Data Evaluation Report on the reproductive effects of Pyroxsulam (XDE 742) to avian species mallard duck (*Anas platyrhynchos*)

PMRA Submission Number 2006-4727 EPA MRID Number 469084-20 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:	71-4b (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/X666742

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

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

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

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

22/2/08

T. Steeger 4/13/06

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:** 01/03/2007

Barbara Martinovic for Barbara Martinovic 05/03/08

Company Code: DWE [DM1]

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 mallard duck *(Anas platyrhynchos)*. Springborn Smithers Laboratories, Wareham, Massachusetts. Dow AgroSciences, unpublished report, Study No. 040130. 13th June 2005 [DM2].

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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:** 01/03/2007

Émilie Larivière (PMRA, EAD) **Date:** 10/9/2007

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EXECUTIVE SUMMARY:

The one-generation reproductive toxicity of pyroxsulam to groups of 64 pairs of 23 weeks old mallard duck (*Anas platyrhynchos*) was assessed over 22 weeks in accordance with the FIFRA Guideline 71-4, OPPTS 850.2300 and OECD Guideline 206. The pre-egg laying exposure was 10 weeks and the egg-laying exposure was 12 weeks. Pairs were housed in cages (76 x 83 x 44 cm) and kept at 15-26°C, 39-96% RH, and a 7 hour light cycle up through week 8, and then changed to a 17 hour light cycle. Eggs were collected daily and incubated at 37°C, 53 % humidity until day 23 at which time they were transferred to the hatcher. Eggs hatched around day 27. Offspring were housed in poultry brooders and maintained at 36-37°C, 65-76% humidity and a 14 hour light cycle. 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 22 (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 of weight were made at days 0 and 14.

No adult mortalities were observed during the study. Apart from the female birds' weights at test termination, no significant differences were found in adult body weight, mean food consumption or any other reproductive parameters for treatment groups compared with the control. With respect to the female birds' weights at test termination, the mean weight of the birds exposed to 1000 mg pyroxsulam/kg feed in the diet was identified as statistically significantly less than the mean weight of the controls. Consequently, the NOEC for adverse effect on female body weight is set at 500 mg pyroxsulam/kg feed (499 mg pyroxsulam/kg feed, mean measured).

The reproductive **NOEC observed during this study was 500 mg/kg feed** (based on weights of the day 14 ducklings and of the body weights of female ducks at test termination) Using mean measured concentrations, the NOEC/NOAEC was and the LOEC were-499 and 1142 mg/kg feed-respectively.

This toxicity study is classified as acceptable and is consistent with the guideline requirement for a mallard duck reproductive toxicity study.

Results Synopsis

Test Organism Size/Age:	23 weeks at experimental start, weight range of 853-1414 g
NOEC/NOAEC (nominal):	500 mg ac/kg diet
LOEC (nominal):	1000 mg ac/kg diet (95.2 mg ac/kg body weight/day)

LOEC (mean measured): 1142 mg ac/kg diet (108.7 mg ac/kg body weight/day)

Endpoint(s) Effected: Reduced body weight of 14 day old ducklings (4%) and adult females (7.5%) at 1000 mg/kg feed treatment level on termination.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

Springborn protocol "Reproductive toxicity test with mallard duck *(Anas platyrhynchos)*, following FIFRA Guideline 71-4, OPPTS 850.2300 and OECD 206 (Avian Reproduction Test), Springborn Smithers Laboratories Protocol No: 022304/FIFRA/OECD/OPPTS/Mallard/repro. The methods in the protocol were generally based on:

- *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms.* Subsection 71-4. U.S. EPA, Office of Pesticide Programs, October 1982;
- 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.; and
- Organization for Economical Cooperation and Development (OECD) Guidelines for Testing of Chemicals, 206, Avian Reproductive Toxicity Test.

Deviations from Guidelines:

No major deficiencies were noted. Minor deficiencies noted were as listed on page 16 of this DER.

