Data Evaluation Report on the acute dietary toxicity of IM-1-4, a metabolite of acetamiprid insecticide, to the Mallard Duck (Anas platyrhynchos)

PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090
EPA MRID Number 446518-62

Data Requirement: : PMRA DATA CODE: 9.6.2.5-2
EPA DP Barcode:
OECD Data Point: IIA 8.1.2
EPA Guideline: US EPA Subdivision E Guideline 71-2

Test material: **IM-1-4 (Alias - RPA 539373)** **Purity (%):** 99.6%
Common name: a metabolite of acetamiprid
Chemical name: NA
IUPAC: NA
CAS name: NA
CAS No.: not available
Synonyms: RPA 539373

Primary Reviewer: Alison McLaughlin **Date:** January 30th 2001
For PMRA

Secondary Reviewer(s): Hemendra Mulye, PhD **Date:** June 5, 2001
{EPA/OECD/PMRA}

Company Code: [For PMRA]
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EFED Shared drive Copy

CITATION: Brewer, L.W., Taliaferro, M.C. and V. Miller. 1998. 5-Day Dietary Toxicity Test with IM-1-4 in the Mallard Duck (Anas platyrhynchos), EBA Inc., 2900 Quakenbush Rd., Snow Camp, NC 27349. Laboratory Project Identification No. 019803, Sponsor: Rhone-Poulenc Ag Company, NC, USA. September 1 1998. Unpublished.

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EXECUTIVE SUMMARY:

The acute dietary toxicity of IM-1-4, a metabolite of acetamiprid insecticide, to 10 day old Mallard Duck (Anas platyrhynchos) was assessed over 5 days in accordance with an experimental protocol based on the US EPA Avian dietary LC_{50} test (OPPTS 850.2200). IM-1-4 was administered to controls and five treatment groups, each with ten birds. The treatment groups received nominal dosages of 5, 50, 500, 2500 and 5000 mg a.i/kg dw (ppm) in diet respectively. These dosages were equivalent to measured concentrations of 5.93 $\pm$ 0.57 for the 5 ppm level; 43.09 $\pm$ 0.46 for the 50 ppm level; 436.71 $\pm$ 75.82 for the 500 ppm level; 2260.74 $\pm$ 177.10 for the 2500 ppm level; and 4717.14 $\pm$ 707.13 for the 5000 ppm dose level. The 5 day acute dietary LC_{50} was >5000 mg a.i/kg dw of diet (nominal). The 5 day NOEC of IM-1-4 based on mean body weights was 500 mg a.i/kg dw of diet (nominal).

Sublethal effects at the two highest dose levels included depression of normal bodyweight increase. Birds fed nominal dosages of 2500 and 5000 mg a.i/kg dw (ppm) in diet demonstrated depression of normal bodyweight increase by about 10 grams during the treated feed trial. There was no significant reduction in food intake which would have accounted for the reduced mean bodyweight of these groups, thus, the reduction in bodyweight may be a direct sublethal effect of the test substance rather than an indirect effect of starvation. This difference in bodyweights rapidly disappeared, however, after the three day follow-up with clean feed. No signs of intoxication or aberrant behaviour were reported amongst the observations. There was no necropsy data.

This toxicity study is classified acceptable and satisfies the guideline requirement for an acute dietary toxicity study for Mallard Duck (Anas platyrhynchos). The LC_{50} value was found to be > 5000 mg ai/kg bw. On the basis of this result, this metabolite (IM-1-4) would be classified as practically non-toxic to Mallard Duck (Anas platyrhynchos) on an acute dietary basis. There were sub-lethal signs of toxicity, such as depression of bodyweight increase, reported in all birds treated at the 2500 and 5000 mg ai/kg levels. The NOEC value was 500 mg ai/kg bw based on mean bodyweight.

Results Synopsis

Test Organism: Mallard Duck (Anas platyrhynchos), 10 days old, mean initial weight 185 g.

