This study is a corrected version of MRID 402613-03
11/8/92

7-4

403350-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Endosulfan 079402

2. **TEST MATERIAL:** Endosulfan Technical Substance. 96% active ingredient.

3. **STUDY TYPE:** Avian Reproduction.
Species Tested: Colinus virginianus

4. **CITATION:** Beavers, J. B., P. Frank and M. J. Jaber. 1987. Endosulfan Technical Substance (Code: HOE 002671 OI ZD95 0005) A One Generation Reproduction Study with the Bobwhite Colinus virginianus. Proj. No. 125-134. 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:**

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Wildlife Toxicologist
KBN Engineering and
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Signature: Michael L Whitten

Date: 3-2-89

Daniel Rieder 8-8-89

Henry T. Craven, M.S.
Supervisor, EEB/HED
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Signature:

Date: Henry T. Craven 8/8/89

7. **CONCLUSIONS:** There were no treatment related effects upon bobwhite exposed to dietary concentrations of 15, 30, or 60 ppm Endosulfan Technical. The number of cracked eggs in the 120 ppm group was significantly lower than in the control group ($p<.05$). The no-observed-effect concentration for bobwhite in this study was 60 ppm. This is equivalent to a daily intake of approximately 6 mg/kg of body weight. This study appears to be scientifically sound and meets the requirements for an avian reproduction test. There was a treatment related decrease in food consumption at the 120 ppm concentration.

8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND: This study is a corrected version of previously submitted study MRID No. 40261303.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Pen-reared bobwhite that were apparently healthy and phenotypically indistinguishable from wild birds, were purchased from Fritts Quail Farm, Phillipsburg, New Jersey. All birds were from the same hatch and were 27 weeks of age at test initiation (first day of exposure to test diets). 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.

Adult birds were identified by individual leg bands. All eggs laid during the study were marked with a permanent ink marking pen for identifi-cation. Hatchlings were identified by leg bands so that the chicks could be traced to their parental pen of origin.

B. Test System: The adult birds were housed indoors in batteries of pens manufactured by Georgia Quail Farm Manufacturing, measuring approximately 30 x 51 cm. The pens had sloping floors which resulted in ceiling height ranging from 21 to 26 cm. 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. Waterers were changed and water added as necessary to provide potable water (generally every one or two days).

The birds were maintained in a separate study room which helped avoid excessive disturbances. The average temperature in the adult bobwhite study room during the course of the study was 19.5°C ± 2.3°C (SD) with an average relative humidity of 45%. 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 bobwhite 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 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 23 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 14 days of age. 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.

Eggs were collected daily and stored in a cold room at 11.8°C ± 1.2°C (SD) and approximately 70% relative humidity. All eggs to be incubated were fumigated with formaldehyde gas to reduce the possibility of pathogen contamination prior to incubation.

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 29.3°C 0.05°C (SD) (relative humidity of approximately 54%). The incubator was equipped with a pulsator fan to eliminate intracabinet temperature and humidity variation 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 each hour through Day 21 of incubation. The eggs were transferred to the hatcher on Day 21. Eggs were not

rotated in the hatcher. The temperature in the hatcher was 37.1°C ± 0.5°C (SD) and the wet bulb temperature was raised to 31.8°C ± 1.0°C (SD) (relative humidity of 70%).

C. Dosage: Each of four groups were fed diets containing either 15, 30, 60 or 120 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. "Treatment levels were based upon known toxicity data and expected environmental concentration (EEC)."

D. Design: The primary phases of the study, and their approximate durations, were:

1. Acclimation - 3 weeks
2. Pre-photostimulation - 8 weeks.
3. Pre-egg laying (with photostimulation) - 4 weeks.
4. Egg laying - 10 weeks
5. Post-adult sacrifice (final incubation, hatching, and 14-day offspring rearing period) - 5 weeks.

