

DATA EVALUATION RECORD

1. CHEMICAL: Metolachlor (108801)
2. FORMULATION: Technical
3. CITATION: Truslow Farms Incorporated (1976). Acute Oral LD₅₀ -- Mallard Ducks, CGA - 24705 Technical, Final Report: Project No. 108 - 117. Received Nov. 23, 1976 under Application for New Registration, (Unpublished report prepared for CIBA-GEIGY Corp., Greensboro, N.C.; CDL226955).
4. REASON FOR REVIEW: Generic Standard for Metolachlor
5. REVIEWED BY: John S. Leitzke
Ecologist
Ecological Effects Branch
Criteria and Evaluation Division
6. DATE REVIEWED: 2/10/78
7. TEST TYPE: Avian Acute Oral LD₅₀
 - A. Test ID: ES. C.1.
 - B. Test Species: Mallards (Anas platyrhynchos)
 - C. Test Material: Technical (CGA - 24705)
 - D. Reported Results: Acute oral LD₅₀ equals 1750 mg/kg. Symptoms preceeding death were loss of coordination, salivation and convulsions. Only at the highest dosage level (4640 mg/kg) were there decreases in average weight gain and average feed consumption; 50% mortality (5 out of 10) occurred in ducklings receiving 4640 mg/kg.
 - E. Summary of Conclusion: The statistical analysis of the data was grossly in a error. A recalculation gives an LD₅₀ of 4640 (3000-7200) mg/kg as opposed to the cited 1750 (1337 - 2289) mg/kg. Due to this and several major deviations from recommended test protocols, this study cannot be accepted as fulfilling the registration requirement for an avian acute oral LD₅₀.

F. Materials and Methods:

- 1) **Test Procedure:** In several instances test procedures did not follow generally accepted guidelines. Generally accepted guidelines give the age of the birds as no less than 16 weeks vs. 14-day old mallard ducklings used in this study; guidelines give a minimum test duration of 14 days vs the 8 days used; guidelines give instructions for fasting test birds 15 hours prior to dosing vs no fasting; finally, birds should be randomly assigned to the test groups so that the average weights across test groups are more or less uniform vs average body weights of 205.2_g in the metolachlor group, 188.2_g in the dieldrin (positive control) group and 179.8_g in the negative control (corn oil only) group. Initial and final body weight, estimated feed consumption, symptoms and mortality were noted.
 - 2) **Statistical Analysis:** Statistical analysis was stated to have been conducted using the method of Litchfield, J.T. Jr. and Wilcoxon, F. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 96:99-113. The data was recalculated using the above method, and a full discussion of this is given below.
- G. **Discussion/Results:** The acute oral LD₅₀ (with 95% confidence limits was originally given as 1750 (1337 - 2289) mg/kg; the LD₁₀ was given as 1000 (650 - 1536) mg/kg and the LD₉₀ as 3063 (2017 - 4651) mg/kg; the Chi-Square value was given as 2.023 with 1 degree of freedom ($p < .05$). However, an examination of the cumulative mortality data showed that 0/10 died at 1000 mg/kg, 1/10 at 2150 mg/kg and 5/10 at 4640 mg/kg. In a recalculation of the given data, the given LD₁₀, 50 and 90 figures were plotted on 1d - p graph paper; the Chi-Square value was recalculated as 177.1. The LD₅₀ was recalculated as 4640 (3000-7200) mg/kg with a Chi-Square value of 0.29 ($p < .05$). The original calculations were grossly in error.

The given LD₅₀ of dieldrin (the positive control) was 64.8 mg/kg (which agrees with the reported mortality data) for the 14-day old mallard ducklings. Tucker and Crabtree (1970) (Tucker, R.K. and Crabtree, D.G. 1970. Handbook of toxicity of pesticides to wildlife. Bur. Sport Fish. Wildl., Resource Publ. No. 84.) give an LD₅₀ of 381 mg/kg for 6-7 month old mallards. This difference in LD₅₀ values is not altogether unexpected in the light of the data of Hudson et al. (1972) (Hudson, R.H., Tucker R.K. and Haegle, M. A. 1972. Effect of age on sensitivity: Acute oral toxicity of 14 pesticides to mallard ducks of several ages. Toxicol. Appl. Pharmacol. 22(4): 556-61.) which showed that chlorinated hydrocarbons can have LD₅₀'s on 7-day and 30-day old mallards that are appreciably less (5-6x) than those on 6-month old mallards. However, since organophosphates were shown to possibly have the opposite age-dependency curve (i.e. increasing sensitivity with age), this differential age susceptibility response varies between chemical groups. Since it is not known how the toxicity of acetanilides varies with age, no such statement can be made about the toxicity of metolachlor to 4 ~~or more month~~ ^{month or more} old mallards as well.

