Data Evaluation Report on the reproductive effects of Pyroxsulam (XDE 742) to avian species Bobwhite Quail (*Colinus virginianus*)

PMRA Submission Number 2006-4727 EPA MRID Number 469084-21 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.6.3.1; 9.6.3.2; 9.6.3.3
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.1.4
	EPA Guideline:	71-4a (850.2300)

Test material: XDE-742 **Purity (%): 98%**

Common name: Pyroxsulam

Chemical name:

IUPAC: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide

CAS name: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide

CAS No.: 422556-08-9

Synonyms: XDE-742/BAS 770 H/X666742

Primary Reviewer: David McAdam **Date:** 08/12/2006

Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

P. Murphy for D. McAdam 22/02/08

Secondary Reviewers: Jack Holland (DEWHA) **Date:** 21/12/2006

22/2/08

T Steeger 4/13/08

Thomas Steeger, Ph.D., Senior Biologist **Date:** 15/01/2007

Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Barbara Martinovic PMRA EAD **Date:** 22/01/2007

Jennifer Lanno for Barbara Martinovic 05/03/08

Company Code: DWE

Active Code: JUA

Use Site Category: 13 and 14

EPA PC Code: 108702

CITATION: Stafford, J. M. 2005. XDE-742: Reproductive Toxicity Test with the Northern Bobwhite Quail (*Colinus virginianus*). Springborn Smithers Laboratories, 2900 Quakenbush Rd. Snow Camp, North Carolina 27349 and 790 Main Street, Wareham, Massachusetts 02571-1037, Study No. 12550.4113. Dow AgroSciences, unpublished report No. 040032, 23 September 2005.

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EXECUTIVE SUMMARY:

The one-generation reproductive toxicity of pyroxsulam to groups of 18 pairs of 29-week old northern Bobwhite quail (*Colinus virginianus*) was assessed over approximately 26 weeks. The pre-egg laying exposure was 15 weeks and the egg-laying exposure was 11 weeks. Pairs were housed in cages (82 x 40 x 26 cm) and kept at 15-29°C, 26-79% RH, and a 7 hour light cycle until photostimulation, and then changed to a 17 hour light cycle. Eggs were collected daily and incubated at 37°C, 50-56% humidity until day 21 at which time they were transferred to the hatcher. Eggs hatched around day 23. Offspring were housed in poultry brooders (91 x 81 x 25 cm. Pyroxsulam was administered to the birds in the diet at 0 (control), 250, 500 and 1000 mg/kg diet (nominal). Observations of parental mortality and sublethal effects were made daily; body weight and food consumption were made at weeks 0, 2, 4, 6, 8, 10 and 26 (study termination). Reproductive effects included number of eggs laid, cracked, egg weight, eggshell thickness, numbers of fertile 11 day old embryos, viable 18 day old embryos, and hatching success. Observations of hatchling mortality were made at day 14, and weight were made at days 0 and 14.

The **reproductive NOEC/NOAEC of pyroxsulam to northern Bobwhite quail was determined to be 1000 mg/kg diet.** The LOEC/LOAEC was determined to be >1000 mg/kg diet.

No treatment related mortality was observed. No significant differences were detected in any of the reproductive performance variables when compared to the control. Significant adult body weight reductions were detected in any treatment group means compared to the control mean for adult birds during the treated feed phase. A significant difference in weekly average food consumption was detected at 2, 15 and 16 weeks between the 250 mg/kg group and control, and at 15 weeks between the 500 mg/kg group and control. Since these differences were not consistent throughout the exposure, and not observed at the highest treatment level, 1000 mg/kg feed, they were not considered biologically relevant. In addition, the reduction in food consumption did not lead to effects on body weight.

Four birds developed mild emprosthotonos (a form of tetanic spasm in which the head and feet are brought forward and the body is rendered tense), which was not dose related. The condition did not seem to otherwise affect their behaviour, their ability to eat or drink, or their reproductive output. One female in the 250 mg/kg diet group was found dead during the study (in week 25 of study). No previous abnormal symptoms had been recorded, nor were any unusual observations made during the post-mortem examination on this bird. The mate was euthanized according to standard procedure.

