

US EPA ARCHIVE DOCUMENT

9-9-85

DATA EVALUATION RECORD

1. Chemical: Glyphosate S #103601
2. Test Material: Technical - 83%
3. Study Type: Colinus virginianus One-Generation Reproduction Study
4. Study ID: Beavers, J.B. and Fink, R. (1978) One-Generation Reproduction Study - Bobwhite quail, Technical Glyphosate, Final Report, Project No. 139-141, Wildlife International Ltd. Submitted by Monsanto Agricultural Products Co. on November 13, 1978, for EPA Registration No. 524-308, CDL Accession No. 235924.

5. Reviewed by: Dennis J. McLane
Wildlife Biologist
EEB/HED

Signature: *Dennis J. McLane*
Date: 9-9-85

6. Approved by: Raymond W. Matheny
Supervisory Biologist
EEB/HED

Signature: *Raymond W. Matheny*
Date: 9-9-85

7. Conclusion:

This study is scientifically sound and meets guideline requirements.

The arc-sin transformation between egg and normal hatchling was statistically significant, but the Duncan Multiple Range test failed to discriminate between the tested levels.

Although, reproductive impairment was shown statistically, the biological effect of these differences are expected to be minimal.

8. Recommendation:

N/A

9. Background:

The first validation of this study was on July 3, 1979, by D. McLane. The present review is the result of the Glyphosate Registration Standard.

10. Discussion of Individual Tests:

N/A

11. Material and Methods: (Definitive Test)

a. Test Procedure: After 5 weeks, at initiation of the study and immediately prior to the onset of egg laying body weights were recorded as well as at the termination of the study. Food consumption was recorded biweekly throughout the study. Eggs were placed in the incubator at weekly intervals. Eggs were candled on days 0, 14, and 19. Day 19 eggs were placed in a hatcher. All eggs on day 21 or 22 were removed from the incubator. Hatchling were housed according to the appropriate parental grouping and maintained on a control diet until 14 days of age. For purposes of egg weight and egg-shell measurements, weekly throughout egg laying one egg from every other pen was taken.

b. Design: (excerpted from citation)

<u>Group No.</u>	<u>Dosage level</u> ppm	<u>No. of Pens</u>	<u>Birds per pen</u>	
			<u>Cocks</u>	<u>Hens</u>
1. Controls	0	12	1	2
2. Glyphosate Technical	50	12	1	2
3. Glyphosate Technical	200	12	1	2
4. Glyphosate Technical	1000	12	1	2

c. Statistics: The author indicated the student's t-test was selected to evaluate the differences between the reproductive parameters.

12. Reported Results: (excerpted from citation)

Mature bobwhite quail were fed dietary levels of Glyphosate Technical at concentrations of 50 ppm, 200 ppm, and 1000 ppm for a period of 17 weeks. [see attached tables].

Mature bobwhite quail receiving Glyphosate Technical at dietary concentrations of 50 ppm, 200 ppm, and 1000 ppm showed no symptoms of toxicity or behavioral abnormalities for the duration of the study. Mortalities occurred as follows: Control group one hen - week 14, one hen - week 13, one hen - week 17. These mortalities occurred during the stress of egg production and no gross compound related abnormalities were noted upon gross necropsy, therefore, these deaths were considered to be incidental and not compound related. With the exception of the above mentioned mortalities, all test and control quail appeared normal throughout the study.

Evaluation of the reproductive data in tables 1A, 1B, 2A, 2B, 3A, 3B, and 4, and statistical analysis of the reproductive parameters: eggs laid, eggs cracked, viable embryos, live 3-week embryos, normal hatchlings, 14-day-old survivors, representative hatchlings body weight, demonstrate

that Glyphosate Technical caused no reproductive impairment at the dose levels tested. Statistically significant impairment was found in eggshell thickness at the 50 ppm dose level. However, the reduction in average eggshell thickness appears to be caused by four abnormally thin eggshells out of a total of 46 eggshells measured. When these four measurements are eliminated from the statistical computations, there is no longer statistically significant impairment. In addition, no effect on eggshell thickness was noted at any other dose level tested. Therefore, the impairment noted at the 50 ppm dose level is not considered to be biologically meaningful.