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

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 dark. The test compound in the diet was determined to be stable for 23 days.

Physicochemical properties of XDE-742

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	<1X 10^{-7} Pa at 20°C	Madsen (2003)
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: Mallard duck (*Anas platyrhynchos*)
Age at study initiation: 23 weeks at experimental start. Ducks were younger than recommended, however, reproduction did not appear impacted by age.
Weight at study initiation: 1107.0 g (871.3-1297.3 g)
Source: Whistling Wings, Inc., Illinois

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. Two birds (one male, one female) had minor injuries. All birds 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, 17-22°C, relative humidity 72-94%	Guideline conditions were met.
Conditions (same as test or not): Feeding: Health (any mortality observed):	Same as test conditions. Basal diet, *ad libitum*, daily (Purina® Game Bird Flight Conditioner (Lot 063DEC2703 and 063JUN2804)). All animals appeared healthy upon test initiation.	*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*
Test duration	Approximately 30 weeks (includes 8 weeks for post-egg collection and final incubation	Guideline conditions were met.

Parameter	Details	Remarks
		Criteria
Pre-laying exposure: Egg-laying exposure: Withdrawal period, if used:	and hatching) 10 week pre-photostimulation 2 weeks pre-egg laying photostimulation 10 weeks NA	*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.*
Pen (for parental and offspring) Size: Construction materials: Number:	Adult: 76 X 83 X 44 cm; polycarbonate-coated galvanized welded-wire. Hatchling: 61 X 91 X 61 cm; galvanized welded wire 64	Floor space is 6308 cm^2 per cage, corresponding to 3154 cm^2 per bird. EPA recommendation is for at least 10 000 cm^2 of floor space per bird. *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 of birds per pen (male:female)	2; 1 male and 1 female	Guideline conditions were met. *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.*
Number of pens per group/treatment Negative control: Solvent control: Treated:	16 (no solvent control used)	Guideline conditions were met. *EPA/OECD require at least 12 pens, but considerably more if birds are kept in pairs. At least 16 is strongly recommended.*
Test concentrations (mg ai/kg diet)		Guideline conditions were met.

Parameter	Details	Remarks *Criteria*
Nominal: Measured:	Control, 250, 500 and 1000 Control, 253, 499 and 1142	*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*
EEC/maximum labeled field residue anticipated and source of information:	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: **Environmental Compartment** / **Concentration fresh weight mg ac/kg feed** **short grass** 3.2 **leaves and leafy crops** 1.8 **forage crops** 1.8 **small insects** 1.8 **grain/long grass** 1.5 **pods with seeds** 0.20 **large insects** 0.20 **fruit** 0.20	*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]*
Solvent/vehicle, if used Type: Amount:	Acetone 200 mL; (0.9% v/wt) Corn oil 360 mL; (1.6% v/wt)	Includes 20 mL of acetone as rinsate. Guideline conditions were met. *EPA /OECD require corn oil or other appropriate vehicle and not more than 2% of diet by weight*
Was detailed description and		

Parameter	Details	Remarks
		Criteria
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	Predetermined amount of basal diet (3 separate aliquots of 22 kg) was measured out for each dosing level. For each 22 kg aliquot for each level, the appropriate amount of test substance was weighed into each beaker. 180 mL acetone, 360 mL corn oil added to each beaker. 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	Feed and water were provided to adult birds, *ad libitum*, during acclimation and test period. Feed and water were provided to the hatchlings, *ad libitum*, during the rearing period. Test birds fed Purina® Layena® Game Bird Ration (Lot 063AUG3104, 063NOV0104 and 063NOV2904). Water was provided from a well.	
<u>Test conditions (pre-laying)</u> Temperature: Relative humidity: Photoperiod and Light intensity:	 15 to26 °C 36-96% 10 weeks with 7 hours light 17 hours dark and then changed to 17 hours of light thereafter	EPA requirements are that temperature and relative humidity should be controlled during the study. The wide range indicates that they weren't.