Since the abnormalities observed did not occur in a pattern corresponding to dose or other abnormal pattern among the groups, they were not considered attributable to the test substance. Also, since the postmortem observations made on the one adult mortality were within normal limits, the mortality was not considered attributable to the test substance.

Results Synopsis

Test Organism Size/Age:	29 weeks 5 days at experimental start; 204.6 g (mean weight; male 206.6 g, female 202.5* g), range 180-237 g.
NOEC/NOAEC:	1000 mg/kg diet (nominal); 1142 mg/kg diet (mean measured)
LOEC/LOAEC:	>1000 mg/kg diet (nominal); >1142 mg/kg diet (mean measured)
Endpoint(s) Affected:	None

*mean weight based on grand mean of treatment means at test initiation.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

Organization for Economical Cooperation and Development (OECD). 1984. OECD Guidelines for Testing of Chemicals, 206, Avian Reproductive Toxicity Test. 10 pp. OECD. 1997. OECD Principles of Good Laboratory Practice. Paris, France., as revised in 1997.

U.S. EPA. 40 CFR, Part 158. Data Requirements for Registration. Federal Insecticide, Fungicide and Rodenticide Act. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

United States Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms.* Subsection 71-4. U.S. EPA, Office of Pesticide Programs, October 1982.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.2300. Avian Reproduction Test. "Public Draft". EPA 712-C-96-141. April 1996. U.S. Environmental Protection Agency, Washington, D.C.

Deviations from Guidelines:

On 14 January 2005, a sensor in the environmental chamber malfunctioned, causing the temperature to fall out of range and below freezing. Eggs in the chamber at the time had been collected from 10 to 13 January 2005, representing the first four days of eggs collected for set 9. All eggs collected on 10 January 2004 were frozen, therefore would not hatch, and so were separated out for eggshell thickness measurements. The remaining eggs were transferred from the primary chamber to a back-up egg cooler. Subsequent egg collections representing set 9 were also stored in the back-up egg cooler while maintenance was conducted on the environmental chamber. All salvageable eggs from set 9 were set and incubated as recorded in the study data. Eggs were maintained at an average of 12°C and 62% relative humidity in the back-up egg cooler. Since not all eggs from set 9 could be incubated, an extra set of eggs (set 11) was added to the study. The environmental chamber was repaired by 18 January 2005.

Additional statistical analyses were conducted on the offspring data, with set 9 data excluded, to determine any potential effect that could have been caused by the small amount of eggs salvaged and set for set 9.

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

A. MATERIALS:

1. Test Material Pyroxsulam (XDE-742)

Description: Solid, white beige
Lot No./Batch No. : E0952-52-01 (ID: TSN 103826)
Purity: 98% active constituent
Stability of Compound under Test Conditions: Determined to be stable under ambient conditions for 23 days.
Storage Conditions of Test Chemicals: Stored at ambient temperature in the original container in the dark. The test compound in the diet was determined to be stable for 23 days.

Physicochemical properties of Pyroxsulam

Parameter	Values	Comments
Water solubility at 20°C	pH 4 0.0164 g/L pH 6 0.0626 g/L pH 7 3.2 g/L pH 9 13.7 g/L	Turner (2004a) Turner (2004a) Turner (2004a) Turner (2004a)
Vapor pressure	1 X 10^{-7} Pa	Madsen (2003 and 2006)
UV absorption	NA	
pKa	4.670	Cathie (2004)
Kow	pH 4 0.097 pH 7 0.024 pH 9 12.10	Turner (2004b) Turner (2004b) Turner (2004b)

2. Test organism:

Species: Bobwhite quail (*Colinus virginianus*)
Age at study initiation: 29 weeks and 5 days old at experimental start. Quail were older than recommended.
Weight at study initiation: 204.6 g (179.9-237.2 g)
Source: Stevenson Game Bird Farm, Riverside, Texas