There are some deviations from normally expected figures, also in body weights and efficiency of feed utilization. As noted above, initial body weights varied markedly across the test groups from 205.2_g in the metolachlor group, 188.2_g in the dieldrin group and 179.8_g in the negative controls.

The normally expected weight of 14-day old mallard ducklings is about 150g. The final body weight of the negative controls was 367.2, as opposed to a normally expected 200,. Efficiency of feed utilization in the negative controls was as high as 55 to 63%, which is rather on the high side.

The deviations of test procedures from normally accepted guidelines, the grossly in error statistical analysis and the discrepancies in body weights and efficiency of feed utilization cast great doubt on the accuracy and acceptability of the study and are enough to invalidate it.

H. Reviewer's Evaluation:

- 1) Test Procedures: Test procedures did not follow generally accepted guidelines.
- 2) Statistical Analysis: The original statistical analysis was grossly in error; the LD₅₀ was recalculated from given cumulative mortality data using the generally accepted method cited originally.
- 3) Validation:
 - a) Category: Supplementary
 - b) Rationale: Although the experimental design was sound in and of itself and although the cumulative mortality data can be regarded as real and true, the deviations of test procedures from normally accepted guidelines, the grossly in error statistical analysis and the discrepancies in body weights and efficiency of feed utilization are enough to invalidate the study as fulfilling the registration requirement for an avian acute oral LD₅₀.
- 4) Conclusions: The study indicates that there might be moderate acute toxicity of methachlor to mallards.

LITCHFIELD-WILCOXON PROBIT ANALYSIS WORK SHEET

Chemical Metolachlor DRC _____ Test Animal Mallards Age _____

Formulation date _____ Test date _____ Observation period _____ days

Type of administration _____ Formulation _____

Dose	No. Dead/ Total No.	Observed % Mortality	Expected % Mortality	$\Delta(O-E)$	Contribution to Chi
1000	0 / 10	0	0.1 (10%)	0.1 (10)	0.001 (11)
2150	1 / 10	10	6 (68%)	4 (58)	0.028 (1.6)
4640	5 / 10	50%	50 (98.6%)	0 (48.6)	0 (16)
	1				
	1				
Total animals = 30					Total 0.029 (11.71)

K = 3

$$\chi^2 = \text{Contribution to Chi} \times \frac{\text{Total animals}}{K} = \frac{177.1}{3} = 0.29$$

Degrees of freedom, $n = K - 2 = 1$

χ^2 (p=.05) for 1 deg. of freedom = 3.84

$$ED_{84} = 7600 \quad S = \frac{ED_{84} / ED_{50} + ED_{50} / ED_{16}}{2} = \frac{16.4 + 16.4}{2} = 16.4$$

$$ED_{50} = 4640 \quad S = 16.4$$

$$ED_{16} = 2850$$

Confidence limits (.05) for ED_{50} :

$$f \cdot ED_{50} = S \cdot 2.77 / \sqrt{N} = 16.4 \cdot 0.876 = 1.55$$

$$ED_{50} / f \cdot ED_{50} = \text{lower limit} = 3000 \quad ED_{50} = 4640$$

$$ED_{50} \times f \cdot ED_{50} = \text{upper limit} = 7200$$

Confidence limits (.05) for S:

$$R = \quad S = \quad A =$$

$$f_s = A^{10(K-1)/K} \sqrt{N} =$$

$$S / f_s = \text{lower limit} =$$

$$S \times f_s = \text{upper limit} =$$

$$S =$$