Parameter	Details	Remarks *Criteria*
	11 footcandles for pre-photostimulation and 13 footcandles after photostimulation.	*Temperature:* *EPA: about 21°C (70°F)* *OECD: 22-25C* *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:	Egg collection began during week 12. daily for 10 weeks 16 °C 65% Up to one week	Guideline conditions were met *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? Incubation conditions	Yes Temperature: approximately 37-38C Humidity: approximately 52-55%	*EPA requires eggs to be candled on day 0*
Were eggs set weekly?	Yes	
When candling was done for fertility?	Day 14 for embryo development and on day 23 for embryo survival	Guideline conditions were met. *EPA requires:* *bobwhite: approx. day 11* *mallard: approx. day 14* *OECD requires: 6-11 day*
When the eggs were transferred to the hatcher?	After 23 days of incubation, viable eggs moved to hatcher	*EPA requires:* *Bobwhite: day 21* *Mallard: day 23*
Hatching conditions Temperature: Humidity:	36-37°C (measured: 36.8 – 37.1°C)	OECD Guideline conditions were met. US Guideline 850.2300 requires 37.5°C for incubation.

Parameter	Details	Remarks
		Criteria
Photoperiod:	65 to 76% (measured: 65 – 76%) 14 hours light: 10 hours dark upon hatch	*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 27 post-incubation	Guideline conditions were met *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	Guideline conditions were met *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).	Guideline conditions were met. *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 test termination. Adult feed consumption daily. Egg collection: daily for 10 weeks. Shell thickness: every 2 weeks for 10 weeks.	Guideline conditions were met. *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 adult mortalities were observed during the study.

B. REPRODUCTIVE AND OTHER ENDPOINTS:

There were no statistical differences in adult feed consumption when weekly average feed consumption was compared among groups. Mean body weight among females in the 1000 mg/kg feed group were 7.5% lower than the controls at study termination, which was statistically significant and for males in the 1000 mg/kg feed group mean body weight was 6.16% lower, which wasn't statistically significant.

Statistically testing (Bonferroni's test) detected a significant difference in mean 14-day chick body weight between the 1000 mg/kg feed group and the control. Overall mean 14-day survivor weights in the 1000 mg/kg feed group, were 4.0% lower than that of the controls. There were no statistically significant reductions in any of the other reproductive parameters for any treatment level compared to the control.

Ten birds were found to have either regressed or underdeveloped gonads, possibly affecting their reproductive activity. Such conditions were present in on female in the control group, three females and one male in the 250 mg/kg feed group and three females in the 500 mg/kg feed group, and two females in the 1000 mg/kg feed group. These birds presented ovarian, follicular, and/or oviduct atresia. (i.e. there was an absence of a normal opening in the oviduct, or a failure of the oviduct to be tubular.)

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	956	856	932	966	1000/>1000
No. eggs laid/hen/day	0.85	0.76	0.83	0.86	1000/>1000
No. eggs cracked	13	13	10	14	1000/>1000
No. eggs set	867	779	840	870	1000/>1000
Shell thickness (mm SD)	0.338 (0.022)	0.347 (0.029)	0.336 (0.031)	0.338 (0.025)	1000/>1000
No. viable embryos	722	640	699	729	1000/>1000
No. of hatchling/hen	40	34.6	38.4	40.5	1000/>1000
No. of normal hatchlings	640	519	614	648	1000/>1000
Hatchling weight (g)	34.3	34.8	35.5	34.2	1000/>1000
No. 14-day old survivors	634	515	612	645	1000/>1000
14-day old survivors weight (g)	296.4	293.7	294.8	284.5*	500/1000
Mean food consumption (g/bird/day)	100.6	107.8	104.3	103.4	1000/>1000
Weight of adult females: at initiation	1005.1	1045.1	1048.7	1003.0	
at onset of egg laying	1004.2	1019.3	1035.1	1011.9	1000/>1000
at test termination:	1238.6	1215.1	1210.5	1146.1*	500/1000
Weight of adult males at initiation	1201.6	1183.3	1195.2	1174.9	
at onset of egg laying/	1185.6	1204.8	1174.9	1134.8	1000/>1000
at test termination	1248.9	1226.8	1222.7	1172.0	1000/>1000

*Statistically significant. Note that the study report (Table 5, page 36 of the study report) indicates that the weight of the female birds at onset of egg laying were statistically significantly different at the 1000 ppm level compared to the controls. This is considered to be an error.