B. STUDY DESIGN:

1. Experimental Conditions

Range-finding Study: A pilot study was conducted at Springborn Smithers at nominal pyroxsulam concentrations of 0, 437, 726, 1205 and 2000 mg/kg diet. The 25 pairs of birds were fed their respective treatment diets for four weeks under low light conditions. At the end of the four week period, light levels were increased (photostimulation) to induce egg production. After approximately seven days of photostimulation, eggs were collected, counted and weighed for four weeks. Eggs were not set. Adult birds were euthanized after four weeks of egg production.

No mortalities were observed in any of the treatment levels during the pilot study. One female bird in the 437 mg ac/kg feed group was observed to have wounds on the head (possible self-inflicted) on 17 May 2004, subsequently antiseptic salve was applied to the top of the head. This bird recovered, as confirmed by subsequent normal observations.

Measurements of feed consumption and body weights of adult birds were determined as outlined in the definitive study. In addition, egg production and egg weights were recorded. Based on these results and consultation with the Study Sponsor, nominal pyroxsulam concentrations of 250, 500 and 1000 mg/kg diet were selected for the definitive exposure.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	**Details**	**Remarks**
		Criteria
Acclimation Period:	14 day duration, 20 to 25°C, relative humidity of 57 to 81%,	Acclimation meets OECD and US EPA 850.2300 Guidelines.

Parameter	Details	Remarks
		Criteria
Conditions (same as test or not):	photoperiod of 7 hours light, 17 hours dark. Same as test conditions.	*EPA recommends 2-3 week health observation period prior to selection of birds for treatment. Birds must be generally healthy without excess mortality. Sickness, injuries or mortality should be noted. Feeding should be ad libitum* *OECD requires acclimation of at least 2 weeks*
Feeding:	Basal diet, *ad libitum*, daily using Purina® Layena Game Bird Ration (Lot 063AUG3104).	
Health (any mortality observed):	All animals appeared healthy upon test initiation.	
Test duration	Approximately 26 weeks	Meets Guideline conditions.
Pre-laying exposure:	15 weeks (pre-photostimulation and 5 weeks pre egg laying photostimulation)	*Pre-laying exposure duration* *EPA /OECD require at least 10 weeks prior to the onset of egg-laying.* *Exposure duration with egg-laying* *EPA requires at least 10 weeks.* *Withdrawal period* *EPA requires if reduced reproduction is evident, a withdrawal period of up to 3 weeks should be added to the test phase.*
Egg-laying exposure:	11 weeks	
Withdrawal period, if used:	NA	
Pen (for parental and offspring)		Floor space is 3280 cm^2 per cage, corresponding to 1640 cm^2 per bird. EPA recommendation is for at least 5000 cm^2 of floor space per bird.
Size: Construction materials:	Adult: 82 X 40 X 26 cm; polycarbonate-coated galvanized welded-wire mesh. Hatchling: 91.4 X 81.3 X 25.4 cm; galvanized welded-wire mesh	*EPA requirements:* *Pens* *Adequate room and arranged to prevent cross contamination* *Materials* *Nontoxic material and nonbinding material, such as galvanized steel.* *Number* *At least 5 replicate pens are required for mallards housed in groups of 7. For other arrangements, at least 12 pens are required, but considerably more may be needed if birds are kept in pairs. Chicks are to be housed according to parental grouping.*
Number:	72	
Number of birds per pen (male:female)	2; 1 male and 1 female	Meets Guideline conditions. *EPA requires one male and 1 female per pen. For Bobwhite, 1 male and 2 females is acceptable. For mallard, 2 males and 5 females is acceptable.*

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td><u>Number of pens per group/treatment</u>

Negative control:

Solvent control:

Treated:</td><td>

18

No solvent control required.

16</td><td>Based on distribution of weights reported in Appendix 6 (of the study report).

