5/6/94

MRID No. 429186-14

DATA EVALUATION RECORD

1. **CHEMICAL:** MB 46030 (Fipronil).
Shaughnessey Number: 129121.

2. **TEST MATERIAL:** M & B 46030 technical; Batch No. PGS 963; 95.4% purity; an off-white powder.

3. **STUDY TYPE:** 71-1A. Avian Single Dose Oral LD_{50} Test.
Species Tested: Red-legged partridge (*Alectoris rufa*).

4. **CITATION:** Hakin, B. and M. Rodgers. 1992. M & B 46030: Acute Oral Toxicity (LD_{50}) to Red-Legged Partridge. Study performed by Huntingdon Research Centre Ltd., Cambridgeshire, England. Laboratory Report No. RNP 377/911083. Submitted by Rhone-Poulenc Agrochimie, Lyon Cedex, France. EPA MRID No. 429186-14.

5. **REVIEWED BY:**

Andrew C. Bryceland, Fishery Biologist **Signature:** [signature]
Review Section 5
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) **Date:** 3/15/94

6. **APPROVED BY:**

Ann Stavola, Supervisory Biologist **Signature:** [signature]
Review Section 5
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) **Date:** 5/6/94

7. **CONCLUSIONS:** The study is scientifically sound but does not meet the guideline requirements for an avian oral LD_{50} test due to choice of test species. The test species was not one of those reqired by the Agency. Based on nominal dosages, the LD_{50} was 34 mg/kg, which classifies the test material as highly toxic to the red-legged partridge. The NOEL could not be determined.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

DATA EVALUATION RECORD

1. **CHEMICAL:** MB 46030 (Fipronil).
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5. **REVIEWED BY:**

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6. **APPROVED BY:**

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Signature:
Date:

7. **CONCLUSIONS:** The study is scientifically sound and meets the requirements for an avian oral LD_{50} test. Based on nominal dosages, the LD_{50} was 34 mg/kg, which classifies the test material as highly toxic to the red-legged partridge. The NOEL could not be determined.

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

9. **BACKGROUND:**

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

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A. **Test Animals:** The birds used in the study were red-legged partridges (*Alectoris rufa)* obtained from a supplier in Kent, England. The birds were young adults over eleven months of age and ranged in weight from 372 to 577 grams. The birds were acclimated to the laboratory for 15 days prior to testing. Water was continuously accessible. Except for a 21-hour fasting period immediately prior to dosing, Standard HRC layer diet in pellet form was offered *ad libitum* during acclimation and testing. No antibiotics were incorporated in the diet.

B. **Test System:** All birds were housed indoors in tiered pens (1.44 x 0.55 x 0.50 meters) constructed of plastic-coated wire. Lights provided seven to eight hours of illumination per day. The average temperature was 17-19°C and the average relative humidity was 75%.

C. **Dosage:** Twenty-one-day single dose oral LD_{50} test. Based on the results of a range-finding test, the five dosages selected were 16, 24, 36, 53, and 80 milligrams of test material per kilogram of body weight (mg/kg). The dosages were not corrected for the percentage purity of the test material. Control birds were dosed with the vehicle (corn oil).

D. **Design:** Fifteen days prior to test initiation, groups of ten birds (five males and five females) were arbitrarily assigned to each treatment and control group by body weight so that all test groups would have similar initial bodyweight means. The birds were separated by sex.

The test substance was dispersed in corn oil and intubated directly into each bird using a plastic catheter and disposable syringe. A 20 milliliter (ml) sample of each dosing solution was taken immediately after preparation. The samples were stored at -20°C for possible future analysis.

Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight. The control birds received a corresponding volume of corn oil only. The birds were dosed at a volume of 10 ml/kg of body weight.

The birds were observed daily during the study and at frequent intervals during the post-treatment period. Mortalities, signs of toxicity, and abnormal behavior were recorded at each observation.

The birds were weighed individually 15 and 7 days prior to the start of the study, at test initiation, on day 7, 14, and on the last day of the study (day 21). Average group food consumption values were recorded several times during the acclimation period and for days 1-7, 8-14, and 15-21 of the study.

Pathological examinations were performed on all birds that died during the study.

E. **Statistics:** The LD_{50} was determined by probit analysis. Analysis of variance was carried out for individual body weight data.

12. **REPORTED RESULTS:** None of the control birds or birds dosed at 16 mg/kg showed any clinical signs during the study (Appendix 2, attached).

All birds dosed at the 24 mg/kg level were subdued on days 7 and 8. One bird died on day 8 and one bird died on day 11. All other birds were normal in appearance and behavior after day 8.