One hundred and sixty (160) bobwhite (80 cocks and 80 hens) were randomly distributed into five groups. Sex of the birds was determined by a visual examination of the feather coat. Each group continued sixteen pairs of birds with one male and one female per pen (see below).

Endosulfan Technical Substance (ppm).

Nominal Concentration	Mean Measured Concentration	Number of Pens	Birds Per Pen Cocks	Hens
1 - Controls	-	16	1	1
2 - 15	15.0	16	1	1
3 - 30	30.6	16	1	1
4 - 60	63.6	16	1	1
5 - 120	134.1	16	1	1

Basal diet 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 test.

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. All offspring received a water soluble vitamin mix (Headstart Poultry, Whitmoyer Laboratories, Inc., Meyerstown, PA) in their water from the day of hatch until the birds were 14 days of age. Feed and water were provided to the offspring ad libitum.

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 the four test concentrations were prepared and presented to the birds weekly. 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.

Prior to initiation of the study, 0, 7.5, 60 and 120 ppm diets were prepared. Samples were collected and shipped to EN-CAS Analytical Laboratories, Winston-Salem, N.C. immediately following preparation for determination of homogeneity. Open air and freezer stability studies were also conducted by EN-CAS Analytical Laboratories. Samples of the control diet and each of the test diets were taken immediately after mixing for Weeks 1, 2, 3, 4, 8, 12, 16 and 23. Samples were frozen immediately after collection and shipped to EN-CAS Analytical Laboratories for analysis of the active ingredient.

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 the adult sacrifice. The first eggs were set for incubation during Week 14.

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. All surviving 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 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 11 of incubation to determine embryo viability; and on Day 21 to determine embryo survival. On Day 21 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 25 or 26 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were leg banded 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 chicks was determined. The chicks were sacrificed with chloroform and disposed of by incineration.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the 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 dry for at least one week at room temperature. The average thickness of the dried shells 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
- Live Three-Week Embryos
- Viable Embryos
- Hatchlings
- Body Weight of Hatchlings
- 14-Day Old Survivors
- Egg Shell Thickness
- 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 trans-formation. 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 of 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 Hatchlings
Eggshell Thickness

12. REPORTED RESULTS:

A. Diet Analyses: "...The results showed values that ranged from 87% to 127% of nominal with an average of 105%. 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	-
15	15.0
30	30.6
60	63.6
120	134.1"

B. Mortalities: "There were no treatment related mortalities. However, there were two mortalities in the control group. One hen died following body weight determination at the end of Week 8. The bird was noted displaying lower limb rigidity after weighing, and died within an hour. Necropsy revealed a small area of bruising on the midline of the cranium, but no other lesions were observed.

Her pen mate was sacrificed, with no lesions other than those associated with cervical dislocation observed.

"The second mortality in the control group, a cock, was first observed with a lesion on the right foot and a slight limp during Week 11. The foot lesions observed appeared to be the result of pecking by either the cock or his pen mate. The bird was ultimately found dead during Week 14. Necropsy of the bird revealed loss of weight (body weight of 106 g), extensive lesions on both feet, with blood matted on abdominal feathers, minor head lesions, loss of body muscle mass and an empty intestinal tract. Necropsy of this bird's pen mate was unremarkable.

"No mortalities occurred during the course of the study among birds in the 15, 30, 60 or 120 ppm groups."

C. Clinical Observations: "No apparent overt signs of toxicity were observed at any concentration tested. One cock at 30 ppm displayed a slightly ruffled appearance for two days during Week 2, and one cock at 15 ppm and one hen at 120 ppm displayed a ruffled

appearance for one day during Week 9. A hen in the 60 ppm treatment group [was] noted as lethargic and displaying lower limb weakness at the end of Week 15. The hen was noted as thinner and staying in the rear of the pen at the beginning of Week 18. During Week 18 this hen improved slightly and was considered normal by the end of Week 19.

"Except for the mortalities and clinical signs previously noted, and aside from lesions or observations normally associated with pen wear and/or interaction among pen mates, all other birds at all concentrations appeared normal throughout the study.