Meets Guideline conditions

EPA/OECD require at least 12 pens, but considerably more if birds are kept in pairs. At least 16 is strongly recommended.</td></tr>
<tr><td><u>Test concentrations (mg pyroxsulam/kg diet)</u>

Nominal:

Measured:</td><td>

Control, 250, 500 and 1000

Control, 253, 499 and 1142</td><td>Meets Guideline conditions.

EPA requires at least two concentrations other than the control; three or more are recommended. The highest test concentrations should show a significant effect or be at or above the actual or expected field residue level.
OECD requires measured concentration in diet should be at least 80% of nominal</td></tr>
<tr><td>EEC/maximum labeled field residue anticipated and source of information:</td><td>15 g ac/ha (based on the proposed Australian label "GF-1674* Herbicide", containing 30 g/L pyroxsulam to be applied at a rate of 500 mL product/ha.)

Using the Plfeeger et al. modified Kenaga nomogram approach; the EECs are:

<table><tr><th>Environmental Compartment</th><th>Concentration fresh weight mg ac/kg feed</th></tr><tr><td>short grass</td><td>3.2</td></tr><tr><td>leaves and leafy crops</td><td>1.8</td></tr><tr><td>forage crops</td><td>1.8</td></tr><tr><td>small insects</td><td>1.8</td></tr><tr><td>grain/long grass</td><td>1.5</td></tr><tr><td>pods with seeds</td><td>0.20</td></tr><tr><td>large insects</td><td>0.20</td></tr><tr><td>fruit</td><td>0.20</td></tr></table></td><td>

EPA requires the highest test concentrations should show a significant effect or be at or above the actual or expected field residue level. The source [i.e., maximum label rate (in lb ai/A & ppm), label registration no., label date, and site should be cited]</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
Solvent/vehicle, if used Type: Amount:	Acetone (200 mL; 0.9% v/wt) Corn oil (360 mL; 1.5% v/wt)	Includes 20 mL of acetone as rinsate. *EPA /OECD require corn oil or other appropriate vehicle and not more than 2% of diet by weight*
Was detailed description and nutrient analysis of the basal diet provided (Yes/No)	Yes	 *EPA requires a commercial breeder feed (or its equivalent) that is appropriate for the test species.*
Preparation of test diet	For each of the 22-kg aliquots (three aliquots) for each level, the appropriate amount of test substance was weighed into a beaker, mixed with 180 mL of acetone for approximately ten minutes, then 360-mL of corn oil was added to the mixture. The resulting solution was sprinkled onto the feed aliquot as it was being mixed. The beaker was rinsed with approximately 10 mL of acetone, twice, and added to the feed. The three 22-kg aliquots were combined and mixed for approximately five additional minutes. Acetone was evaporated during the mixing process. The resulting diet mixed for each treatment was 66 kg.	 The study states that the acetone evaporated during mixing. *A premix containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it must be completely evaporated prior to feeding.*
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes	
Were concentrations in diet verified by chemical analysis (Yes/No)?	Yes	
Feeding and husbandry	During the test, the adult birds were fed the appropriate treatment and control diets for approximately 10 weeks prior to laying and exposure via the diet continued until the adult terminal sacrifice.	Meets Guideline conditions.

