DATA EVALUATION RECORD

1. **CHEMICAL:** Proxel. Shaughnessey Number: 098901.

2. **TEST MATERIAL:** Proxel Press Paste; 1,2-Benzisothiazolin-3-one; Batch No. BN 023378; 73.4% purity; a brown powder.

3. **STUDY TYPE:** Avian Single Dose Oral LD_{50} Test.
Species Tested: Bobwhite quail (Colinus virginianus).

4. **CITATION:** Roberts, N.L, C.N.K. Phillips, C.B. Howlett, A. Anderson, and I.S. Dawe. 1988. The Acute Oral Toxicity of Proxel Press Paste to the Bobwhite Quail. Study performed by Huntingdon Research Centre Ltd., Cambridgeshire, England. Laboratory Study No. ISN 164/88278. Submitted by ICI Americas, Wilmington, Delaware. MRID No. 409913-01.

5. **REVIEWED BY:**

Michael W. Davy
Agronomist
EPA/OPP/EFED/EEB

Signature: Michael Davy
Date: 7-16-92

6. **APPROVED BY:**

Daniel Rieder
Section Head
EPA/OPP/EFED/EEB

Signature: Daniel Rieder 7/21/92
Date: 7-22-92 Henry Craven formulation. for 73.4% a.i. MA

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for an avian oral LD_{50} test. The acute oral LD_{50} of Proxel was estimated to be 617 mg/kg (based on nominal concentrations) with 95% confidence limits of 464 and 816 mg/kg. The no-observed effect level was not determined, due to toxic effects at all dosage levels. With an LD_{50} of 617 mg/kg, the test material is considered slightly toxic to bobwhite quail.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in the study were adult bobwhite quail (Colinus virginianus) approximately 8 months of age. The birds were obtained from D.R. and R.E. Wise, Cambridgeshire, England. All birds were acclimated to the facilities for 14 days prior to dosing. All birds were in apparent good health at the start of the pre-treatment period.

B. **Test System:** All birds were housed indoors in pens constructed of plastic coated wire cages measuring 78 x 39 x 25 cm. Artificial lights provided 7 hours of light per day. Maximum and minimum temperatures and the relative humidity of the animal room were recorded once daily. The average temperature ranged from a minimum of 10°C ± 1°C (SD) to a maximum of 14°C ± 2°C (SD). The average relative humidity was 70% ± 7% (SD).

C. **Dosage:** 14-day single dose oral LD_{50} test. Based upon an initial range-finding study, nominal dosages selected for the study were 125, 250, 500, 1000, and 2000 milligrams of Proxel per kilogram of body weight (mg/kg). The dosages and reported LD_{50} value were not corrected for purity of the test substance. Analyses were conducted by Huntingdon Research Centre Ltd. to determine the concentration, heterogeneity, and stability of the test substance as administered. Actual dose levels were 118, 243, 477, 1020, and 2040 mg/kg.

D. **Design:** Groups of ten birds (five males and five females) were allocated to each of five treatment groups and one control group, so that all groups initially had similar mean bodyweights. Water was available at all times and food was offered ad libitum with the exception of an overnight starvation period prior to dosing. The birds were offered standard Huntingdon Research Centre pelleted layer diet from Joseph Odam Limited, Cambridgeshire, England. The test substance was administered as a suspension in 0.5% HPMC in 0.1% Polysorbate 80. The suspensions were prepared immediately prior to use by HRC. Dosages were administered by oral intubation, using a disposable syringe and a plastic catheter. The control birds received a corresponding dose of vehicle only.

Bird health and mortalities were observed daily throughout the study period. The birds were individually weighed on a weekly basis throughout the

pretreatment, treatment and post-treatment periods. Group mean food consumption was also determined weekly throughout the acclimation period and the study period.

Macroscopic post-mortem examinations were performed on all birds at the termination of the study.

E. Statistics: The LD_{50} value and 95% confidence limits were determined by the method of probit analysis, using the Maximum Likelihood Program.

12. REPORTED RESULTS: There were no mortalities in the control group. Mortalities of 30, 90, and 100% occurred in the 500, 1000, and 2000 mg/kg treatment groups, respectively (Table 1, attached).

At the 125 mg/kg dosage level, all birds appeared subdued and had ruffled feathers by the end of the first day following dosing. All birds recovered soon thereafter and showed no adverse effects for the remainder of the study.