D. Gross Necropsy "All surviving adults were necropsied at adult terminal sacrifice (Week 23). All lesions observed were considered to be incidental and not related to treatment...."

E. Adult Body Weight and Feed Consumption "When compared to the control group, there were no apparent treatment related effects upon body weight among adult birds at any concentration tested...."

"There was no apparent treatment related effect upon feed consumption among birds at 15, 30 or 60 ppm. When compared to the control there was an increase in feed consumption at 30 and 60 ppm which was statistically significant at $p<.05$ at 30 ppm for Week 13, and at 60 ppm for Weeks 13 and 14, and statistically significant ($p<.01$) at 30 ppm for Week 14. However, these differences were considered to be related to the onset of egg production, and did not appear to be treatment related.

"At 120 ppm there appeared to be a treatment related effect upon feed consumption. When compared to the controls, there was a statistically significant ($p<.05$) decrease in feed consumption during Week 1, followed by an increase in feed consumption from Week 2 through Week 11. This increase was statistically significant at $p<.05$ during Weeks 2 and 3, and at $p<.01$ during Weeks 4, 5, 7, 8, 10 and 11. An increase in feed consumption also was observed during Weeks 13 and 14 which was statistically significant at $p<.01$. However, these differences were considered to be related to the onset of egg production and did not appear to be treatment related...."

F. Reproductive Results: "There were no apparent treatment related effects upon reproductive parameters at 15, 30, 60 or 120 ppm. At 60 ppm, when compared to the control group, there was a statistically significant ($p<.01$) increase in the number of hatchlings as a percentage of live three-week embryos (91% for controls vs. 95% at 60 ppm). In the 120 ppm group there was a statistically significant ($p<.05$) increase in the number of cracked eggs as a percentage of eggs laid. The differences observed was [sic], in part, the result of one pen in the 120 ppm group with 33% cracked eggs. Mean egg shell thickness for this pen was 0.184 mm, compared to a control mean of 0.223 mm. No other statistically significant difference was seen between the control group and 15, 30, 60 or 120 ppm treatment groups...."

G. Egg Shell Thickness "There were no apparent treatment related effects upon eggshell thickness at any concentration tested. When compared to the control group, there were no statistically significant differences in egg shell thickness at 15, 30, 60 or 120 ppm...."

H. Offspring Body Weights "There was no apparent 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 15, 30, 60, or 120 ppm...."

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

"Dietary concentrations of Endosulfan Technical Substance at 15, 30, 60 and 120 ppm did not result in treatment related mortality, overt signs of toxicity or effects upon reproductive parameters among bobwhite during the 23 week exposure period. There were no effects upon adult body weight or feed consumption at the 15, 30, or 60 ppm concentrations. At 120 ppm there was a slight decrease in adult feed consumption during Week 1, followed by a slight increase in adult feed consumption through the remainder of the pre-egg laying period. The no-observed-effect concentration for Endosulfan Technical Substance in this study was 60 ppm. This is equivalent to a daily intake of approximately 6 mg/kg of body weight."

"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 nineteen (19) 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 diet analysis, mortalities, clinical observations, gross necropsies, adult body weight and feed consumption, reproductive results, egg shell thickness and offspring body weights (at hatching and 14 days) supported text.

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

a. Recommended relative humidity for pens is 55% (SEP, pg. 3). Study provided 45% relative humidity.

b. Study photoperiod for first 8 weeks was 8 hours per day (vs. 7 hours per day recommended by SEP, pg 4).

c. Study provided 12 footcandles of light for adult birds (vs. 6 footcandles recommended by SEP, pg 4).

d. Neither palatability not feed spillage was reported or accounted for.

e. Eggs were stored at 11.8°C and 70% relative humidity (vs. 16°C and 65% relative humidity required by SEP, pg 5).

f. Hatchlings were removed from hatcher on Day 25 or 26 (vs. Day 24, required by SEP, pg 5).