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. Data sets were first tested for normality using a Chi-square test and for homogeneity of variance using Levene's test. Proportional data was 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 or Kruskal-Wallis test. All statistical tests were conducted with TOXSTAT® v3.5 (West and Gulley, 1996) software.

The endpoints statistically analysed 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 22 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: The mean 14-day old survivor body weights was analyzed by ANOVA, which showed a statistically significant effect, by F-test and use of the statistical package ToxCalc™ which showed that 500 mg/kg group was not statistically significant but the1000 mg/kg group was; mean 14-day chick body weight was 4% lower than controls.

There was a significant ($p<0.05$) difference in mean female body weight between the 1000 mg/kg diet treatment group and controls at test termination. Mean female weight was 7.5% lower than controls (see page 22 of this DER).

Table 5 (page 36 of the study report) indicates that the female mean adult body weight at 1000 ppm and at week 10 (start of photostimulation) was 1011.9 g with that value statistically significantly less than the control mean based on Dunnett's test where the t value calculated was 2.3199 and the T (critical) was 2.10.

However, Page 27 of the study report indicates that the statistically significant result was at test termination (Table 5 refers to this time as "Adult termination", which took place on 5 January 2005, 22 weeks after the study commenced on 3 August 2004).

The reviewer's statistical analysis of the relevant female body weight data confirmed that the statistically significant difference was at test termination, not at week 10 (pages 14 and 22 of this DER refer). Consequently, the Table 5 entry with respect to statistical significance at week 10 for the female birds is incorrect, the statistically significant body weight result is that for the adult female birds killed on 5 January 2005 after 22 weeks exposure to pyroxsulam at 1000 ppm in their diet.

Visual examination of the feed consumption and the remaining reproduction variables (given

above) indicated that there was no apparent dose response and no significant variation between control and dose groups.

Statistical Method: ANOVA and t-test

NOEC/NOAEC:	500 mg/kg feed nominal; 499 mg ac/kg feed
LOEC/LOAEC:	1000 mg/kg feed nominal; 1142 mg ac/kg feed
Most Sensitive endpoint(s):	14-day duckling body weight and female body weight at termination.

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 per-laying;
- The temperature in the incubator was slightly less (0.5 °C) than given in the US EPA 850.2300 Guideline.
- 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 were deviation from the US EPA (850.2300) and the OECD (206) Guidelines.
- A sensor malfunctioned for one day, which led to a severe temperature drop in the egg chamber. Therefore an extra set of eggs (11) was added to the study.

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

F. REVIEWER'S COMMENTS: The study is scientifically sound. Under the exposure conditions tested, 14-day survivor weight was significantly different (4% reduction) in the 1000 mg/kg diet treatment relative to controls. Adult female body weights were also significantly different (7.5% lower) in the 1000 mg/kg diet treatment at test termination relative to controls.

Although post-mortem examinations revealed several birds with regressed or underdeveloped gonads that possibly affected their reproductive activity, the frequency of these observations was low [albeit elevated compared to controls].

The PMRA's statistical evaluation of the body weight data for the ducklings and for the female birds at test termination resulted in no statistically significant differences in duckling weights or the weights of the adult ducks at test termination (see PMRA comments under "Reviewer's Statistical Analyses" below). However, the PMRA agrees to a NOEC of 500 mg/kg feed nominal. Biologically significant effects of this product on birds are not expected within the

range of concentrations tested.