A statistically significant reduction in egg weight occurred at the 1000 ppm dose level. However, no effect was seen in the overall reproductive success of the birds at this dose level. Therefore, this reduction in egg weight is not considered to be biologically meaningful.

A high incidence of eggshell cracks was noted during the course of this reproduction study. The bobwhite quail utilized for this study were inadvertently not debeaked prior to the initiation of the study. Caged quail have a natural propensity to peck at their eggs, causing cracks in the eggshell. By debeaking the birds (removing the sharp point of the upper beak) this problem is usually alleviated.

13. Study Author's Conclusion/QA Measures:

No mention was made of quality assurance methods or inspections.

14. Reviewer's Discussion and Interpretation of the Study:

a. Test Procedures: The following items did not meet the guideline requirements:

1. Duration of administration prior to egg laying was 9 weeks rather than 10 weeks.
2. The percentage of the test diet which was corn oil was not reported.
3. The body weight was not taken at biweekly intervals of 0, 2, 4, 6, and 8 weeks but on weeks 0, 6, and 10.
4. Eggs were stored at 55% relative humidity rather than 65%.

- b. Statistical Analysis: The study results report three areas which involve statistical interpretation of the data. The statistical method student's t-test chosen by Wildlife International Ltd., is not as accurate as the ANOVA. EEB has two approaches to the ANOVA. Both are derived from SAS programs. One is a comprehensive group of ANOVAs and arc-sin transform ANOVAs for the following parameters egg laid, eggs cracked, viable embryos, live 3-week embryos, and normal hatchlings. The other is a single ANOVA program which may be used on other parameters.

Egg Shell Thickness

The author points out the t-test indicated statistical difference was found in eggshell thickness at the 50 ppm level. However, the ANOVA results did not find a difference at the 50 ppm but at the 1000 ppm level. The following results of the Duncan's Multiple Range test separate the highest level because of its increased thickness and not thinning.

Grouping	Mean	N	Treatment
	0.22327	11	1000 ppm
	0.24273	11	Control
	0.20927	11	200 ppm
	0.20445	11	50 ppm

These results indicates the within group variation was not taken into account. The student's T was incorrect indicating a statistical significant difference between the 50 ppm level and other group. Whereas the ANOVA indicates the opposite situation of eggshell thickening at the highest level. (McLane has the ANOVA printout)

Eggs Cracked

The author further refers to a high incidence of cracked eggs, however, the ANOVA indicated no statistical difference between the control and test levels.

Egg Weight

Concerning egg weight, EEB ANOVA results agreed with the authors', in that, statistical difference was evident between the 1000 ppm level and the other levels.

Egg to Normal Hatchling Reproduction

Two of the ANOVA's indicated a statistical difference. The normal hatchling/live embryos demonstrated the probability of the F value, 3.1, as greater than 0.0364.

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Similarly, for normal hatchlings/egg laid, the probability of the F value, 3.16, is greater than 0.0338. In both cases the Duncan's Multiple Range Test was unable to discriminate between test levels. In both cases, the lowest concentration, 50 ppm was the smallest of the four means showing the greatest difference from the control group.

- c. Discussion/Results: The study did not use the appropriate statistical method nor was the duration of administration of the treatment adequate. The acceptance of EEB results will allow the study to meet guideline requirements for statistical analysis. The duration of administration of 9 weeks is also acceptable, in view of the slightly toxic nature of this compound and its half-life.
- d. Adequacy of Study:
 - 1. Category: Core based on EEB statistical results.
 - 2. Rationale: The study meets the guidelines provided that the EEB statistical results can be used in place of the author's.
 - 3. Repairability: None

15. Completion of One-Liner for Study:

Completed August 6, 1985.