B. Statistical Analysis: (See attached printouts) Statistics were verified using EPA's BIGBIRD program (ANOVA and Duncan's test) and Dunnett's Test. These tests supported the author's analyses, with the exception of the number of cracked eggs in the 120 ppm group.

C. Discussion/Results: There were no treatment related effects upon bobwhite exposed to dietary concentrations of 15, 30, or 60 ppm Endosulfan Technical. The number of cracked eggs in the 120 ppm group was significantly lower than in the control group ($p<.05$). The no-observed-effect concentration for bobwhite in this study was 60 ppm. This is equivalent to a daily intake of approximately 6 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 2/9/89.

REFERENCES

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

2. Anonymous, 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 (Based on study results and confirmed for those parameters addressed by BIGBIRD)

Parameter	Concentrations of Endosulfan in the Diet				
	0 PPM	15 PPM	30 PPM	60 PPM	120 PPM
Eggs Laid	579	704	802	610	762
Eggs Cracked	8	13	23	12	47*
Eggs Set	512	614	684	525	635
Viable Embryos	475	530	663	490	597
Live 3-Week Embryos	466	526	652	480	586
Hatchlings	425	505	627	469	554
14-Day Old Survivors	395	475	584	440	507
Eggs Laid/Hen	41	44	50	38	48
14-Day Old Survivors/Hen	28	30	37	28	32
Eggs Laid/Max. Laid (%)	59	63	72	54	68
Eggs Cracked/Max. Laid (%)	1	2	3	1	6*
Viable Embryos Set (%)	93	87	97	93	94
Live 3-Week Embryos/Viable (%)	98	99	98	98	98
Hatchlings/3-Week (%)	91	96	96	97	95
14-Day Old Survivors/Hatch (%)	93	94	93	93	90
Hatchlings Set (%)	83	83	92	89	88
14-Day Old Survivors/Set (%)	77	78	85	83	80
Hatchlings/Max. Set (%)	51	53	66	50	59
14-Day Old Survivors/Max. Set (%)	48	50	62	47	54
Average Hatchweight (g)	7	7	6	6	6
Average 14-Day-Old Survivor Weight (g)	27	27	27	26	26
Adult Body Weight (G/bird)					
Females	234	235	237	223	230
Males	210	205	206	207	199
Adult Body Weight (Increase compared with Day 0)					
Females	42	38	43	33	38
Males	12	10	10	10	3
Mean Eggshell Thickness	0.223	0.220	0.223	0.219	0.225
Mean Feed Consumption	-	ns	t	tt	ttt

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* Difference from the control statistically significant at $p < .05$.

** Difference from the control statistically significant at $p < .01$.

t Significantly different (greater) than control for weeks 13 ($p<.05$) and 14 ($p<.01$).

tt Significantly different (greater) than conrol for weeks 13 & 14 ($P< .05$) .

ttt Significantly different from control for weeks 1-3 ($p<.05$) and for weeks 4,5,7,8,10,11,13 and 14. Week 1 was characterized by a decrease; followed by an increase.

ONE LINER SHEET

Shaughnessey No. 079402 Chemical Name Endosulfan Tech. Chemical Class _____ Page 1 of 1

Study/Species/Lab Accession #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status

Avian Reproduction
Species: Bobwhite

96.

Lab: Wildlife Int. Ltd.
Project #: 125-134
AC #: 403350-02

Group	Dose (ppm)	Affected/Parameters	Mort.(%)	% ChE Inh.
Control	0	0	6	N/A
Treatment I	15	0	0	N/A
Treatment II	30	0	0	N/A
Treatment III	60	0	0	N/A
Treatment IV	120	6%/eggs cracked[1]	0	N/A

Study Duration: 30 weeks

Lincer/ CORE 2/9/89

(1) Based on eggs cracked/eggs laid (%), the 120 ppm group had 6% (vs. control at 1%). This was, apparently, partially a function of one pen of 120 ppm group that showed unusually high breakage.