LC_{50}: >5000 mg a..i./kg diet	Endpoint(s) Effected: mortality
NOEC: 500 mg a.i./kg diet	Endpoint(s) Effected: mean body weight

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The method followed was an EBA Inc. laboratory protocol based on the US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, Series 71-1 Avian dietary LC_{50} test, dated Oct. 1982 and draft revised guideline dated Mar 1988. The protocol was provided in Appendix L and deviations to the protocol were listed in Appendix M. Deviations included increased room temperature, reduced humidity, no measurement of illumination intensity and lower than recommended analytical concentrations of the test substance in the range-finding study.

COMPLIANCE: It was stated that this study had been conducted according to GLP Standards under the US EPA, FIFRA, 40 CFR Part 160, with the exception that feed analysis for pesticides, PCBs and toxic metals would not be analyzed under GLP compliance as stated in the protocol but would be analysed using standard US EPA procedures. Signed and dated GLP and Quality Assurance were provided. There was also a signed and dated Statement of No Data Confidentiality Claim.

A. MATERIALS:

1. Test Material IM-1-4

Description: Colourless waxy solid

Lot No./Batch No. : NK-97127

Purity: 99.6 %

Stability of Compound Under Test Conditions: Results of the analytical chemistry report (Appendix D) indicate that IM-1-4 was stable at nominal

concentrations of 5 ppm and 5000 ppm in the avian diet formulation assessed over a period of 7 days during storage under ambient conditions.

Storage Conditions of Test Chemicals: Prior to testing, IM-1-4 was stored at -10°C in darkness in an airtight container; the test substance analysis certificate reported that IM-1-4 is stable for 20 months in a freezer in the dark.

Physicochemical properties of [test material].

Parameter	Values	Comments
Water solubility at 20°C	not reported	* reported elsewhere as 0.4% at 25°C
Vapour pressure	not reported	* reported elsewhere as <1.0 x 10-6 Pa at 25°C
UV absorption	not reported	
pKa	not reported	
Kow	not reported	

* These results come from the Salinity Challenge Study in this same data submission

2. **Test organism**:

Species: Mallard Duck (Anas platyrhynchos)

Age at study initiation: 10 days old at the introduction of the test diet

Weight at study initiation: (mean and range): mean 185 g, range 175 - 197 g.

Source: Whistling Wings, Hanover, IL, USA

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A preliminary range finding test had been performed on birds at measured concentrations of 3.41 ± 0.34 for the 5 ppm level; 31.21 ± 0.54 for the 50 ppm level;

364.65 ± 53.77 for the 500 ppm level; 2054.95 ± 96.73 for the 2500 ppm level; and 4694.87 ± 987.73 for the 5000 ppm dose level. No mortality occurred in the range-finding study.

b) Definitive Study: The definitive study was performed on birds at measured concentrations of 5.93 ± 0.57 for the 5 ppm level; 43.09 ± 0.46 for the 50 ppm level; 436.71 ± 75.82 for the 500 ppm level; 2260.74 ± 177.10 for the 2500 ppm level; and 4717.14 ± 707.13 for the 5000 ppm dose level. Nominal dosages were prepared by mixing the test material directly into the feed using a Hobart mixer.

Table 1: Experimental Parameters

Parameter	**Details**	**Remarks**
		Criteria
Acclimation Period: Conditions (same as test or not): Feeding: Health (any mortality observed):	 9 days acclimation yes Purina Startena basal diet *ad libitum* none reported	acceptable *OECD requires at least 7 days of acclimation*
Pen size and construction materials	epoxy-coated wire floor pens 56 in (l) x 28 in (w) x 30 in (h), in randomized order	 *EPA requires: about 35 x 100 x 24 cm; OECD requires: 300 cm^2 for bobwhite and 600 cm^2 for mallard*
Test duration	9 days acclimation, 5 days treatment, 3 days post-treatment	acceptable *EPA/OECD requires: 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations Nominal: Measured:	 5 *mg ai/kg diet* 50 *mg ai/kg diet* 500 *mg ai/kg diet* 2500 *mg ai/kg diet* 5000 *mg ai/kg diet* 5.93 ± 0.57 *mg ai/kg diet* 43.09 ± 0.46 *mg ai/kg diet* 436.71 ± 75.82 *mg ai/kg diet* 2260.74 ± 177.10 *mg ai/kg diet* 4717.14 ± 707.13 *mg ai/kg diet*	acceptable *Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC_{50} > 5000 mg ai/kg diet.* Measured conc. should be 80% of the nominal