Parameter	Details	Remarks *Criteria*
Test conditions (pre-laying) Temperature: Relative humidity: Photoperiod and Light intensity:	15 to 29 °C 26 to 79% 7 hours light: 17 hours dark during first 10 weeks of treated Feed 15 footcandles for pre- photostimulation and 25 foot candles after photostimulation.	EPA requirements are that temperature and relative humidity should be controlled during the study. The wide range indicates that they weren't. Temperature and relatively humidity were lower than Guideline requirements. *Temperature:* *EPA: about 21°C (70°F)* *OECD: 22 5°C* *Relative humidity:* *EPA: about 55%* *OECD: 50-75%* *Lighting:* *EPA/OECD: first 8 weeks: 7 h per day* *Thereafter:* *EPA: 16-17 h per day.* *At least 6 footcandles at bird level* *OECD: 16-18 h per day*
Egg Collection and Incubation		
Egg collection and storage Collection interval: Storage temperature: Storage humidity: Storage period:	daily for 11 weeks 15.1 to 16.8°C 64.4 to 69% Up to one week	Following breakdown of the egg cooler, the temperature of eggs during storage in back-up egg cooler was 12°C rather than 16°C as required by Guidelines. This did not affect the study as an extra week of egg collection was added to the Guideline to give 10 weeks of egg laying. Apart from the mechanical breakdown, meets Guidelines conditions. *EPA requires eggs to be collected daily; egg storage temperature approximately 16°C (61°F); humidity approximately 65%.* *Collection interval: daily*
Were eggs candled for cracks prior to setting for incubation?	Yes	*EPA requires eggs to be candled on day 0*
Were eggs set weekly?	Yes	

Parameter	Details	Remarks *Criteria*
When candling was done for fertility?	Day 11	Meets Guideline conditions. *EPA requires:* *Bobwhite: approx. day 11* *mallard: approx. day 14* *OECD requires: 6-11 day*
When the eggs were transferred to the hatcher?	Weekly (after 21 days of incubation) Eggs were collected beginning week 15	*EPA requires:* *Bobwhite: day 21* *Mallard: day 23*
Hatching conditions Temperature: Humidity: Photoperiod:	36.8 to 37°C 72-76% 14 hours light: 10 hours dark upon hatch	Meets Guideline (850.2300 and OECD 206) conditions. *Temperature:* *EPA requires: 39°C (102°F)* *OECD requires: 37°C* *Humidity* *EPA requires: 70%* *OECD requires: 70-85%*
Day the hatched eggs were removed and counted	Day 23 and day 24	*EPA requires Bobwhite: day 24* *Mallard: day 27*
Were egg shells washed and dried for at least 48 hrs before measuring?	Yes	
Egg shell thickness No. of eggs used: Intervals: Mode of measurement:	All eggs newly laid on a single day Once every two weeks Digital micrometer	*EPA requires newly hatched eggs be collected at least once every two weeks. Thickness of the shell plus membrane should be measured to the nearest 0.01 mm; 3 - 4 measurements per shell.*
Reference chemical, if used Name: Concentration tested:	Not applicable	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured		
Parental: (mortality, body weight, mean feed consumption)	Observed daily: mortality, general condition, signs of toxicity, abnormal behaviour. Recorded: mortality and signs of morbidity, or symptoms of intoxication, body weight (7 times during study), feed consumption (weekly).	Meets Guideline conditions. *OECD requires that the mortality in the controls is not exceed 10% at the end of the test. The average number of 14 day-old survivors per pen in controls at least 14 and 12 for mallard and Bobwhite, respectively. OECD requires average egg shell thickness for control group 0.34 and 0.19 for mallard and Bobwhite, respectively* *EPA requires:* *body weight should be recorded at test initiation and a biweekly intervals up to week eight or up to the onset of egg laying and at termination.* $ *Eggs laid/pen* $ *Eggs cracked/pen* $ *Eggs set/pen* $ *Viable embryos/pen* $ *Live 3-week embryos/pen* $ *Normal hatchlings/pen* $ *14-day-old survivors/pen* $ *Weights of 14-day-old survivors (mean per pen)* $ *Egg shell thickness* $ *Food consumption (mean per pen)* $ *Initial and final body weight (mean per pen)*
Egg collection and subsequent development: (no. of eggs laid, no. of eggs cracked, shell thickness, no. of eggs set, no. of viable embryos, no. of live 3 week embryos, no. hatched, no. of 14-day survivors, average weight of 14-d old survivors, mortality, gross pathology, others)	Recorded: Number of eggs laid per cage (pair). Number of eggs cracked to number of eggs laid. Defective eggs of total laid per hen. Number of fertile eggs to number of eggs in incubator. Number of viable embryos to number of fertile eggs. Number of hatchlings to number viable embryos. Number of 14-day old survivors to number of eggs hatched. Hatchling body weights. 14-day old survivor body weights. Eggshell thickness.	
Indicate if the test material was regurgitated	No	
Observation intervals (for various parameters)	Adult body weight: just prior to test initiation, 2, 4, 6, 8, 10 weeks and at study termination. Egg collection: daily for 10 weeks. Shell thickness: every 2 weeks for 10 weeks.	*Body weights and food consumption must be measured at least biweekly.*

