This study is a corrected version of MRID 402613-02 @ 11/8/92

71-4

EPA ACC. NO. 403350-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Endosulfan 079402

2. **TEST MATERIAL:** Endosulfan Technical Substance, 96% (w/v) pure. Assigned Wildlife International Ltd. identification number WIL-1069.

3. **STUDY TYPE:** Avian Reproduction
   Species Tested: Mallard Duck (Anas platyrhynchos)

4. **CITATION:** Beaver, J. B., P. Frank and M. Jaber. 1987. Endosulfan Technical Substance (Code: HOE 002671 OI ZD95 0005) A One Generation Reproduction Study with the Mallard (Anas platyrhynchos). Proj. No. 125-137. Corrected Version of Previously Submitted Study Report MRID No. 40261302. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Hoechst Celanese Corp., North Somerville, NJ.

5. **REVIEWED BY:**

   Jeffrey L. Lincer, Ph.D.
   Eco-Analysts, Inc.
   Sarasota, Florida

   Signature:

   Date:

6. **APPROVED BY:**

   Michael L. Whitten, M.S.
   Wildlife Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: Michael L. Whitten

   Date: 3-1-89

   8-P-89

   Henry T. Craven, M.S
   Supervisor, EEB/HED
   USEPA

   Signature:

   Date: Henry T. Craven 8/18/89

7. **CONCLUSIONS:** Based on the data submitted, Endosulfan Technical Substance is expected to cause reproductive impairment for number of eggs laid, number of eggs set, embryo viability, hatching success and number of 14-day-old survivors at 60 ppm. The NOEL was 30 ppm, which according to the author, is equivalent to approximately 4 mg/kg of body weight. This study appears to be scientifically sound and meets the requirements for an avian reproductive study.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND: This is a corrected version of a previously submitted report (MRID No. 40261302).

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Pen-reared mallards that were apparently healthy and phenotypically indistinguishable from wild birds, were purchased from Whistling Wings, Hanover, Illinois. All birds were from the same hatch and were 19 weeks of age at test initiation (first day of exposure to test diet). The birds were approaching their first breeding season and had not been used in previous testing. At test initiation, all birds were examined for physical injuries and general health. Birds that did not appear healthy were discarded.

B. Test System:

Study Phases. The primary phases of the study and their approximate durations were:

1. Acclimation - 14 days.

2. Pre-photostimulation - 8 weeks.

3. Pre-egg laying (with photostimulation) - 2 weeks.

4. Egg laying - 8 weeks

5. Post-adult sacrifice (final incubation, hatching, and 14-day offspring rearing period) - 6 weeks.

Identification. Adult birds were identified by individual leg bands. All eggs laid during the study were marked with a permanent ink marking pen for identification. Hatchlings were identified by toe and web clipping so that the ducklings could be traced to their parental pen of origin.

Animal Diet. Basal diets for the adult birds and their offspring was formulated to Wildlife International Ltd. specifications by Agway, Inc. Water was supplied by the town of Easton. Feed and

water are analyzed periodically as per Wildlife International Ltd. SOP No. 4.7. Neither the adults nor offspring received any form of medication during the study.

The adults were fed a game bird ration formulated for breeding birds. During the study the birds received the appropriate test or control diet from study initiation to terminal sacrifice. Water and feed were provided ad libitum during acclimation and during the testing.

All offspring received a game bird ration formulated for young growing birds (identical to adult diet, but without the addition of limestone). The test substance was not mixed into the diet of the offspring. Feed and water were provided to the offspring ad libitum.

Housing and Environmental Conditions. The adult birds were housed indoors in batteries measuring approximately 75 x 90 x 45 cm high. The pens were constructed of galvanized wire grid and galvanized sheeting.

"Each pen was equipped with a feeder. Each week, sufficient feed for seven days was placed in feeders for each pen and presented to the birds. During the week additional feed was added to the feeders where excessive wastage by the birds made it necessary. Water supplied by nipple type waterers...."

"The birds were maintained in a separate study room which helped avoid excessive disturbances. The average temperature in the adult mallard study room during the course of the study was 18.6°C ± 1.5°C (SD) with an average relative humidity of 54%. The air handling system in the study room was designed to vent up to fifteen room air volumes every hour and replace it with fresh air."

"The photoperiod in the adult mallard study room was maintained by a time clock. The photoperiod for the first 8 weeks of the study was eight hours of light per day. The photoperiod was then increased during Week 9 to seventeen hours of light per day and was maintained at that length until sacrifice of adult birds. The birds received approximately 129 lux (12 footcandles) of

3

illumination throughout the study, provided by Chroma 50 fluorescent lights which closely approximate noon-day sunlight...."