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Parameter	Details	Remarks
		Criteria
Solvent/vehicle, if used Type: Amount:	The test substance, IM-1-4, was added as the solid test material.	The EPA guidelines are not clear on policy for the addition of dry powder to a test diet.
		EPA requires: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. Solvent not more than 2%.
Diet preparation and feeding	Treated diets were prepared by mixing the test substance with the untreated basal diet. Specific information on when the diets were prepared was supposed to have been reported in Appendix C: Reference Substance Tracking, but this information was not clearly indicated.	
		EPA requires: Control group tested with diet containing the maximum amount of vehicle used in treated diets
Was detailed description and nutrient analysis of the basal diet provided (Yes/No)	Yes. The composition of the diet was described.	acceptable
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes. Homogeneity and stability were acceptable.	acceptable
Feed withholding period	none	
Number of birds per replicate/groups For negative control: For vehicle control: For treated:	 10 for each of three replicates (n=30) NA 10 for each treatment level	
		EPA requires: 10 birds each (strongly recommended)
Number of replicates/group (if used) For negative control: For vehicle control: For treated:	 3 NA 1 at each treatment level	
Test conditions Temperature:	 *32-40 °C* in the animal room An infra-red heat lamp was suspended over each box to provide additional	The laboratory temperature was out of the range recommended by EPA (i.e. 22-27 degrees C)
		Brooder temperature:

Parameter	Details	Remarks
		Criteria
	heat	*EPA: about 35°C (95°F)* *Room temperature:* *EPA: 22-27°C (71-81°F);* *OECD: range of 22-38 °C based on bird age and species (see OECD 205)* *Relative humidity:* *EPA: 30-80%* *OECD: 50-85% based on bird species (see OECD 205)* *Photoperiod:* *EPA: Minimum of 14 h of light* *OECD: 12-16 h of light*
Relative humidity (%):	34-45 %	
Photoperiod:	14 hour light / 10 hour dark	

b) Analytical Chemistry Report analysis for the Measured Dose Concentrations:

Table 2. Calculation of the Measured Dose

Nominal conc. (mg ai/kg dw) (ppm):	Mean analyzed conc. (mg ai/kg dw) (ppm):	Measured concentration deviation from nominal value	% of Nominal
5	5.93 ± 0.57 *mg ai/kg diet*	+ 0.93	118.6
50	43.09 ± 0.46 *mg ai/kg diet*	- 6.91	86.1
500	436.71 ± 75.82 *mg ai/kg diet*	- 63.29	87.3
2500	2260.74 ± 177.10 *mg ai/kg diet*	- 239.26	90.4
5000	4717.14 ± 707.13 *mg ai/kg diet*	- 282.86	94.3

Results of the Analytical chemistry report confirmed that the nominal concentrations of the dietary formulation were very accurate. The report confirmed that the dietary formulations were stable over a period of seven days. The report also showed that the dietary formulations were homogeneous at the time of preparation, however, there were no results for homogeneity at study completion. Average concentrations of IM-1-4 found in the homogeneity verification samples were 4.08 ppm ± 0.36 for the 5 ppm level and 5398.84 ppm ± 371.05 for the 5000 ppm level.

2. Observations:

Table 3: Observations

	Details	Remarks

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Parameters		*Criteria*
Parameters measured (mortality/body weight/ mean feed consumption/ others)	mortality, intoxication body weight feed consumption	*OECD : the mortality in the controls should not be exceed 10% at the end of the test.*
Indicate the stability and homogeneity of test chemical in the diet	Stable over 7 days Homogeneous at study initiation	
Indicate if the test material was regurgitated	No regurgitation was reported	
Treatments on which necropsies were performed	There was no macroscopic post mortem examination following the euthanasia of the birds at the conclusion of the test.	
Observation intervals (days)	Bodyweight: -4, 0, 5, 8 Food consumption: -4 to -1 pre-test, daily during testing (1,2,3, 4, 5), and 6 to 8 post-testing	
Were raw data included?	Raw analytical data, raw data for bodyweight, and food consumption were included.	

II. RESULTS AND DISCUSSION:

A. MORTALITY: No mortalities were noted either in the range finding study or the definitive study.

Table 4: Effect of IM-1-4 on mortality of Mallard Duck (Anas platyrhynchos).