G. CONCLUSIONS: The study is rated as acceptable. The following NOEC/NOAEC etc. have been based on the results of the ToxCalc™ statistical analyses of the weights of the female adult birds at test termination and the weights of the 14 day old ducklings which identified statistically significant differences at the 1000 ppm exposure level for these two parameters.

NOEC/NOAEC: 500 mg/kg feed nominal; 499 mg ac/kg feed
LOEC/LOAEC: 1000 mg/kg feed nominal; 1142 mg ac/kg feed
Most Sensitive endpoint(s): 14-day duckling body weight and female body weight at test termination.

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Reviewer's Statistical Analyses

Data for 14-day old ducklings.

0	250	500	1000
303.6	285.8	284.6	275.1
298.6	318.4	300.5	302.4
265.9	278.5	283.7	253.4
301.6	276.5	274.3	236.5
305.6	324.1	269.1	303.6
297.2	309.9	332.1	250.3
291.4	267.4	316.5	310.7
300.2	280.8	330.7	283.5
307.9	279.9	295.6	292.1
275.6	276.8	303.8	269.1
298.4	301.5	263	284.5
303.4	320.3	295.8	281.1
280.1	284.1	270	263.7
310.3	305.2	323.7	268.9
285.3		287.1	269.1
301.1		286.3	315.2

PMRA comment: A p value of 0.054 is borderline and should not be considered significant. However, the PMRA agrees to a NOEC of 500 mg/kg diet. Biologically significant effects are not expected within the range of concentrations tested.

ANOVA

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0	16	4726.2	295.3875	156.2918
250	14	4109.2	293.5143	362.4967
500	16	4716.8	294.8	477.8093
1000	16	4459.2	278.7	501.7173

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3033.083	3	1011.028	2.696106	0.054166	2.763552
Within Groups	21749.73	58	374.9954			
Total	24782.82	61				

Weights of the 14 days old mallard ducklings (ToxCalc™ analysis)

The weights of the 14 days old duckling from ducks and drakes exposed to pyroxsulam (and the control birds also) at various times in the study were analysed by use of the ToxCalc™ statistical package (v5.0.23j, © Tidepool Scientific Software). Bonferroni's test (1 tail, 0.05) was selected for the hypothesis testing because the data sets were of unequal size. The results are shown in the following ToxCalc™ output:

ToxCalc™ Analyses of the 14 days old ducklings' weights

Conc-ppm	1	2	3	4	5	6	7	8	9	10
B-Control	303.60	298.60	265.90	301.60	305.60	297.20	291.40	300.20	307.90	275.60
B-Control	298.40	303.40	280.10	310.30	285.30	301.10				
250	285.80	285.80	285.80	285.80	285.80	285.80	285.80	285.80	285.80	285.80
250	301.50	320.30	284.10	305.20						
500	284.60	300.50	283.70	274.30	269.10	332.10	316.50	330.70	295.60	303.80
500	263.00	295.80	270.00	323.70	287.10	286.30				
1000	275.10	302.40	253.40	236.50	303.60	250.30	310.70	283.50	292.10	269.10
1000	284.50	281.10	263.70	268.90	296.10	315.20				

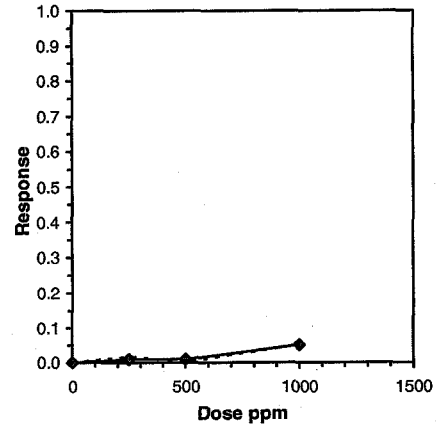
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
B-Control	295.39	1.0000	295.39	265.90	310.30	4.232	16				295.39	1.0000
250	290.65	0.9840	290.65	284.10	320.30	3.679	14	0.721	2.180	14.32	292.73	0.9910
500	294.80	0.9980	294.80	263.00	332.10	7.415	16	0.093	2.180	13.84	292.73	0.9910
*1000	280.39	0.9492	280.39	236.50	315.20	8.076	16	2.364	2.180	13.84	280.39	0.9492