16. CBI Appendix:

N/A

Table 1A

REPRODUCTIVE DATA - BOBWHITE QUAIL

	Controls	<u>Glyphosate Technical (ppm)</u>		
		50	200	1000
Eggs laid	764	673	673	781
Eggs cracked	74	51	62	49
Eggs set	645	576	567	687
Viable embryos	593	464	520	598
Live 3-week embryos	577	451	507	577
Normal hatchlings	470	317	372	429
14-day-old survivors	449	295	356	201

Table 1B

REPRODUCTIVE SUCCESS DATA - BOBWHITE QUAIL

	Controls	<u>Glyphosate Technical (ppm)</u>		
		50	200	1000
Eggs laid per hen in 8-weeks*	32	28	28	33
Eggs cracked of eggs laid (%)	10	8	9	6
Viable embryos of eggs set	92	81	92	87
Live 3-week embryos of viable embryos (%)	97	97	98	96
Normal hatchlings of live 3-week embryos (%)	81	70	73	74
14-day-old survivors of normal hatchlings (%)	96	93	96	93
14-day-old survivors hen*	19	12	15	17

* Based on 24 Hens

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Table 2A

EGG WEIGHT DATA - BOBWHITE QUAIL

	Controls	<u>Glyphosate Technical (ppm)</u>		
		50	200	1000
No. of eggs analyzed	45	46	44	44
Mean egg weight (g)	10.3	9.9	10.2	9.5

Table 2B

EGG SHELL THICKNESS DATA - BOBWHITE QUAIL

	Controls	<u>Glyphosate Technical (ppm)</u>		
		50	200	1000
No. of eggs analyzed	45	46	44	44
Mean shell thickness (mm)	0.214	0.204*	0.211	0.224

* Statistically significant impairment (p. 05)

Table 3A

BODY WEIGHT DATA - REPRESENTATIVE HATCHLINGS - BOBWHITE QUAIL

	Controls	<u>Glyphosate Technical (ppm)</u>		
		50	200	1000
No. of chicks analyzed	72	80	76	73
Mean body weight (g)	7	7	7	7

Table 3B

BODY WEIGHT DATA - REPRESENTATIVE 14-DAY-OLD SURVIVORS

BOBWHITE QUAIL

	Controls	Glyphosate Technical (ppm)		
		50	200	1000
No. of chicks analyzed	72	80	75	72
Mean body weight (g)	22	22	23	22

Table 4

BODY WEIGHT AND FOOD CONSUMPTION DATA - ADULT BOBWHITE QUAIL

Week	Glyphosate Technical (ppm)							
	Controls		50		200		1000	
	B.W.	F.C.	B.W.	F.C.	B.W.	F.C.	B.W.	F.C.
0	192	-	192	-	203	-	180	-
2	-	23	-	20	-	20	-	23
4	-	21	-	18	-	17	-	19
6	216	17	207	18	213	18	211	19
8	-	22	-	21	-	21	-	21
10	232	21	224	20	227	20	223	19
12	-	21	-	21	-	20	-	20
14	-	22	-	23	-	21	-	21
16	-	22	-	22	-	21	-	21
17	234	21	209	20	230	20	233	21

The body weight data are presented as a group mean.
The food consumption data are presented as the group mean feed consumed per bird per day.

B.W. - Body weight in grams.

F.C. - Food consumption/bird/day in grams.