Hatchlings were placed in batteries of brooding pens manufactured by Beacon Manufacturing. Each pen measured approximately 72 x 90 x 24 cm high. The external walls and ceilings of each pen were constructed of galvanized wire mesh and galvanized sheeting. Floors were of galvanized wire mesh. Thermostats in the brooding compartment of each pen were set to maintain a temperature of approximately 38°C from the time of hatching until the birds were 5 to 7 days of age. Hatchlings were then maintained at ambient room temperature of 21.5°C ± 1.9°C (SD). The photoperiod for the hatchlings was maintained by a time clock at 16 hours of light per day.

"Housing and husbandry practices were conducted so as to adhere to the "Guide for the Care and Use of Laboratory Animals," NIH Publications No. 85-23, 1985."

Incubation and Hatching. Eggs were collected daily and stored in a cold room at 12.4°C ± 1.8°C (SD) and approximately 69% relative humidity. All eggs were washed to reduce the possibility of pathogen contamination before storing them in the cold room. Eggs were washed in a commercial egg washer (Kuhl Egg Washer) with a chlorine based detergent. Water in the washer was warmed to approximately 46°C (115°F). The eggs were placed in the wash water and soaked for approximately 15 seconds. The washer's circulation motor was then turned on for approximately 3 minutes. The eggs were removed from the washer, allowed to cool to approximately room temperature and rinsed with fresh water. The eggs were then ready for storage in the cold room.

"Eggs were set for incubation on a weekly basis. The eggs were placed in the incubator where the temperature was maintained at 37.5°C ± 0.1°C (SD) with a wet bulb temperature of 29.4°C ± 0.5°C (SD) (relative humidity of approximately 54%). The incubator was equipped with a pulsator fan and blades that produced a mild breathing air movement...designed to eliminate intracabinet temperature and humidity variations during incubation. In order to prevent adhesion of the

embryo to the shell membrane, the incubator was also equipped with an automatic egg rotation device, designed to rotate the eggs from 50° off of vertical in one direction to 50° off of vertical in the opposite direction (total arc of rotation is 100°) each hour through Day 24 of incubation. The eggs were transferred to the hatcher on Day 24. Eggs were not rotated in the hatcher. The temperature in the hatcher was 37.1°C ± 0.4°C (SD) and the wet bulb temperature was raised from 31.8°C ± 1.1°C (SD) (relative humidity of 67%)."

C. Dosage: "Test diets were prepared by mixing Endosulfan Technical Substance into a pre-mix which was used for weekly preparation of the final diet. Control diet and four test concentrations (7.5, 15, 30, & 60 ppm) were prepared weekly beginning on October 9, 1986 and presented to the birds on Thursday of each week, except for Christmas week when feed was presented to the birds on Wednesday. When necessary during the study, additional feed was prepared. Dietary concentrations were adjusted for purity of the test material and are presented as ppm of active ingredient."

Samples of control and each of the test diets were collected, immediately following preparation, to determine homogeneity. Similarly, samples from each group were collected for Weeks 1, 2, 3, 4, 8, 12 and 16 to be analyzed for the active ingredient.

D. Design: Treatment levels were defined by the Sponsor based upon known toxicity data and the expected environmental concentrations (EEC). One hundred and sixty (160) mallards (80 drakes and 80 hens) were randomly distributed into five groups. Sex of the birds was determined by a visual examination of the feather coat. Nominal and measured dietary concentrations and bird distribution over treatment groups were as follows:

Endosulfan Technical Substance (ppm).

Nominal Concentration	Mean Measured Concentration	Number of Pens	Birds/Pen Drakes	Hens
1 - Controls	-	16	1	1
2 - 7.5	7.8	16	1	1
3 - 15	15.0	16	1	1
4 - 30	30.3	16	1	1
5 - 60	63.6	16	1	1

"Each group contained sixteen pairs of birds with one male and one female per pen. Each of four groups were fed diets containing either 7.5, 15, 30, or 60 parts per million (ppm) of Endosulfan Technical Substance, respectively. The fifth group was fed control diet containing an amount of the solvent (acetone) and carrier (corn oil) equivalent to the amount in the treated diets. Each of the five groups of adult birds was fed the appropriate diet from the initiation of the test until the terminal sacrifice."

