect concentration" (NOEC) expressed in mg/kg diet and mg/kg body weight per day should be determined for all health and reproductive parameters evaluated. The mg/kg/day value

should be presented as the mean and range for each treatment group.

### **Test report**

58. The final report should include the following information:

Test substance:

- identification, including chemical name;
- batch or lot number;
- degree of purity;
- chemical stability under the conditions of the test;
- volatility.

Test species:

- name of species tested (scientific name), strain or origin, source and age of birds at start of pre-treatment (in weeks if possible).

Test conditions:

- housing conditions (type, size and material of pens for adults and for chicks, additional floor covering, adjustments made to pen floors to facilitate egg collection and prevent breakage, temperature, humidity, ventilation, illumination intensity and photoperiod, water supply); any changes to these during the test; measures taken to minimise food spillage;
- description of the untreated diet, manufacturer, composition, caloric content of diet and carrier, results of periodically performed contaminant and nutrient analysis of diet and drinking water contaminant analysis;
- test groups (number of concentrations used, nominal concentrations); details of type of carrier used and concentration as percentage of diet; test substance concentrations must be reported as mg/kg diet and as mg/kg body weight.
- specific analytical method used to determine the concentrations of test substance in the diet as well as actual values of homogeneity, stability and accuracy of preparation in diet under test conditions, as recorded in the study;
- description of the kind and frequency of the procedure used to prepare the test diets, description of the manner of administering the test diets, storage conditions;
- description of acclimation, stabilisation and pre-treatment procedures, and method of assigning pairs to test groups, arrangement of pens; number of pairs per dose group; measures taken, if any, to reduce pen-mate aggression.
- frequency, duration and methods of observation;
- information on the period and conditions of storage of eggs and on the incubation method;
- method of marking all birds and eggs.

Results:

- Data on adult birds:
  - \* description of incidence of mortality, indicating the number of dead animals and the time of death during the test;
  - \* description of all clinical signs of toxicity and other abnormal behaviour, including time of onset, duration and severity, and the number of affected birds per test group and in the

- control group; any injuries sustained and subsequent treatment
  - \* macroscopic pathological findings and organ weights and, if conducted, results of histopathological investigations;
  - \* mean body weights of males and females per pen at start of pre-treatment, at start of treatment and at the end of the test; individual weights of the birds that died or were killed during the test;
  - \* weekly food consumption per test group during the pre-treatment and treatment periods and extrapolated mean food consumption per pair; and expressed as mg/kg body weight per bird per day; an indication of any apparent food spillage.
  - \* results of range finding test (if conducted);
  - \* lowest-observed-effect concentration (LOEC), if available;
  - \* no observed effect concentration (NOEC).
- At least the following data on reproduction during pre-treatment and treatment periods must be reported:
    - \* number of eggs laid per hen and per day
    - \* all eggs should be traceable to their pen of origin;
    - \* all egg abnormalities should be described
    - \* number of eggs set and the percentage eggs set related to eggs laid;
    - \* numbers of eggs cracked and the percentage eggs cracked related to eggs laid;
    - \* number of broken eggs
    - \* eggshell thickness or eggshell strength measurements;
    - \* number of fertile eggs and the percentage fertility related to eggs set;
    - \* number of early viable embryos and the percentage viability related to fertile eggs;
    - \* number of late viable embryos and the percentage related to fertile eggs;
    - \* number of normal hatchlings as percentage of egg set;
    - \* number of normal hatchlings as percentage of fertile eggs;
    - \* number of normal hatchlings and the percentage related to total number of hatchlings;
    - \* number of 14-day old chicks per hen and per day;
    - \* number of 14-day old chicks as a percentage of (normal) hatchlings;
    - \* number of 14-day old chicks as a percentage of eggs set;
    - \* number of 14-day old chicks as a percentage of fertile eggs;
    - \* mean body weights of chicks on the day of hatching and after 14 days per pen, per test group;
    - \* description of abnormal behaviour of chicks, of severe birth defects and their general state of health, during the first 14 days after hatching, clinical signs of toxicity;
    - \* observations of hatchling mortality;
    - \* NOEC (and LOEC) per parameter that was evaluated statistically.
  - A description of statistical methods used in the analysis of data.