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ATTACHMENT I
 REPRODUCTIVE DATA BY PEN - BOBWHITE QUAIL
 GLYPHOSATE TECHNICAL

	Eggs Laid	Eggs Cracked	Eggs Set	Viable Embryos	Live Three-Week Embryos	Normal Hatchlings
	74	10	60	47	47	35
	71	5	62	61	57	52
	20	2	15	15	15	14
	92	0	88	83	81	68
	84	11	69	61	58	50
Controls	43	2	38	31	31	27
	32	9	19	16	15	11
	71	2	65	62	59	37
	76	5	67	64	64	56
	83	7	73	71	71	58
	59	13	42	40	39	27
	59	8	47	42	40	35
Totals	764	74	645	593	577	470
	54	7	43	38	36	22
	62	6	52	50	50	33
	49	5	40	32	30	22
	56	3	49	29	27	18
50 ppm	75	7	64	60	60	41
	56	2	50	50	47	27
	47	1	43	31	31	17
	17	1	13	11	11	6
	58	8	46	12	12	11
	38	2	32	29	29	27
	72	4	64	52	49	36
	89	5	80	70	69	57
Totals	673	51	576	464	451	317

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ATTACHMENT I

REPRODUCTIVE DATA BY PEN - BOBWHITE QUAIL

GLYPHOSATE TECHNICAL

	Eggs Laid	Eggs Cracked	Eggs Set	Viable Embryos	Live Three-Week Embryos	Normal Hatchlings
	34	2	29	29	29	24
	74	8	62	58	56	31
	65	0	61	51	51	31
	44	1	39	34	34	27
200 ppm	58	3	51	48	47	38
	49	10	35	30	28	21
	18	4	12	11	8	6
	80	14	62	60	60	46
	67	5	58	58	57	43
	63	4	55	50	49	40
	31	4	24	24	23	16
	90	7	79	67	65	49
Totals	673	62	567	520	507	372
	58	10	45	33	31	24
	68	2	62	43	42	36
	65	2	59	52	43	31
	24	0	20	19	17	14
1000 ppm	102	4	94	92	91	62
	70	2	64	60	60	44
	67	13	51	51	50	40
	78	3	71	55	53	39
	65	1	60	57	57	39
	78	8	66	50	50	32
	22	1	19	16	16	9
	84	3	76	70	67	59
Totals	781	49	687	598	577	429

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ATTACHMENT I

EGG WEIGHT DATA (g) - BY PEN - BOBWHITE QUAIL

GLYPHOSATE TECHNICAL

													<u>Average</u>
Controls	10.1	10.4	9.8	10.4	10.8	9.6	9.8	10.2	10.8	10.5	10.1	10.6	10.3
50 ppm	10.2	10.3	9.0	8.8	10.2	10.6	10.2	9.7	10.1	10.5	10.1	9.4	9.9
200 ppm	10.0	10.0	10.1	10.3	10.5	11.0	9.8	10.0	10.3	10.0	10.2	9.7	10.2
1000 ppm	9.8	9.4	8.7	9.7	10.3	9.2	9.4	9.2	9.4	9.2	9.2	10.4	9.4

EGGSHELL THICKNESS DATA BY PEN - BOBWHITE QUAIL

GLYPHOSATE TECHNICAL

(Thickness Measured in Millimeters)

													<u>Average</u>
Controls	0.225	0.205	0.212	0.236	0.2	0.213	0.209	0.203	0.225	0.225	0.203	0.209	0.214
50 ppm	0.216	0.20	0.211	0.19	0.209	0.212	0.212	0.181	0.195	0.209	0.214	0.214	0.204
200 ppm	0.222	0.188	0.184	0.205	0.215	0.221	0.221	0.204	0.225	0.213	0.204	0.204	0.211
1000 ppm	0.215	0.216	0.23	0.215	0.229	0.221	0.221	0.226	0.220	0.235	0.228	0.228	0.224

ATTACHMENT I

BODY WEIGHT DATA(g) - BY WEEK -
 REPRESENTATIVE HATCHLINGS - BOBWHITE QUAIL
 GLYPHOSATE TECHNICAL

Week #	1	2	3	4	5	6	7	8	Average
Controls	6.0	6.6	7.2	7.1	7.2	6.6	7.1	6.9	6.8
50 ppm	7.0	7.0	6.9	7.3	7.2	6.5	6.8	6.8	6.9
200 ppm	6.8	6.7	6.9	6.9	6.8	6.9	6.9	7.0	6.9
1000 ppm	7.3	7.1	5.8	6.3	6.5	6.6	6.9	6.8	6.7