The test birds were acclimated to the facilities for 14 days prior to initiation of the test. During acclimation and upon initiation of the study, the birds were maintained under a photoperiod of eight hours of light per day. During Week 9 the photoperiod was increased to seventeen hours of light per day to induce egg laying. The photoperiod was maintained at 17 hours of light per day until adult sacrifice. The first eggs were set for incubation during Week 12.

All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. A record was maintained of all mortalities and observations. All birds that died during the study were necropsied. In addition, at the conclusion of the adult exposure period all birds were sacrificed by cervical dislocation, necropsied and disposed of by incineration.

Adult body weights were measured at study initiation, on Weeks 2, 4, 6, 8, and at terminal sacrifice. Body weights were not measured during egg laying because of the possible adverse effects

handling may have on egg production. Feed consumption was measured for each pen for a seven day period every week throughout the study, except during Christmas week when feed consumption was measured for a six day period, followed by an eight day period."

Eggs were collected daily from all pens and marked according to the pen of origin. The eggs were then washed to prevent pathogen contamination. The eggs were then stored in a cold room until incubated. At weekly intervals all eggs were removed from the cold room and candled with a Speed King egg candling lamp to detect egg shell cracks. Cracked eggs were discarded. All eggs that were not cracked or used for egg shell thickness measurements were placed in a Petersime Incubator. Eggs were candled again on Day 14 of incubation to determine embryo viability; and on Day 21 to determine embryo survival. On Day 24 of incubation, the eggs were placed in a Petersime Hatcher and allowed to hatch. Pedigree baskets constructed of galvanized steel wire mesh were used to keep hatchlings separated by pen.

"All hatchlings, unhatched eggs and egg shells were removed from the hatcher on Day 27 or 28 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were toe and web clipped for identification by pen of origin and then housed according to the appropriate parental concentration grouping in brooding pens until 14 days of age. The hatchlings were fed untreated diet. At 14 days of age, the average body weight by parental pen of all surviving ducklings was determined. The ducklings were sacrificed with chloroform and disposed of by incineration."

Weekly throughout the egg laying period, one egg was collected, when available, from each odd numbered pen during odd numbered weeks (1, 3, 5, etc.) and from each of the even numbered pens during the even numbered weeks (2, 4, 6, etc.). The eggs were opened, the contents removed, and the shell thoroughly washed. The shells were then allowed to air day for at least one week at room temperature. The average thickness of the dried shell plus the membrane was determined by measuring five points around the waist of the egg using a

micrometer. Measurements were made to the nearest 0.005 mm."

Records were maintained by pen for each of the following reproductive parameters:

- Eggs Laid
- Eggs Cracked
- Eggs Set
- Viable Embryos
- Live Three-Week Embryos
- Egg Shell Thickness
- Hatchlings
- Body Weight of Hatchlings
- 14-Day Old Survivors
- Body Weight of 14-Day Old Survivors

E. Statistics: Upon completion of the study, Dunnett's method (3,4) was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data. Each of the following parameters was analyzed statistically:

Adult Feed Consumption
Adult Body Weight
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of Viable Embryos
Hatchlings 3-Week Embryos
Hatchlings of Eggs Set
Offspring's Body Weight
Hatchlings of Maximum Set
14-Day Old Survivors of Maximum Set
14-Day Old Survivors of Eggs Set
14-Day Old Survivors of of Hatchlings
Eggshell Thickness

12. REPORTED RESULTS:

"Results of Diet Analysis. Samples of the diet fed to mallards were analyzed for Endosulfan Technical Substance. The results showed values that ranged from 87% to 127% of nominal with an average of 103% of nominal....The test material was stable for one week at room temperature and four weeks in the freezer. The homogeneity studies indicated that the preparation technique was adequate. Nominal and mean measured concentrations were as follows:

Endosulfan Technical Substance (ppm)

Nominal Concentration	Mean Measured Concentration
0	-
7.5	7.8
15	15.0
30	30.3
60	63.6

"Mortalities. There were no mortalities in the 0, 7.5 or 30 ppm treatment groups. Two mortalities occurred in the 15 ppm treatment group. One female was found dead during Week 2. The birds had appeared normal prior to death. The bird was light in weight (584 g) with no external lesions noted. Necropsy of this birds revealed an empty gastro-intestinal tract, chalk-like plaques in the pericardium and on the surface of the liver. The pen-mate of this bird was sacrificed at end of Week 2, with necropsy results unremarkable.