Treatment (mg a.i. kg diet)	**No. of birds per treatment**	**Mortality (Days of Study)**							
		-9 to -1	**1**	**2**	**3**	**4**	**5**	**6 to 8**	**Total**
control	10								0
control	10								0
control	10								0
Test concentration 5	10								0

Test concentration 50	10								0
Test concentration 500	10								0
Test concentration 2500	10								0
Test concentration 5000	10								0
LC_{50}	estimated at > 5000 ppm								

B. SUB-LETHAL TOXICITY ENDPOINTS:

A sublethal effect at the two highest dose levels was indicated by depression of normal bodyweight increase. Birds fed nominal dosages of 2500 and 5000 mg a.i/kg dw (ppm) in diet demonstrated depression of normal bodyweight increase by about 10 grams during the treated feed trial. Examination of the food consumption data would not suggest that food avoidance was a factor. There were no clinical signs of intoxication reported at any dose level.

Table 5: Group mean bodyweights and bodyweight changes (g)

Treatment (mg a.i. kg diet)	**No. of birds per treatment**	**Bodyweight (g)**		**Bodyweight increase (g)**		
		Day 0	**Day 5**	**Mean change days 0 to 5**	**% Change days 0 to 5**	**% Change within 3 days post testing**
control	10	191.4	238.3	47.0	25	15
Test concentration 5	10	178.3	233.5	55.3	31	19
Test concentration 50	10	197.4	243.8	46.4	24	16
Test concentration 500	10	184.4	231.4	47.1	26	17
Test concentration 2500	10	175.1	213.0	37.9	22	22
Test concentration 5000	10	180.7	217.2	36.6	20	18
NOEC	500 mg a.i./ kg diet					

Table 6: Group mean food consumption (g/bird/day)

Treatment	**No. of**	**Study Days**

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(mg a.i. kg diet)	birds per treatment	1	2	3	4	5	1 to 5	6 to 8
control	10	35.6	35.4	35.5	34.3	33.0	173.8	152.2
control	10	34.1	34.9	32.8	35.1	38.4	175.3	151.1
control	10	35.8	36.6	35.7	36.3	37.6	182.0	157.6
Test concentration 5	10	35.6	37.1	35.8	35.2	36.7	180.4	158.6
Test concentration 50	10	35.8	37.7	36.4	36.3	36.4	182.6	146.4
Test concentration 500	10	35.8	33.7	35.4	35.3	37.4	177.6	145.7
Test concentration 2500	10	35.7	35.7	34.5	37.2	36.9	180.0	156.8
Test concentration 5000	10	35.4	35.4	35.8	35.6	38.4	180.6	145.4

C. REPORTED STATISTICS: No statistical analysis was performed for mortality because none was observed. Statistical analysis was performed for bodyweight changes. A t-test comparison of the values of mean bodyweight change indicated that there was a statistical difference ($\leq$0.05) between the 2500 ppm and 5000 ppm treatment groups and the controls. A t-test comparison of the arc sine(square root (y)) values of percent bodyweight change indicated no statistical differences between any of the treatment groups and the controls. Based on transformed data for % bodyweight gained between day 0 and day 5, a NOEL of 5000 ppm was proposed. There were no statistical differences noted in feed consumption.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER: There were no mortalities reported, hence, the LC50 value > 5000 ppm. Using Excel, a two sample student's t-test was performed on the proponent's raw data for % bodyweight change as well as mean bodyweight change of control birds versus birds treated with doses of 500 ppm, 2500 ppm and 5000 ppm. The test was set to be two tailed with an assumption of equal variance. This t-test is normally used to determine whether or not a significant difference exists between two sample means. There was no compelling justification for transforming the raw data for this test. A copy of the work sheet for these tests is shown in Appendix 1 of this study review.

Based on non-transformed data for % bodyweight gained between day 0 and day 5, there is a statistical difference between data at the control and 5000ppm levels (p=0.048). If the data for mean bodyweight is compared the results indicate a significant difference of the means for control versus birds in the 5000 ppm group (p=0.016), as well as for the control versus birds in the 2500

ppm group (p=0.016). The results indicated no significant difference between the means for control versus birds in the 500 ppm group (p=0.965). The NOEC was determined to be 500 ppm on the basis of changes in bodyweight during the test period.