Five birds died at the 36 mg/kg level. There were three mortalities by day 4, one death on day 9 and one death on day 14. The birds that died by day 4 did not show any clinical signs of toxicity prior to death. All other birds in the treatment group showed subdued behavior for varying lengths of time during the study.

There were eight deaths and one necessary sacrifice by day 7 in the 53 mg/kg group. The bird that was sacrificed on day 7 had been unable to stand and it was flapping its wings against the cage floor. Since it was considered that the bird would not recover, it was included in the LD_{50} calculation. All of the birds in this group showed subdued behavior, and some of the birds were unsteady in their movements.

All birds dosed at 80 mg/kg died before the completion of the study. Nine birds died by day 8 after showing signs of toxicity including subdued behavior and unsteadiness. The tenth bird started to show signs of recovery, but then relapsed and died on day 16.

On day 7, males dosed at the 16, 24, and 36 mg/kg levels had significantly lower body weights when compared with the controls (Table 2, attached). On days 14 and 21, only the male birds in group 4 were significantly lighter in weight than the controls. Data from the two highest dosage level

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groups were not included in the statistical analysis do to the high amount of mortality.

Females dosed at the 24 and 36 mg/kg levels had significantly lower body weights on day 7 when compared with the control group.

A clear reduction in food consumption during days 1 to 7 was observed in all treated groups, although only a small reduction was observed in the 16 mg/kg group (Table 3, attached).

The only finding that resulted from the pathological examination was that several of the birds were thin. There were no physiological abnormalities in any of the birds examined.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The LD_{50} for the test material was calculated to be 34 mg/kg with 95% confidence limits of 28 to 42 mg/kg. The no-observed-effect-level (NOEL) for mortality and clinical signs of toxicity was 16 mg/kg.

Quality Assurance and Good Laboratory Practice statements were included in the report indicating conformance with GLP regulations as set forth in 40 CFR Part 160.

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

A. **Test Procedure:** The test procedures were in accordance with Subdivision E and SEP guidelines except for the following deviations:

The test species is not a species recommended by SEP guidelines, but parallel studies (MRID Nos. 429186-16 and 429186-17) were performed using recommended species (mallard ducks and bobwhite quail).

The birds were not randomly assigned to groups. Instead, they were assigned to groups based on body weight. The groups were then randomly assigned treatment levels.

The photoperiod was only seven to eight hours, which is less than the recommended ten hours of light. The shorter photoperiod was used in order to lessen aggressive behavior in the birds.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the LD_{50} value (attached

printout). The LD_{50} (34 mg/kg) and confidence interval were the same as reported by the authors. The slope of the probit dose response curve was 6.8.

C. **Discussion/Results:** The authors failed to report two of the deaths that occurred in the 36 mg/kg group in the written report of results. The deaths were reported in Appendix 2 (attached). One of the birds in the group died on day 9 and one of the birds died on day 14.

Pathological examinations were performed only on birds from the 80 mg/kg treatment group. A cross-section of test groups may have provided more definitive evidence that there were also no abnormalities at lower dose levels.

The reviewer does not agree that the NOEL was 16 mg/kg. Although no clinical signs were observed at 16 mg/kg, the authors stated that on day 7, "males in group 2 - 4 (M&B 46030 at 16 - 36 mg/kg) had significantly lower bodyweights when compared with the controls." The reviewer concludes that an NOEL was not established in this study.

This study is scientifically sound but does not meet the guideline requirements for an oral LD_{50} test. With an LD_{50} of 34 mg/kg, the test material is classified as highly toxic to red-legged partridges. An NOEL could not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** This test was performed using a non-standard test species, the SEP states that "testing must be done on either a waterfowl species, preferably mallard duck, or an upland game species, preferably bobwhite quail".

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes; January 10, 1994.

Figwell Revised

Page _____ is not included in this copy.

Pages 7 through 11 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

√ FIFRA registration data.

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_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Nicole Jurczyk FIPRONIL ALECTORIS RUFA 01-10-94

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
80	10	10	100	9.765625E-02
53	10	9	90	1.074219
36	10	5	50	62.30469
24	10	2	20	5.46875
16	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 16 AND 53 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 35.99999

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1144044	34.41878	28.26784	41.4913

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.2161265	1	.9076186

SLOPE = 6.786438
95 PERCENT CONFIDENCE LIMITS = 3.631464 AND 9.941412

LC50 = 34.35248
95 PERCENT CONFIDENCE LIMITS = 28.30963 AND 41.57258

LC10 = 22.32648
95 PERCENT CONFIDENCE LIMITS = 14.34758 AND 27.32078

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