"Another hen in the 15 ppm treatment group was found dead during Week 9. The hen had first been observed exhibiting conjunctivitis in her left eye during Week 3. The severity of the lesions observed increased with time and during Week 5 the right eye also showed lesions. By Week 9, the bird was unable to see, soft tissue swelling was observed around the left orbit, the right eye was clouded and both eyes were tearing. The bird was also observed exhibiting a ruffled appearance, lethargy and weight loss prior to death. The hen was submitted to the Maryland Department of Agriculture, Animal Health Laboratory, Salisbury, Maryland for pathological examination. A gross necropsy revealed tumor-like lesions on the heart, left kidney and one eye. Following histological examination of the eye, kidney and heart tissue a diagnosis of granulomatous mycostic (fungal) panophthalmitis and myocarditis was made....The pen mate of this bird was sacrificed at the end of Week 10. Necropsy of this bird was not remarkable.

"At the 60 ppm concentration one mortality, a hen, was found dead during Week 2. The birds was light in weight (604 g), with no external lesions noted. Necropsy revealed a partially empty gastro-intestinal tract, with no feed present in the crop. Chalk-like plaques were observed in the pericardium. No other lesions were observed. The drake from this pen was sacrificed at the end of Week 2. Results of this necropsy were not remarkable.

"These mortalities were considered incidental and not related to treatment. No other mortality occurred during the course of the study.

"Clinical Observations. No apparent overt signs of toxicity were observed at any concentration tested. There were no apparent central nervous system related findings....Except for the mortalities previously noted and aside from lesions or observations normally associated with pen wear and/or interaction among pen mates, all birds at all concentrations appeared normal throughout the study.

"Gross Necropsy. All surviving adults were necropsied at adult terminal sacrifice (Week 18). All lesions observed appeared to be incidental and not related to treatment....

"Adult Body Weight and Feed Consumption. When compared to the control group, there was no apparent treatment related effect upon body weight of adult hens or drakes at 7.5, 15 or 30 ppm during the course of the study. In the 60 ppm group a slight treatment related loss of body weight was observed in both hens and drakes between the initial body weight and the Week 2 body weight....

"Because of excessive feed wastage by some birds, feed consumption is variable between pens. However, when compared to the control group, there was no apparent treatment related effect upon feed consumption at 7.5, 15 or 30 ppm. In the 60 ppm treatment group a statistically significant decrease ($p<.01$) in mean feed consumption was observed during Week 1. From Week 3 through Week 11 there was a slight increase in the mean feed consumption in the 60 ppm group. When compared to the control group this difference was statistically significant....

"Reproductive Results. There were no apparent treatment related effects upon reproductive parameters at 7.5, 15 or 30 ppm. At 15 ppm, the number of 14-day old survivors as a percentage of hatchlings was statistically different from the control at $p< .01$. The difference was slight and not attributed to treatment. The 97% value at 15 ppm was identical to the historical control value of 97% $\pm$ 2....The difference observed was considered to be a reflection of the fortuitous 100% survivability seen in the control group.

At 60 ppm there were statistically significant reductions in the number of eggs laid ($p<.01$), and in the number of hatchlings as a percentage of live 3-week embryos ($p<.05$). The effects on egg production and hatchability also were reflected in the number of hatchlings and 14-day old survivors as a percentage of eggs set ($p<.01$), and the number of hatchlings and 14-day old survivors as a percentage of maximum set ($p<.01$). The effects observed at 60 ppm were considered to be treatment related....

"Egg Shell Thickness. There were not apparent treatment related effects upon eggshell thickness at any treatment level tested. When compared to the control group, there were no statistically significant differences in egg shell thickness at 7.5, 15, 30 or 60 ppm....

"Offspring Body Weights. There was no apparent treatment related effect upon body weight of hatchlings or 14-day old survivors at any concentration. There were no statistically significant differences in body weights of offspring at 7.5, 15, 30 or 60 ppm...."

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

"This study was examined for conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs (Federal Register, Volume 48, No. 230, November 29, 1983, pages 53946 - 53969). The final report was determined to be an accurate reflection of the results obtained. The dates of all audits and the ...results of those audits were reported to the Study Director/Laboratory Management...." A total of fourteen (14) audits were performed during the test and reporting phases of this study.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure:

(1) Raw data for mortality, diet analyses, clinical observations, gross necropsies, adult body weight, feed consumption, reproductive results, egg shell thickness, and offspring body weights supported text.

The text reported statistical significance (for the 60 ppm group) for number of eggs laid and

hatchlings but Table 5 did not reflect this significance nor that of several other parameters (see 14B).