## **LITERATURE**

- (1) Report of the SETAC/OECD Workshop on Avian Toxicity testing. (1996). Environment Health and Safety Publication - Series on Testing and Assessment: No. 5. OECD/GD(96)166, Paris.
- (2) OECD Guidelines for the Testing of Chemicals (1993). Section 2 - Effect on Biotic Systems: Test Guideline 206: Avian Reproduction Test (adopted April 1984).

- (3) Leopold, A., Springer, T., Beavers, J. and Jaber, M. (1997) Avian Reproduction Testing in Northern Bobwhite - An evaluation of novel elements in the revised guidelines. Posters presented at the 18<sup>th</sup> annual meeting of SETAC, (November 1997) and at the 8th annual meeting of SETAC-Europe in Bordeaux, April 1998.
- (4) Schlatterer, B. et al. (1993). Effects of bis(tri-n-butyltin)oxide in Japanese quail exposed during egg laying period: an interlaboratory comparison study. *Arch. Environ. Contam. Toxicol.* 24: 440-448.
- (5) Baus, G; Krauskopf, S; Ebert, E. and Zok, S. (1999) Report from HMR Deutschland GmbH. Analysis of Avian Reproductive Toxicology Studies with the Japanese Quail - Some Statistical Power Examinations.
- (6) Solecki, R., Faqi, A.S., Pfeil, R. and Hilbig, V. (1996). Effects of methyl parathion on reproduction in the Japanese quail. *Bull. Environ. Contam. Toxicol.* 57: 901-908.
- (7) Mineau P.; Boersma, D.C. and Collins, B. (1994). An Analysis of Avian Reproduction Studies Submitted for Pesticide Registration. *Ecotoxicol. and Environ. Safety* 29: 304-329.
- (8) OECD Guidance document on the Classification of Substances and Mixtures based on Aquatic Environmental Hazards. Environmental Health and Safety Publication. Series on Testing and Assessment No X (to be published in 2000). ??? Still needed?
- (9) Macleod, D.A. Statistical analysis methods for avian reproduction experiments. Technical report series No. 211, Canadian Wildlife Service, 1994.
- (10) Springer, TA, Collins, BT and Baus, G. (1999). Statistical Power of Tests in the Avian Reproduction Study: A Summary and Interpretation of Available Information. In press.

## ANNEX 1

### Definitions

For the purpose of this Guideline the following definitions are used:

NOEC (No Observed Effect Concentration) is the highest concentration tested of a chemical, which produces no observable effects on the adult birds nor on any of the reproductive parameters or the offspring.

LOEC (Lowest Observed Effect Concentration) is the lowest concentration of a chemical tested, which produces an observed effect in the adult birds, in one or more reproductive parameters or on the offspring.

Acclimation: period of physiological and behavioural adaptation to environmental conditions (e.g. housing and diet) associated with the test procedure.

Photostimulation: Change of light regime to stimulate the start of egg-laying.

Hatch: birds of the same age and from the same breeding population.

Hatch rate : number of viable chicks at the end of incubation as a percentage of eggs fertile.

Fertility: number of eggs with embryonic development as a percentage of eggs set as observed by candling/opening up of eggs for macroscopic observation/ opening up of eggs for microscopic observation.

Viability: embryos that are considered to be alive when observed through candling.

Early viability: embryos that are considered to be alive when observed through candling on approximately day 8 and day 11 of incubation for Japanese quail and northern bobwhite respectively.

Late viability: embryos that are considered to be alive when observed through candling on approximately day 15 and day 20 of incubation for Japanese quail and Northern bobwhite respectively.

Early embryonic death: Embryos that die prior to the first candling of viable embryos (about 8/11 days after start incubation).

Late embryonic death: Embryos that die between the first and second candling of viable embryos (about 15/20 days after start incubation)

**ANNEXE 2**

**Typical values for reproductive parameters in  
Japanese Quail and Northern Bobwhite**

	Japanese Quail	Northern Bobwhite
Number of eggs laid per hen per day	0.66 - 0.89	0.40 - 0.81
Percentage of cracked/broken eggs	0 to 10 %	0 to 6 %
Viability (percentage fertile, live embryos of eggs set)	85 to 96 %	72 to 98%
Hatchability (percentage hatching of fertile eggs)	70 to 98 %	70 to 98 %
Percentage hatchlings that survive to 14 days	85 to 97%	69 to 98 %
Mean number of 14-day old survivors per hen per day	0.34 to 0.71	0.24 to 0.38
Eggshell thickness (mm)	0.19 to 0.22	0.20 - 0.25