All birds in the 250 mg/kg group appeared subdued and had ruffled feathers toward the end of Day 1. All birds had recovered by the end of Day 2.

At 500 mg/kg, all birds were found to be unsteady and subdued with ruffled feathers on Day 1. Two birds in the group died overnight on Day 2, and a third bird died on Day 3. All other birds recovered and showed no further adverse effects.

At the 1000 mg/kg dosage level, 5 birds died on Day 1 after dosing. Several surviving birds showed signs of unsteadiness, subdued behavior, wing droop and ruffled feathers. Four birds died overnight on Day 2. The surviving bird in the group was very unsteady, with ruffled feathers at the end of Day 2, but recovered by the end of Day 3.

At the highest dosage level (2000 mg/kg), all birds died overnight on Day 1.

Bodyweight changes during the pre-treatment period were considered to be within normal limits in all groups (Table 2, attached). Birds at the 250 mg/kg level showed a mean decrease in bodyweight over the 7-day period after dosing. Surviving birds at the 500 mg/kg level showed marked mean decreases in body weight. The one surviving bird in the 1000 mg/kg group also showed a marked decrease in

bodyweight. With the exception of control group males, all surviving groups showed mean bodyweight increases between Days 7 and 14.

There was evidence of reduced food consumption in females in the 250 mg/kg dosage group for the 7-day period after dosing (Table 3, attached). Birds at the 500 mg/kg dosage level showed a more marked decrease over the same period, while birds at the two highest dosage levels showed a very marked depression of food consumption.

Post-mortem examination revealed no abnormalities in any of the birds that died during the study. Examinations of ten surviving birds from the three highest dose groups showed no abnormalities.

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

The acute oral LD_{50} of Proxel was determined to be 617 mg/kg with 95% confidence limits of 464 to 816 mg/kg.

The report stated that the study was conducted in conformance with Good Laboratory Practice regulations. The statement was signed by the study director and laboratory manager for HRC.

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

A. Test Procedure: The test procedures were in accordance with Subdivision E and SEP guidelines with the following exceptions:

Birds were not randomly allocated to treatment groups, as stipulated by the SEP guidelines. Rather, birds were selected and allocated so that all groups had similar mean bodyweights at the study initiation.

B. Statistical Analysis: The reviewer calculated the LD_{50} and 95% confidence limits using EPA's Toxanal computer program (attached). Using the probit method, an LD_{50} of 617 mg/kg (based on nominal concentrations) was calculated for Proxel in bobwhite quail. The calculated 95% confidence limits are 465 and 818 mg/kg. These values are similar to those presented by the author, and are therefore accepted.

C. Discussion/Results: The deviation from recommended protocol (listed in Section 14-A) was minor and did not affect the validity of the study. The study is scientifically sound and meets the requirements for an avian oral LD_{50} test.

With an LD_{50} of 617 mg/kg (based on nominal concentrations), Proxel is considered to be slightly toxic to bobwhite quail. The no-observed effect level was not established, due to clinical signs of toxicity at all levels.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes; June 12, 1992.

MORTALITIES AND LD_{50} VALUE

The distribution of mortalities following dosing is summarised in Table 1.

TABLE 1

Distribution of mortalities

Group	Treatment	Dose level (mg/kg)	Sex	No. of birds	Days after dosing													Total	
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	Control	0 *	♂	5															0
			♀	5															0
2	PROXEL Press Paste	125	♂	5															0
			♀	5															0
3	PROXEL Press Paste	250	♂	5															0
			♀	5															0
4	PROXEL Press Paste	500	♂	5			1												1
			♀	5		2													2
5	PROXEL Press Paste	1000	♂	5	3	2													5
			♀	5	2	2													4
6	PROXEL Press Paste	2000	♂	5	5														5
			♀	5	5														5

The acute oral LD_{50} value was calculated using the method of Probit Analysis[1], using Maximum Likelihood Program[2], and the results were as follows:

	♂♂	♀♀	♂ + ♀
LD_{50} value (mg/kg)	609	624	617
95% confidence limits	404 - 935	420 - 935	464 - 816
Slope of line	6.43	6.43	6.42
Standard error	1.93	1.93	1.91

* ♂ and ♀ LD_{50} values estimated after fitting parallel lines

1 Finney, D.J. (1978) Statistical Method in Biological Assay, Griffin & Co., London

2 Ross, G.J.S (1980) Maximum Likelihood Program, Rothamsted Experimental Station, Harpenden, U.K.

TABLE 2

Group mean bodyweights and bodyweight changes (g/bird)