14-DAY-OLD SURVIVORS - BY WEEK

BOBWHITE QUAIL
 GLYPHOSATE TECHNICAL

Week	1	2	3	4	5	6	7	8	Totals
Controls	2	17	52	69	66	85	77	81	449
50 ppm	18	29	58	49	33	46	37	25	295
200 ppm	5	24	53	59	44	63	54	54	356
1000 ppm	2	16	35	58	56	76	73	85	401

ATTACHMENT I

BODY WEIGHT DATA (g) - BY WEEK

REPRESENTATIVE 14-DAY-OLD SURVIVORS - BOBWHITE QUAIL

GLYPHOSATE TECHNICAL

Week #	1	2	3	4	5	6	7	8	Average
Controls	18.0	20.0	21.0	22.0	22.2	24.4	23.9	24.7	22.0
50 ppm	19.8	20.2	26.4	20.4	22.7	20.7	23.0	24.2	22.2
200 ppm	23.0	22.6	22.2	22.4	20.4	21.0	23.8	25.5	22.6
1000 ppm	21.0	22.2	20.7	21.0	21.9	20.8	24.2	24.5	22.0

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Data Evaluation Record

1. Chemical Glyphosate

2. Formulation

Technical, 83% active ingredient

3. Citation

Beavers, J.B., and R. Fink, One-generation reproduction study
-Bobwhite Quail Glyphosate Technical Final Report, Wildlife
International LTD. for Monsanto Agricultural Products Co.
(1978) Acc. No. 235924.

4. Reviewed by

Name Dennis J. McLane
Title Biologist
Organization EEB/HED

Signature
Date

Dennis J. McLane
7-3-79

5. Test Type

Avian Reproduction (upland game bird)

6. Conclusion

The study is scientifically sound and revealed no toxicological effects at the levels tested. The study does fulfill the requirement for a upland game bird reproduction study and is acceptable as core data.

7. Material and Methods

A. Three tests levels, 50, 200, 1000 ppm and one control level were established. Protocol followed that recommended by USEPA (1978).

B. Statistical Analysis:

The ANOVA statistical method was used to verify the comparisons for eggs laid, eggs cracked, egg weight, and eggshell thickness. The ANOVA proved that the student's test used was conservative, since no significance was indicated for egg shell thickness or egg weight as reported by the author.

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8. Reported Resulted

Mature bobwhite quail receiving glyphosate technical showed no symptoms of toxicity or behavioral abnormalities for the duration of the study. Mortalities occurred as follows: Control groups; one hen-week 15, one hen-week 16, 50 ppm group; two hens - weeks 13, one hen-week 14, one hen-week 15, 200 ppm group; two hens - week 13, 1000 ppm group; one hen-week 17.

A statistically significant reduction in egg weight occurred at the 1000 ppm dose level. However, no effect was seen in the overall reproductive success of the birds at this dose level.

A high incidence of eggshell cracked was noted during the course at this reproduction study.

9. Discussion

The author reported two parameters were statistically significant in their difference from the controls, egg weight and egg shell thickness. However, the ANOVA method did not indicate any significant difference. In addition, the egg weight difference did not effect any of the other parameters measuring reproductive success. Egg shell thickness and a high number of cracked eggs would seem to indicate a problem. However, the egg shell thickness of 0.204 mm could be the normal thickness for some populations. The percent of hatchlings of live three week embryos was 70 at the affected level (50 ppm). This percentage fell in the middle of the range suggested by EPA protocol. Also, it was noted that the higher levels did not demonstrate similar thinning.