Parameter	Details	Remarks *Criteria*
	Embryo development: day 11. Embryo survival: day 21. Hatch weight: day of hatch. Survival weight: day 14.	
were raw data included?	Yes for parental data and reproductive data.	No raw data for temperature and relative humidity for adults, brooders, egg storage, incubator and hatcher. A summary table with range over the whole study only.

II. RESULTS AND DISCUSSION:

A. MORTALITY: No mortality in adult birds except for one female in the 250 ppm group, cage 81, was found dead on 22 January 2005. No previous abnormal symptoms had been recorded, nor were any unusual observations made during the post-mortem examination on this bird. The one mortality observed was not considered attributable to the test substance. The mate was euthanized according to standard procedures.

B. REPRODUCTIVE AND OTHER ENDPOINTS:

No significant adult body weight reductions were detected in any treatment group means compared to the control mean for adult birds during the treated feed phase. A significant difference in weekly average food consumption was detected at 2, 15, and 16 weeks between the 250 mg/kg feed group and the control and at 15 weeks between the 500 mg/kg feed group and the control. Since these differences were not consistent throughout the exposure, and not observed at the highest treatment level, 1000 mg/kg feed, they were not considered biologically relevant. No significant differences were detected in any of the reproductive performance variables analyzed when compared to the controls.

Table 3. Reproductive and Other Parameters

Parameter	Control	250 mg/kg diet	500 mg/kg diet	1000 mg/kg diet	NOEC/LOEC (mg/kg diet)
No. eggs laid/pen	1200	1243	1185	1281	1000/>1000
No. eggs laid/hen/day	0.87	0.90	0.85	0.92	1000/>1000
% of eggs cracked	7	10	3	8	1000/>1000
No. eggs set	1080	1116	1070	1160	1000/>1000
Shell thickness (mm SD)	0.200 (0.02)	0.201 (0.02)	0.203 (0.01)	0.199 (0.02)	1000/>1000
No. viable embryos	1024	1058	986	1023	1000/>1000
No. live (viable) 3-week embryos	1024	1058	986	1023	1000/>1000
No. of hatchling/hen	56.7	58.8	54.8	56.8	1000/>1000
No. of normal hatchlings	56.7	58.8	54.8	56.8	1000/>1000
Hatchling weight (g)	7.5	7.5	7.5	7.5	1000/>1000
# hatchlings per eggs incubated	0.93	0.92	0.89	0.85	1000/>1000
No. 14-day old survivors	992	1018	944	969	1000/>1000
14-day old survivors weight (g)	30.7	29.6	30.2	29.9	1000/>1000
Mean food consumption (g/bird/day)	17.9	17.3	17.7	18.2	1000/>1000
Weight of adult females at test initiation	201.7	199.9	200.6	207.9	1000/>1000
at onset of egg laying/other:	209.3	210.1	211.5	215.0	
test termination	251.4	252.6	251.3	256.1	1000/>1000
Weight of adult males at test initiation	204.5	208.5	203.4	210.0	1000/>1000
at onset of egg laying/other:	211.2	214.5	210.1	216.4	
test termination	223.7	222.7	225.0	225.2	1000/>1000