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.7415	1.035	0.04905	0.04018
Bartlett's Test indicates equal variances (p = 0.01)	11.1219	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs B-Control	500	1000	707.107		13.835	0.04684	770.653	322.224	0.07779	3, 58

Linear Interpolation (200 Resamples)

Point	ppm	SD	95% CL	Skew
IC05	990.65			
IC10	>1000			
IC15	>1000			
IC20	>1000			
IC25	>1000			
IC40	>1000			
IC50	>1000			



The ToxCalc™ calculations were based on the reported day 14 weights of the ducklings in each

test group with there being 16 ducklings in the control, 500 and 1000 ppm parental groups and 14 ducklings in the 250 ppm parental exposure group.

The PMRA's One Way Analysis of Variance USING SIGMA STAT provided the following outcome with respect to duckling weights:

Chick weight

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.128)

Group	Name	N	Missing	Mean	Std Dev	SEM
control	16	0	295.388	12.502	3.125	
low	14	0	293.514	19.039	5.088	
med	16	0	294.800	21.859	5.465	
high	16	0	278.700	22.399	5.600	

Source of Variation	DF	SS	MS	F	P
Between Groups	3	3033.083	1011.028	2.696	**0.054**
Residual	58	21749.735	374.995		
Total	61	24782.817			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.054).

Power of performed test with alpha = 0.050: 0.412

The PMRA agrees with setting the NOEC at 500 mg/kg diet. Biologically significant effects on birds are not expected within the range of concentrations tested.

Changes in weights of the female adult mallard ducks

The weights of the female and male mallard ducks exposed to pyroxsulam (and the control birds also) at various times in the study were analysed by use of the ToxCalc™ statistical package. Dunnett's test (1 tail, 0.05) was selected for the hypothesis testing with normality of the distribution and equivalence of variance being determined by, respectively, the Kolmorogov D test and Bartlett's test. In all cases, the data were identified as normally distributed and having respective equalities of variance.

Based on the Dunnett's test results, the analyses showed that, with respect to initial weights and weights at the end of the photostimulation period, there were no statistically significantly lower mean values in the either the female or male birds exposed to pyroxsulam compared to their respective control means. The NOEC/NOAEC in each case was identified as 1000 mg pyroxsulam/kg of feed. The results for these comparisons are summarised in Table 4.

With respect to the weights at test termination, no statistically significant differences were found in the mean weights of the male birds exposed to the three pyroxsulam feed concentrations compared to the control mean. In contrast, the analysis identified the mean weight of the female ducks fed 1000 ppm in their diet as statistically significantly lower when compared to the control mean. The means for the 250 and 500 ppm exposed birds were not identified as statistically significant from the control mean. These results are also summarised in Table 4.

Table 4. Summary of the statistical analyses of the adult mallard ducks body weights at various times in the mallard duck reproduction study.

Bird weight at:	Bird's gender	t Statistic values for the 250, 500 and 1000 ppm exposures	1 tailed Critical t value	Dunnett's test F probability*	NOEC/NOAEC mg pyroxsulam/kg feed (ppm)
Test initiation	Female	-1.220, -1.327 & 0.062	2.100	0.340	1000
	Male	0.524, 0.175 & 0.725	2.100	0.885	1000
End of the photostimulation period	Female	-0.477, -0.981 & -0.243	2.100	0.789	1000
	Male	-0.456, 0.254 & 1.207	2.100	0.406	1000
Test termination	Female	0.588, 0.705 & 2.320**	2.100	0.127	500
	Male	0.551, 0.653 & 1.919	2.100	0.278	1000
Overall average weight	Female	-0.838, -0.962 & 0.504	2.100	0.411	1000
	Male	-0.020, 0.280 & 1.275	2.100	0.530	1000

*** 3, 60 degrees of freedom. ** Identified as statistically significantly less than the control mean.**

The ToxCalc™ output for the female ducks' weights at test termination was as follows:

ToxCalc™ Analysis of Adult (female) Duck Body Weights at Test Termination:
Individual body weights, means etc are shown in the following summary.