(bodyweight changes based on means of only those birds alive between the relevant time points)

Group	Treatment	Dose level (mg/kg)	Sex	Days of study											
				-14	-7	-1	0	7	14	-14 to -7	-7 to -1	-1 to 0	0 to 7	7 to 14	0 to 14
1	Control	0	♂	201	197	200	192	200	197	-4	-3	-8	+8	-3	+5
			♀	199	200	204	198	203	205	+1	+4	-6	+5	+2	+7
2	PROXEL Press Paste	125	♂	203	200	202	192	197	199	-3	+2	-10	+5	+2	+7
			♀	201	198	202	195	196	201	-3	-4	-7	-1	+5	+6
3	PROXEL Press Paste	250	♂	204	200	204	198	190	199	-4	-4	-6	-8	+9	+1
			♀	202	200	204	196	193	199	-2	-4	-8	-3	+6	+3
4	PROXEL Press Paste	500	♂	202	201	205	197	174	191	-1	+4	-8	-23*	+17*	-6*
			♀	200	192	199	192	175	184	-2	+1	-7	-13[3]	-8[3]	-4[3]
5	PROXEL Press Paste	1000	♂	204	201	202	194	-	-	-3	-2	-9	-	-	-
			♀	201	197	201	194	182	200	-4	-4	-7	-27[1]	-18[1]	-9[1]
6	PROXEL Press Paste	2000	♂	201	198	200	190	-	-	-3	-2	-10	-	-	-
			♀	201	200	204	197	-	-	-1	-4	-7	-	-	-

Superscript numbers refer to number of birds alive if less than 5

FOOD CONSUMPTION

Group mean food consumption results are given overleaf in Table 3.

There was evidence of a slight depression of food consumption in Group 3 females (PROXEL Press Paste at 250 mg/kg) in the 7-day period after dosing. Birds in Group 4 (PROXEL Press Paste at 500 mg/kg) showed a more marked decrease over the same period, while birds in Groups 5 and 6 (PROXEL Press Paste at 1000 mg/kg and 2000 mg/kg, respectively) showed a very marked depression of food consumption. Food consumption in Groups 2 - 5 showed an increase between Days 8 and 14 and this increase was most marked in surviving Group 4 birds and in the one surviving female bird in Group 5. There were no survivors in Group 6.

TABLE 3

Group mean food consumption (g/bird/day)

Group	Treatment	Dose level (mg/kg)	Sex	Days of study			
				-14 to -8	-7 to -1	1 to 7	8 to 14
1	Control	0	♂	16	16	19	19
			♀	18	18	21	18
2	PROXEL Press Paste	125	♂	17	18	18	19
			♀	17	17	17	20
3	PROXEL Press Paste	250	♂	18	18	17	21
			♀	19	18	15	20
4	PROXEL Press Paste	500	♂	20	22	15	31
			♀	18	18	12	24
5	PROXEL Press Paste	1000	♂	16	17	2	-
			♀	16	18	8	22
6	PROXEL Press Paste	2000	♂	17	17	3	-
			♀	18	19	3	-

GROSS MACROSCOPIC POST-MORTEM EXAMINATION

All birds which died as a result of dosing were examined and no abnormalities were detected in any birds. At termination of the study ten surviving birds from the high dose groups (i.e. the remaining bird from Group 5, the seven survivors from Group 4 and two birds from Group 3) were examined. No abnormalities were detected.

RD

Carolyn Poppell Proxel Bobwhite quail 06-12-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2000	10	10	100	9.765625E-02
1000	10	9	90	1.074219
500	10	3	30	17.1875
250	10	0	0	9.765625E-02
125	10	0	0	-9.765625E-02

THE BINOMIAL TEST SHOWS THAT 250 AND 1000 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 620.8041

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1144042	625.0656	457.7282	904.2618

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.3341712	1	.9917213

SLOPE = 6.412292

95 PERCENT CONFIDENCE LIMITS = 2.705504 AND 10.11908

LC50 = 617.3069

95 PERCENT CONFIDENCE LIMITS = 465.469 AND 817.822

LC10 = 391.2405

95 PERCENT CONFIDENCE LIMITS = 191.1778 AND 506.0497

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