(2) Study followed guidelines, with the following exceptions:

(a) Ducks were kept at 8 hours of light per day for the first eight weeks (vs. 7 hours recommended by SEP; pg. 4).
(b) Eggs were stored in a cold room at 12.4°C (vs. 16°C recommended by SEP; pg. 5).
(c) SEP (pg. 6) recommends that a withdrawal study period be added to the test phase if reduced reproduction is evident. This was not done.
(d) Neither palatability nor feed spillage were reported or accounted for.

B. Statistical Analysis: An independent statistical analysis, using EPA BIGBIRD program, confirmed the applicant's results with the following exceptions:

(a) The independent analysis indicated that number of 14-day old survivors, eggs set, viable embryos, hatchlings, and live 3-week embryos, for the 60 ppm group was significantly ($p<.05$) lower than that of the controls.

C. Discussion/Results: Based on the data submitted, Endosulfan Technical Substance is expected to cause reproductive impairment for number of eggs laid, number of eggs set, embryo viability, hatching success and number of 14-day-old survivors at 60 ppm. The NOEL was 30 ppm, which according to the author, is equivalent to approximately 4 mg/kg of body weight.

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: N/A

(3) Reparability: N/A

15. COMPLETION OF ONE-LINER: Yes, on 2-4-89

LITERATURE

1. ASTM Draft, "Standard Practice for Conducting Avian Reproduction Tests," Draft Number 8, American Society for Testing and Materials, 1983.

2. Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, subsection 71-4, Environmental Protection Agency, Office of Pesticide Programs, October 1982.

3. Dunnett, C. W. "A Multiple Comparison Procedure for Comparing Several Treatments with a Control," Jour. Amer. Statis. Assoc. 50:1096-1121, 1955.

4. Dunnett, C. W. "New Tables for Multiple Comparisons with a Control," Biometrics 20: 482-491, 1964.

Table A. Analysis of Reproductive Effects.

Parameter	Concentrations of Endosulfan in the Diet				
	0 PPM	7.5 PPM	15 PPM	30 PPM	60 PPM
Eggs Laid	549	437	489	531	294 *
Eggs Cracked	21	14	9	10	9
Eggs Set	477	377	420	467	254 *
Viable Embryos	453	354	376	437	236 *
Live 3-Week Embryos	444	352	367	433	229 *
Hatchlings	370	292	281	329	150 *
14-Day Old Survivors	369	288	269	325	146 *
Eggs Laid/Hen	34	27	35	33	20
Eggs Laid/Hen/Day	0.61	0.49	0.62	0.59	0.35
14-Day Old Survivors/Hen	23	18	19	20	10
Eggs Laid/Max. Laid (%)	64	51	65	61	36 **
Eggs Cracked/Max. Laid (%)	4	4	2	2	3
Viable Embryos Set (%)	95	92	90	92	84
Live 3-Week Embryos/Viable (%)	98	99	98	99	97
Hatchlings/3-Week (%)	83	81	72	77	65 *
14-Day Old Survivors/Hatch (%)	100	98	97 **	99	98
Hatchlings Set (%)	77	75	63	71	54 **
14-Day Old Survivors/Set (%)	77	74	61	70	53 **
Hatchlings/Max. Set (%)	47	37	41	42	20 **
14-Day Old Survivors /Max. Set (%)	47	37	39	41	20 **
Average Hatchweight (g)	37	37	37	37	35
Average 14-Day-Old Survivor Weight (g)	212	210	214	229	204
Adult Body Weight (g/bird)t					
Females	1219	1238	1257	1244	1193
Males	1118	1135	1150	1108	1086
Adult Body Weight (Increase compared with Day 0)					
Females	+209	+232	+228	+238	+180
Males	-3	+41	+22	+22	+2
Mean Eggshell Thickness	0.388	0.388	0.384	0.376	0.385

---

* Difference from the control statistically significant at $p < .05$.
** Difference from the control statistically significant at $p < .01$.
t at study termination.

ONE LINER SHEET

Shaughnessey No. 079401 Chemical Name Endosulfan Chemical Class _____ Page ____ of ____

Study/Species/Lab Accession #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
Avian Reproduction Species: Mallard 96. Lab: Wildlife Int. Ltd. Project #: 125-137 AC #: 403350-01			Lincer/ CORE 2/4/89	

Group	Dose (ppm)	Affected/Parameters	Mort.(%)	% ChE Inh.
Control	0	none	0	N/A
Treatment I	7.5	none	0	N/A
Treatment II	15	none	0	N/A
Treatment III	30	none	0	N/A
Treatment IV	60	(1)	3	N/A

Study Duration: 26 weeks

(1) Eggs laid, eggs set, embryo viability, hatchling success and number of 14-day-old survivors.