Conc-mg/Kg	1	2	3	4	5	6	7	8	9	10
D-Control	1189	1151.7	1248.3	1179.7	1259.2	1109	1299.7	1238.4	1266.9	1212
D-Control	1234.2	1306.5	1161	1279.8	1247.3	1434.1				
250	1316.4	1398	998.1	1322.5	1434.9	1263.7	1058.1	1097.8	1085.8	1332.8
250	1194.5	1221.4	1130.3	1295.3	1202.9	1089.3				
500	1142.7	1262	1174.6	1234	1123.5	1195.9	1201.3	1135.2	1321.7	1135.9
500	1117.9	1326.4	1141.8	1406.8	1233.3	1214.2				
1000	1002.9	992.9	1054.3	1151.8	1238	998.1	1283.6	1205.9	1417.5	1027.6
1000	1079	1241.3	1088.2	1001.7	1114.7	1439.5				

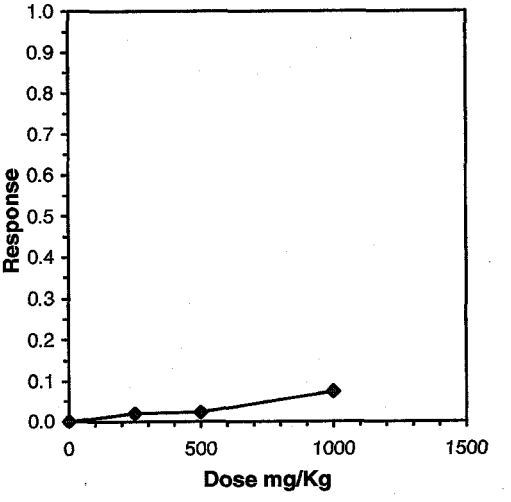
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/Kg	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1238.55	1.0000	1238.55	1109	1434.1	6.138	16				1238.55	1.0000
250	1215.113	0.9811	1215.113	998.1	1434.9	10.668	16	0.588	2.100	83.7203	1215.11	0.9811
500	1210.45	0.9773	1210.45	1117.9	1406.8	6.956	16	0.705	2.100	83.7203	1210.45	0.9773
*1000	1146.063	0.9253	1146.063	992.9	1439.5	12.701	16	2.320	2.100	83.7203	1146.06	0.9253

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.61121	1.035	0.57736	0.07102
Bartlett's Test indicates equal variances (p = 0.04)	8.51177	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	500	1000	707.107		83.7203	0.0676	25104.3	12714.9	0.12742	3, 60

Linear Interpolation (200 Resamples)

Point	mg/Kg	SD	95% CL	Skew
IC05	762.69			
IC10	>1000			
IC15	>1000			
IC20	>1000			
IC25	>1000			
IC40	>1000			
IC50	>1000			



The PMRA's One Way Analysis of Variance – USING SIGMA STAT provided the following outcome:

Female body weights at test termination – with all treatment groups

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.030)

Group Name	N	Missing	Mean	Std Dev	SEM
control	16	0	1238.550	76.018	19.005
low	16	0	1215.112	129.629	32.407
medium	16	0	1210.450	84.203	21.051
high	16	0	1146.063	145.558	36.390

Source of Variation	DF	SS	MS	F	P
Between Groups	3	75313.023	25104.341	1.974	**0.127**
Residual	60	762894.815	12714.914		
Total	63	838207.838			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.127).

Power of performed test with alpha = 0.050: 0.245

The PMRA agrees with setting the NOEC at 500 mg/kg feed. Biologically significant effects on birds are not expected within the range of concentrations tested.