C. **REPORTED STATISTICS:** Statistical analyses were conducted to determine whether statistically significant ($p \leq 0.05$) mean differences existed between the control group and any of the treatment groups for adult weight, feed consumption, or reproductive variables. Analysis of reproduction parameters was conducted two ways: first with all 11 egg sets, and secondly with egg set 9 excluded. Data sets were first tested for normality using a Chi-square test and for homogeneity of variance using Levene's test. Proportional data were Arcsine or Anscombe Arcsine transformed. Normal and homogeneous data were analyzed by analysis of variance (ANOVA) and an appropriate pair-wise mean comparison or means separation test. Dunnett's test was used for data sets of equal size, and Bonferroni's test was used for data sets of unequal size. If the data set was not normal and/or not homogenous, they were analyzed with a non-parametric Steel's Many One-Rank (equal replicates) or Kruskal-Wallis (unequal replicates) test. All statistical tests were conducted with TOXSTAT® v3.5 (West and Gulley, 1996) software.

The endpoints statistically analyzed during the reproduction toxicity test included:

- Adult male body weight: seven intervals
- Adult female body weight: seven intervals
- Adult feed consumption: weekly totals for each of 26 weeks and overall total
- Number of eggs laid: sum of eggs laid per cage (pair)
- Number of eggs set into the incubator: sum of eggs per pair set in incubator
- Number of eggs cracked of number eggs laid: proportion of number of eggs
- cracked out of the total number of eggs laid
- Defective eggs of total laid per hen: proportion of number of defective eggs out of the total number of eggs laid, including any eggs broken during handling.
- Number of fertile eggs of number of eggs set in incubator: proportion of number of fertile eggs out of total number of eggs set
- Number of viable embryos of number of fertile eggs: proportion of number of viable embryos (live 3-week embryos) out of total number of fertile eggs
- Number of hatchlings of number viable embryos: proportion of number of eggs hatched out of total number of viable embryos
- Number of 14-day old survivors of number of eggs hatched: proportion of number of 14-day survivors out of total number of eggs hatched
- Hatchling body weights: individual hatchling weights taken at time of hatch, by cage of origin
- Mean 14-day old survivor body weights: individual 14-day survivor weights, by cage of origin
- Mean eggshell thickness: average of all eggshells measured by cage of origin.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER: Statistics not verified as there were no claims of statistical effects and visual examination of the body weight, feed consumption and reproduction variables (given above) indicated that there was no significant variation between control and dose groups nor was there any apparent dose response.

Statistical Method: NA, visual

NOEC/NOAEC: 1000 mg/kg feed nominal; 1142 mg ac/kg feed, mean measured.
LOEC/LOAEC: >1000 mg/kg feed nominal; >1142 mg ac/kg feed, mean measured.
Most Sensitive endpoint(s): None

E. STUDY DEFICIENCIES:
No major deficiencies were noted. Minor deficiencies noted were:

- Floor space was less than recommended;
- Temperature and relative humidity apparently not controlled during pre-laying;
- Temperature of eggs during storage in back-up egg cooler was 12°C rather than 16°C as required by Guidelines;
- The study reports deviations from protocol for temperature in brooding compartments (29.1-37.7°C during first week and 25-33°C in second week protocol requirements were 32-35 and 28-32°C for first and second week respectively) and very minor deviations for relative humidity in both incubator and hatcher (49-58% and 69-76% for incubator and hatcher respectively; protocol requirements were 50-55 and 75-76%

respectively).

These minor deviations from Guidelines and protocol requirements did not affect the acceptability study.

F. REVIEWER COMMENTS: The reviewer notes that apart from the breakdown of the egg cooler, the study was well conducted.
PMRA comment: Eggs hatched/eggs set were slightly lower (not significantly; p=0.148) due to an outlier for # eggs set for 500 ppm and # hatchlings for 1000 ppm.

G. CONCLUSIONS: The study is rated as acceptable.

NOEC/NOAEC: 1000 mg/kg feed nominal; 1142 mg ac/kg feed, mean measured.
LOEC/LOAEC: >1000 mg/kg feed nominal; >1142 mg ac/kg feed, mean measured.
Most Sensitive endpoint(s): None

No effect on reproductive parameters.

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