E. STUDY DEFICIENCIES:

1) There were minor deviations from the standard EPA protocol as noted in the experimental parameters.

F. REVIEWER'S COMMENTS: None.

G. CONCLUSIONS: This toxicity study is classified acceptable. The LC_{50} value was found to be > 5000 mg ai/kg bw. On the basis of this result, this metabolite (IM-1-4) would be classified as practically non-toxic to Mallard Duck. There were sub-lethal signs of toxicity, such as depression of bodyweight increase, reported in all birds treated at the 2500 and 5000 mg ai/kg levels. The NOEC value was 500 mg ai/kg bw based on mean bodyweight. There were no statistical differences noted in feed consumption, hence, the difference in bodyweight may be a direct effect of the test substance rather than an indirect effect of starvation.

III. REFERENCES:

Anderson, A., Dawe, I.S., and L. Martin. Analytical Chemistry Report. IM-1-4: The Analysis in Avian Diet Formulations. NPS 59/932525.

Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50: 1096-1121.

Stephan, C. E. 1977. Methods for calculating an LC_{50} , Aquatic Toxicology and Hazard Evaluations. ATSM Publication No. STP 634. pp.65-84

TOXSTAT, 1994. *Users Manual for Toxstat 3.4,* D.D.Gulley, ED., West, Inc., Cheyenne, WY.

U.S. Environmental Protection Agency. January 1995. *Ecological Effects Test Guidelines.* OPPTS 850.2200: Avian Dietary Toxicity Test.

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildelife and Aquatic Organisms. Series 71:*Avian and Mammalian Testing.

pp. 37-42.

Zar, J.H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J., U.S.A.

Body weight Changes in grams

Start 5000ppm	End 5000ppm	Diff 5000ppm	Start 0ppm	End 0ppm	Diff 0ppm	Start 2500ppm	End 2500ppm	Diff 2500ppm	Start 500ppm	End 500ppm	Diff 500ppm
188	219	31	191	236	45	151	187	36	199	255	56
202	234	32	201	251	50	155	190	35	169	219	50
155	183	28	165	217	52	185	201	16	217	236	19
186	235	49	188	240	52	204	240	36	186	227	41
151	187	36	189	251	62	166	214	48	176	224	48
181	241	60	186	231	45	185	220	35	180	231	51
168	185	17	192	243	51	173	209	36	151	203	52
184	228	44	191	251	60	190	246	56	202	258	56
183	200	17	195	238	43	166	209	43	189	232	43
211	263	52	199	244	45	177	215	38	178	231	53
			214	276	62						
0.015975			194	232	38		0.015818			0.965201	
t-test, 2 tails equal variance			167	224	57		t-test, 2 tails equal variance			t-test, 2 tails equal va	
			179	230	51						
			176	234	58						
			162	216	54		% bodyweight changes				
			213	260	47		0ppm	500ppm	2500ppm	5000ppm	
			181	219	38		19.0678	21.96078	17.64706	14.15525	
			174	223	49		19.92032	22.83105	18.42105	13.67521	
			208	270	62		23.96313	8.050847	7.960199	15.30055	
			207	237	30		21.66667	18.06167	15	20.85106	
			218	248	30		24.7012	21.42857	22.42991	19.25134	
			172	211	39		19.48052	22.07792	15.90909	24.89627	
			217	258	41		20.98765	25.61576	17.22488	9.189189	
			188	235	47		23.90438	21.70543	22.76423	19.29825	
			200	226	26		18.06723	18.53448	20.57416	8.5	
			226	262	36		18.44262	22.94372	17.67442	19.77186	
			197	260	63		22.46377				
			173	215	42		16.37931	0.697992	0.152829	0.047654	
			185	222	37		25.44643				
							22.17391				
							24.78632				
							25				
							18.07692				
							17.3516				
							21.97309				
							22.96296				
							12.65823				
							12.09677				
							18.48341				
							15.89147				
							20				
							11.50442				
							13.74046				
							24.23077				
							19.53488				
							16.66667				

Appendix 1: