UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

PC Codes: 005100
DP Barcode: D301682

MEMORANDUM

June 29, 2005

SUBJECT: **Aminopyralid Ecological Effects Date Evaluation Records (DERs)**

TO: Joanne Miller, Product Manager
Registration Division (7505C)

FROM: Brian Kiernan, Biologist 6/24/05
Environmental Fate and Effects Division (7507C)

THRU: Elizabeth Behl, Branch Chief for
Environmental Fate and Effects Division (7507C)

The Environmental Fate and Effects Division (EFED) has completed its review of ecological effects studies for aminopyralid, after secondary review by PMRA.

Table 1 lists all of the available ecological effects studies, and the acceptability of each study. In general, all but two of the studies contained sufficient information on the ecological effects of aminopyralid for EFED to complete an ecological risk assessment of the chemical.

2030229

Table 1. Status of ecological effects data adequacy for aminopyralid.

Guideline	Date Requirements	Are Data Adequate for Ecological Risk Assessment?	MRID	Study Classification
71-1(a)(b)	Avian Acute Oral LD_{50} Bobwhite Quail	Yes	462358-08 462358-09	Acceptable Supplemental[1]
71-2(a)	Avian Subacute Dietary Bobwhite Quail	Yes	462358-10	Acceptable
71-2(b)	Avian Subacute Dietary Mallard Duck	Yes	462358-11	Acceptable
71-4(a)	Avian Reproduction Bobwhite Quail	No	462358-12	Supplemental[2]
71-4(b)	Avian Reproduction Mallard Duck	Yes	462358-13	Acceptable
72-1(a)	Warmwater Fish Acute Toxicity LC_{50} Bluegill sunfish	Yes	462358-15	Supplemental[3]
72-1(c)	Coldwater Fish Acute Toxicity LC_{50} Rainbow Trout	Yes	462358-14	Acceptable
Non-guideline (based on 72-1a)	Amphibian Larvae Acute Toxicity LC_{50} Northern leopard frog, *Rana pipiens*	Not required	462358-16	Supplemental[4]
72-2(a)	Freshwater Invertebrate Acute Toxicity EC_{50} Water flea	Yes	462358-17	Acceptable
Non-guideline	Midge Chronic Toxicity	Not required	462358-23	Supplemental[4]
72-3(a)	Estuarine/Marine Fish Acute Toxicity LC_{50} Sheepshead Minnow	Yes	462358-20	Acceptable
72-3(b)	Estuarine/Marine Invertebrate Acute Toxicity EC_{50} Eastern Oyster	Yes	462358-18	Acceptable
72-3(c)	Estuarine/Marine Invertebrate Acute Toxicity LC_{50} Mysid Shrimp	Yes	462358-19	Acceptable
72-4(a)	Freshwater Fish Early Life Stage Fathead minnow	Yes	462358-21	Supplemental[5]
72-4(a)	Estuarine/Marine Fish Early Life Stage Silverside or Sheepshead Minnow	No		
72-4(b)	Freshwater Invertebrate Life Cycle Water flea	Yes	462358-22	Supplemental[6]
72-4(c)	Estuarine/Marine Invertebrate Life Cycle Mysid Shrimp	No		
123-1(a)	Tier II Terrestrial Plant Seedling Emergence (GF 871)	Yes	462358-24	Supplemental[7]
123-1(b)	Tier II Terrestrial Plant Vegetative Vigor (GF 871)	Yes	462358-25	Supplemental[8]
123-2	Tier II Aquatic Plant Growth Green Algae, *Pseudokirchneriella subcapitata*	Yes	462358-30	Supplemental
123-2	Tier II Aquatic Plant Growth (Vascular) Duckweed, *Lemna gibba*	Yes	462358-26	Acceptable

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Guideline	Date Requirements	Are Data Adequate for Ecological Risk Assessment?	MRID	Study Classification
123-2	Tier II Aquatic Plant Growth Marine diatom, *Skeletonema costatum*	Yes	462358-28	Acceptable
123-2	Tier II Aquatic Plant Growth Freshwater diatom, *Navicula pelliculosa*	Yes	462358-27	Supplemental
123-2	Tier II Aquatic Plant Growth Blue-Green algae, *Anabaena flos-aquae*	No	462358-29	Unacceptable[9]
141-1	Honey Bee Acute Contact Toxicity	Yes	462358-31	Acceptable
Non-guideline	Honey Bee Acute Oral Toxicity	Not required	462358-32	Supplemental[4]

[1] The study was submitted in support of MRID 462358-08.
[2] Statistically significant differences found in the lowest dose tested for two survival endpoints (hatchling survival per eggs set and 14-day hatchling survival), but it is unclear whether these were treatment-related effects. Together with apparent downward trends in hatchling per live embryos and hatchlings per pen, it is uncertain that the study authors conclusion that these effects are not treatment related can be supported.
[3] Study classified as supplemental since the size of fish (0.18-0.92 g) used was less than the recommended range of 0.5 to 5 g.
[4] Non-guideline study; does not fulfill an OPP guideline.
[5] Replicate data for the days-to-mean hatch and sub-lethal effects were not submitted and could not be verified by EFED
[6] Study classified as supplemental due to excessive water hardness, low dissolved oxygen (31%) and reduced replicate size.
[7] Study classified as supplemental because soil surface watering occurred without report of test substance mobility characteristics and Thiram was applied to sugar beet without further explanation.
[8] Study classified as supplemental because Thiram was applied to sugar beet without further explanation. Both corn and radish were grown under very low light conditions, which may have affected the results.
[9] Study classified as unacceptable because the ability to detect treatment-related effects was compromised by high variability in the controls.

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Data Evaluation Report on the Acute Oral Toxicity of XDE-750 (Aminopyralid) on Northern Bobwhite Quail (*Colinus virginianus*)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-08

Data Requirement:	PMRA DATA CODE	9.6.2.1-2
	EPA DP Barcode	D301682
	OECD Data Point	II A 8.1.1
	EPA MRID	462358-08
	EPA Guideline	§71-1

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: Not reported
CAS name: 3,6-Dichloro-4-amino-2-pyridinecarboxylic acid
CAS No.: Not reported
Synonyms: XDE-750/XR-750

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature:
Date: 9/28/04

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/10/04

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB - IV
Signature: BDK 6/14/05
Date: 11/02/04

Secondary Reviewer(s): Brigitte Lavallée
PMRA (1595)
Signature:
Date: February 2, 2005

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

CITATION: Gallagher, S.P., *et al.* 2001. XDE-750: An Acute Oral Toxicity Study with the Northern Bobwhite. Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 379-106. Study submitted by Dow Chemical Company, Midland, MI for Dow AgroSciences LLC, Indianapolis, IN. Study initiated May 17, 2001 and submitted August 9, 2001.

EXECUTIVE SUMMARY:

The acute oral toxicity of XDE-750 (aminopyralid) to 19-week-old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 14 days. XDE-750 was administered to the birds by oral intubation at nominal concentrations of 0 (deionized water vehicle control), 63, 292, 486, 810, 1350, and 2250 mg a.i./kg bw (adjusted for 94.5% purity).

No mortality occurred at any test level and no treatment-related effects were observed upon terminal necropsy. The acute LD_{50} was >2250 mg a.i./kg bw, the highest level tested, which categorizes XDE-750 (aminopyralid) as practically non-toxic to Northern Bobwhite quail on an acute oral basis. Clinical signs of toxicity (the most sensitive endpoint) were observed in birds from all treatment levels. Effects included reduced reaction to external stimuli (sound and movement), ruffled appearance, lethargy, wing droop, loss of coordination, lower limb weakness, prostrate posture, lower limb rigidity, minor muscle fasciculation, convulsions, loss of righting reflex, depression, and/or gaping. Effects subsided from all affected birds by the morning of Day 1 at the 63 mg a.i./kg level, by the afternoon of Day 3 at the 292 mg a.i./kg level, by the morning of Day 5 at the 486 mg a.i./kg level, by the afternoon of Day 7 at the 810 mg a.i./kg level, and by the morning of Day 8 at the 1350 and 2250 mg a.i./kg levels. The NOEL for sub-lethal effects was <63 mg a.i./kg.

Treatment-related effects on body weight gain were observed for both sexes at the 1350 and 2250 mg a.i./kg treatment levels. From Days 0 to 3, control males increased an average of 9 g, compared to 8, 9, 8, 4, 0, and -4 g for the 63, 292, 486, 810, 1350, and 2250 mg a.i./kg treatment levels, respectively; and control females increased an average of 7 g, compared to 12, 8, 6, 7, 3, and -10 g for the treatment levels, respectively. Body weight changes from 3-7 Days and from 7-14 Days were comparable among all control and treatment groups. The NOEL for body weight changes was 810 mg a.i./kg bw.

A treatment-related effect on feed consumption was observed for both sexes at the 2250 mg a.i./kg treatment level. From Days 0 to 3, mean feed consumption was 19 g/bird/day for control males, compared to 20, 23, 17, 20, 19, and 14 g/bird/day for the 63, 292, 486, 810, 1350, and 2250 mg a.i./kg treatment groups, respectively; and 26 g/bird/day for control females, compared to 21, 21, 30, 20, 23, and 15 g/bird/day for the treatment groups, respectively. Data for this treatment level between Days 4-7 was not available due to a technical error. However, data were comparable between the control and remaining treatment levels from Days 4-7, and between the control and all treatment groups from Days 8-14. The NOEL for food consumption was 1350 mg a.i./kg bw.

This toxicity study is scientifically sound and fulfills the guideline requirements for an acute toxicity study using the Northern Bobwhite quail (§71-1). This study is classified as Acceptable.

EAD Conclusion:
The EAD is in agreement with the conclusions reported by the US EPA reviewer. No mortality occurred during the study. Therefore, the 14-d acute oral LD_{50} for XDE-750 (aminopyralid) is > 2250 mg ai/kg bw, which categorize aminopyralid as practically non-toxic to the bobwhite quail according to the US EPA classification scheme of avian acute oral toxicity (US EPA, 1985). Based on sublethal effects (clinical signs of toxicity), the NOEL value is < 63 mg ai/kg bw the lowest concentration tested. Accuracy of the NOEL value was assessed in a supplementary study (MRID 462358-09).

Results Synopsis

Test Organism Size/Age: Approximately 19-weeks old, 220-304 g (combined sexes)

LD_{50}: >2250 mg a.i./kg bw
NOEL: <63 mg a.i./kg bw
LOEL: 63 mg a.i./kg bw
Endpoint(s) Affected: Clinical signs of toxicity, body weight changes, and feed consumption
Most sensitive endpoint: Clinical signs of toxicity

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The protocol followed procedures of the U.S. EPA Pesticide Assessment Guidelines, Subsection 71-1 (1982). The following deviations from §71-1 were noted:

1. Mortality observed during acclimation (if any) was not reported.
2. The photo-period (8 hours of light) was less than recommended (10 hours of light).
3. A NOEL was not established due to sublethal effects at all treatment levels.

These deviations did not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA, OECD, and Japan MAFF with the following exceptions: stability of the test substance under storage conditions at the test site has not been determined in accordance with GLP Standards, and verification of concentrations, stability, and homogeneity of the test substance in the diluent were not determined (p. 3).

A. MATERIALS:

1. Test Material XDE-750 (aminopyralid)

Description: Cream-colored powder

Lot No./Batch No.: F0031-143 (TSN 102319)

Purity: 94.5%

Stability of Compound Under Test Conditions: N/A

Storage conditions of test chemicals: Ambient

OECD requires water solubility, stability in water and light, pK_a, P_{ow} and vapor pressure of the test compound. OECD requirements were not reported.

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2. **Test organism**:

Species:	Northern Bobwhite quail (*Colinus virginianus*)
Age at study initiation:	Approximately 19 weeks old
Weight at study initiation:	220-304 g (combined sexes)
Source:	Barrett's Quail Farm, Houston, TX

B. STUDY DESIGN:

1. **Experimental Conditions**

a. Range-finding Study: None reported. The test dosages were established based upon known toxicity data provided by the Sponsor (p. 9).

b. Definitive Study:

Table 1: Experimental Parameters.

Parameter	Details	Remarks *Criteria*
Acclimation period:	3 weeks	Beginning 2 days following arrival in the laboratory, test birds were given water soluble antibiotics in their drinking water for 7 consecutive days (p. 11).
Conditions (same as test or not):	Same as test	
Feeding:	Game bird ration (Wildlife International, Ltd., Appendix II, p. 21) and public water from the town of Easton were provided *ad libitum*, except during approximately 16 hours prior to testing.	*EPA recommends that birds be pre-conditioned to the test facilities for at least 15 days.*
Health (any mortality observed):	Birds exhibiting abnormal behavior or physical injury were not used; not otherwise specified.	*OECD recommends that birds be pre-conditioned to the test facilities for at least 7 days.*

Parameter	Details	Remarks *Criteria*
Pen size and construction materials	Battery pens were 78 x 51 x 20/25 (sloping floors) cm, and were constructed with galvanized wire (ceilings and floors) and galvanized sheeting (side walls).	*EPA requires: pens must conform to good husbandry practices and should not create crowding stress.* *OECD lists no criteria for pen construction other than stating that pens should be suitable for the captive rearing of that species.*
Test duration	14 Days	*EPA requires a day for dosing and at least 14 days observation.*
Dose preparation Indicate method of confirmation of dose	Test substance was dispersed in deionized water using a magnetic stirrer (Appendix III, p. 22). Certificate of Analysis included	
Mode of dose administration	Orally intubated into the crop or proventriculus using a stainless steel 14 gauge cannula.	*Gavage or gelatin capsule.*
Dose levels nominal: measured:	0, 63, 292, 486, 810, 1350, and 2250 mg a.i./kg of body weight N/A	The dosages were adjusted to 100% a.i. (p. 11). *EPA requires a minimum of 5 treatment levels unless LD_{50} is demonstrated to be greater than 2250 mg ai/kg.*

Parameter	Details	Remarks *Criteria*
Solvent/vehicle, if used type: amount/bw:	 Deionized water 0.5% (mL/g x 100)	The stock solutions were administered at a constant dosing volume of 5 mL/kg bw (or 0.5%; Appendix III, p. 22). *EPA recommends that the test material be administered without a vehicle if possible. Maximum vehicle should not exceed 0.1 to 1.0% of body weight.*
Number of birds per groups/treatment for negative control: for solvent/vehicle control: for treated:	 N/A 10 10/level	5 males and 5 females per treatment group. *EPA recommends 10 birds per treatment group and 10 birds for each control and vehicle group.*
No. of feed withholding days before dosing	Birds were fasted for at approximately 16 hours prior to dosing.	 *EPA recommends that food should be withheld for at least 15 hours prior to dosing.*
Test conditions Temperature: Relative humidity: Photo-period:	 23.51 ± 0.51°C 61 ± 11% 8-hours light/16-hours dark.	The photo-period was less than recommended. The birds received an average 207 lux of illumination (p. 12). *EPA recommends that a 10 hr light/14 hr dark photo-period.*
Refcrence chemical, if used name: concentrations tested:	None used.	

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2. Observations:

Table 2: Observations.

Parameter	Details	Remarks/Criteria
Parameters measured		
Parameters measured (mortality/individual body weight at test initiation and termination/ mean feed consumption/others)	- Mortality - Clinical signs of toxicity - Individual body weight - Average feed consumption - Gross necropsy	*EPA recommends:* *Body weight measured at test initiation, on Day 14 and at end of the test if the test is extended beyond 14 days.* *Calculation of mortality. Mortality must NOT be more than 10% in controls.* *Feed consumption may be measured as average daily food consumption.*
Indicate if the test material was regurgitated	None reported.	*Regurgitation is an indication that the dose was rejected. The test may have to be repeated if the problem persists.*
Groups on which necropsies were performed	All birds were subjected to gross necropsy.	*EPA recommends that gross necropsies be performed with inspections of the GI tract, liver, kidneys, heart, and spleen.*
Observation intervals	Mortality and signs of toxicity: at least once daily. Body weight: Days 0 (prior to dosing), 3, 7, and 14. Feed consumption per pen: Days 0-3, 4-7, and 8-14.	
Were raw data included?	Yes, sufficient.	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality occurred in any control or treatment group during the 14-day study (Table 1, p. 17). The acute LD_{50} was >2250 mg a.i./kg bw, the highest level tested.

Table 3: Effect of XDE-750 (aminopyralid) on mortality of *Colinus virginianus*.

Treatment (mg a.i./kg bw)		No. of birds	Cumulative mortality							
			day 0	day 2	day 4	day 6	day 8	day 10	day 12	day 14
Vehicle control		10	0	0	0	0	0	0	0	0
63		10	0	0	0	0	0	0	0	0
292		10	0	0	0	0	0	0	0	0
486		10	0	0	0	0	0	0	0	0
810		10	0	0	0	0	0	0	0	0
1350		10	0	0	0	0	0	0	0	0
2250		10	0	0	0	0	0	0	0	0
NOEL		2250 mg a.i./kg bw								
LD_{50}		>2250 mg a.i./kg bw								
Reference chemical	mortality	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LD_{50}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	NOEL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. SUB-LETHAL TOXICITY ENDPOINTS:

Clinical signs of toxicity were observed in birds from all treatment levels (p. 13). Effects included reduced reaction to external stimuli (sound and movement), ruffled appearance, lethargy, wing droop, loss of coordination, lower limb weakness, prostrate posture, lower limb rigidity, minor muscle fasciculation, convulsions, loss of righting reflex, depression, and/or gaping. Effects subsided from all affected birds by the morning of Day 1 at the 63 mg a.i./kg level, by the afternoon of Day 3 at the 292 mg a.i./kg level, by the morning of Day 5 at the 486 mg a.i./kg level, by the afternoon of Day 7 at the 810 mg a.i./kg level, and by the morning of Day 8 at the 1350 and 2250 mg a.i./kg levels. In addition, one female each from the 1350 and 2250 mg a.i./kg groups suffered a leg injury on Day 3 of the test, which was reported to likely have occurred during convulsions. The NOEL for sub-lethal effects was <63 mg a.i./kg.

Treatment-related effects on body weight gain were observed for both sexes at the 1350 and 2250 mg a.i./kg treatment levels (p. 15 and Table 2, p. 18). From Days 0 to 3, control males increased an average of 9 g, compared to 8, 9, 8, 4, 0, and -4 g for the 63, 292, 486, 810, 1350, and 2250 mg a.i./kg treatment levels, respectively; and control females increased an average of 7 g, compared to 12, 8, 6, 7, 3, and -10 g for the treatment levels, respectively. Body weight changes from 3-7 Days and from 7-14 Days were comparable

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among all control and treatment groups. Statistical evaluations were not performed for body weight data. The NOEL based on visual inspection of the data was 810 mg a.i./kg bw.

A treatment-related effect on feed consumption was observed for both sexes at the 2250 mg a.i./kg treatment level (p. 15 and Table 3, p. 19). From Days 0 to 3, mean feed consumption was 19 g/bird/day for control males, compared to 20, 23, 17, 20, 19, and 14 g/bird/day for the 63, 292, 486, 810, 1350, and 2250 mg a.i./kg treatment groups, respectively; and 26 g/bird/day for control females, compared to 21, 21, 30, 20, 23, and 15 g/bird/day for the treatment groups, respectively. Data for this treatment level between Days 4-7 was not available due to a technical error. However, data were comparable between the control and remaining treatment levels from Days 4-7, and between the control and all treatment groups from Days 8-14. Statistical evaluations were not performed for feed consumption data. The NOEL based on visual inspection of the data was 1350 mg a.i./kg bw.

No treatment-related findings were observed upon necropsy of all test birds (p. 15). One control male was noted with a friable liver, a distended gizzard, and a malformed foot. Areas of hyperemia in the small intestines were observed in three control birds, and in one bird each in the 63, 292, 810, and 1350 mg a.i./kg treatment groups. In addition, a male from the 292 mg a.i./kg group was noted with a small cyst attached to the left testis. No other remarkable findings were observed.

Table 4: Sub-lethal effects of XDE-750 (Aminopyralid) on *Colinus virginianus*.

Mean Body Weight (and Change[1]), g									
Treatment, mg a.i./kg bw		**Males**				**Females**			
		Day 0	**Day 3**	**Day 7**	**Day 14**	**Day 0**	**Day 3**	**Day 7**	**Day 14**
Vehicle control		259	268 (9)	271 (3)	265 (-6)	257	264 (7)	269 (4)	269 (1)
63		279	291 (8)	292 (1)	293 (0)	276	288 (12)	293 (5)	290 (-3)
292		258	267 (9)	268 (1)	266 (-2)	237	245 (8)	248 (3)	244 (-4)
486		255	264 (8)	264 (1)	263 (-1)	260	265 (6)	269 (4)	266 (-4)
810		251	255 (4)	261 (6)	258 (-4)	250	257 (7)	260 (3)	258 (-2)
1350		246	247 (0)	253 (6)	253 (0)	255	258 (3)	265 (7)	265 (0)
2250		267	262 (-4)	268 (5)	265 (-2)	248	238 (-10)	244 (7)	251 (7)
NOEL		810 mg a.i./kg				810 mg a.i./kg			
EC_{50}		Not determined				Not determined			
Reference chemical	effect: NOEL: LD_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[1] The mean change is calculated separately from the mean body weights using the individual changes in body weights.

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Mean Feed Consumption, g/bird/day							
Treatment, mg a.i./kg		**Males**			**Females**		
		Days 0-3	**Days 4-7**	**Days 8-14**	**Days 0-3**	**Days 4-7**	**Days 8-14**
Vehicle control		19	24	21	26	28	25
63		20	25	24	21	28	26
292		23	25	26	21	27	25
486		17	20	19	30	29	26
810		20	27	27	20	24	20
1350		19	28	26	23	28	27
2250		14	--	25	15	--	32
NOEL		1350 mg a.i/kg			1350 mg a.i./kg		
EC_{50}		Not determined			Not determined		
Reference chemical	effect NOEL LD_{50}	N/A			N/A		

– No data available due to a technical error.

C. REPORTED STATISTICS:

The LD_{50} and NOEL were visually determined based on mortality, body weight, and feed consumption data.

LD_{50}: >2250 mg a.i./kg bw
NOEL: <63 mg a.i./kg bw
LOEL: 63 mg a.i./kg bw
Endpoint(s) Affected: Clinical signs of toxicity, body weight changes, and feed consumption
Most sensitive endpoint: Clinical signs of toxicity

D. VERIFICATION OF STATISTICAL RESULTS:

Mortality did not exceed 50% during the study, so the acute LD_{50} was determined visually. Statistical analyses were not conducted to compare body weight and food consumption data, as results for these endpoints could also be verified visually.

LD_{50}: >2250 mg a.i./kg bw
NOEL: <63 mg a.i./kg bw
LOEL: 63 mg a.i./kg bw
Endpoint(s) Affected: Clinical signs of toxicity, body weight changes, and feed consumption
Most sensitive endpoint: Clinical signs of toxicity

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §71-1 affecting the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with those of the study authors.

Supplemental data were submitted to established the NOEL in acute toxicity testing to Northern Bobwhite quail. In a follow-up study (MRID 462358-09), the NOEL, based on clinical signs of toxicity, was 14 mg a.i./kg bw.

EAD Comments:
After review of the study data and the US EPA DER, the reviewer is in agreement with the conclusion reached by the US EPA.

A NOEL value could not be determined, however study authors mention that a supplement study was performed following the present study in order to obtain a NOEL value (MRID 462358-09). The NOEL value obtained from this supplemental study is 14 mg ai/kg bw and based on clinical signs of toxicity.

Values mentioned in the study are nominal concentrations. Doses of aminopyralid were not measured once mixed with the solvent (deionized water) or prior to administration by oral intubation to the birds. Also, homogeneity and stability of the mixture of aminopyralid with the solvent were not determined Thus, the aminopyralid dose given to the birds should be considered approximative.

No statistical verifications were performed by either study authors or US EPA reviewer, they both based the NOEL valuc for sub-lethal effects on visual inspection of the data. Since treatment-related effects were observed at all treatment levels for clinical signs of toxicity, ther was no point in assessing significant differences between treatment level sfor body weight gain and feed consumption.

G. CONCLUSIONS:

This toxicity study is scientifically sound and fulfills the guideline requirements for an acute toxicity study using the Bobwhite quail (§71-1). The 14-day acute oral toxicity LD_{50} was >2250 mg a.i./kg bw (combined sexes), which categorizes XDE-750 (aminopyralid) as practically non-toxic to the Bobwhite quail. Based on treatment-related effects on clinical signs of toxicity (the most sensitive endpoint), the NOEL was <63 mg a.i./kg bw, the lowest concentration tested.

LD_{50}: >2250 mg a.i./kg bw
NOEL: <63 mg a.i./kg bw
LOEL: 63 mg a.i./kg bw
Endpoint(s) Affected: Clinical signs of toxicity, body weight changes, and feed consumption
Most sensitive endpoint: Clinical signs of toxicity

III. REFERENCES:

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Subsection 71-1*. Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.

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Stephan, C.E. 1977. Methods for Calculating an LC50. *Aquatic Toxicology and Hazard Evaluations*. Pages 64-84 in American Society for Testing and Materials, Pub. No. STP634.

Data Evaluation Report on the Acute Oral Toxicity of XDE-750 (Aminopyralid) on Northern Bobwhite Quail (*Colinus virginianus*)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-09

Data Requirement:		
	PMRA DATA CODE	9.6.2.1
	EPA DP Barcode	D301682
	OECD Data Point	II A 8.1.1
	EPA MRID	46235809
	EPA Guideline	§71-1

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: Not reported
CAS name: 3,6-Dichloro-4-amino-2-pyridinecarboxylic acid
CAS No.: Not reported
Synonyms: XDE-750/XR-750

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature:
Date: 9/29/04

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/10/04

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB - IV
Signature: BDK 6/16/05
Date: 11/02/04

Secondary Reviewer(s): Brigitte Lavallée
PMRA (1595)
Signature:
Date: February 2, 2005

Reference/Submission No.:

Company Code:
Active Code:
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Date Evaluation Completed: June 12, 2005

CITATION: Gallagher, S.P., *et al.* 2003. XDE-750 Technical: An Acute Oral Toxicity Study with the Northern Bobwhite. Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 379-130. Study submitted by Dow Chemical Company, Midland, MI for Dow AgroSciences LLC, Indianapolis, IN. Study initiated January 6, 2003 and submitted February 20, 2003.

EXECUTIVE SUMMARY:

The acute oral toxicity of XDE-750 (aminopyralid) to 24-week-old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 14 days. XDE-750 was administered to the birds by oral intubation at nominal concentrations of 0 (deionized water vehicle control), 8, 14, 23, 38, 63, and 292 mg a.i./kg bw (adjusted for 94.5% purity).

This study was submitted to provide supplemental data to the previously-conducted primary acute toxicity study (MRID 46235808), in which a NOEL was not established.

No mortality occurred in any control or treatment group during the 14-day study. The acute LD_{50} was >292 mg a.i./kg bw, the highest level tested; as the highest dose tested was well below the limit concentration of 2000 mg a.i./kg, an accurate Toxicity Category could not be assigned.

Treatment-related clinical signs of toxicity were observed in birds from the ≥23 mg a.i./kg levels. Effects included ruffled appearance, loss of coordination, reduced reaction to external stimuli (sound and movement), lethargy, neck curl, prostrate posture, and/or lower limb weakness. Effects subsided from the single affected bird at the 23 mg a.i./kg level within 5.5 hours of dosing, from the single affected bird at the 38 mg a.i./kg level within 2 hours of dosing, from the four affected birds at the 63 mg a.i/kg level by the morning of Day 1, and from the six affected birds at the 292 mg a.i./kg level by the morning of Day 2. The NOEL based on clinical signs of toxicity was 14 mg a.i./kg bw.

No treatment-related effects on body weight changes or feed consumption were observed. The NOEL based on visual inspection of the data for both endpoints was 292 mg a.i./kg bw.

This toxicity study is scientifically sound. As this study was conducted at dosages far below the limit dose of 2000 mg a.i./kg, this study does not fulfill the guideline requirement for an acute toxicity study using the Northern Bobwhite quail (§71-1), and is classified as SUPPLEMENTAL. However, this study was not designed to fulfill guideline requirements. Rather, data obtained from this study were provided to supplement data obtained from the primary acute toxicity study to Northern Bobwhite quail (MRID 462358-08).

EAD Conclusion:
The EAD is in agreement with the conclusions reported by the US EPA reviewer. No mortality occurred during the study. Therefore, the 14-d acute oral LD_{50} for XDE-750 (aminopyralid) is > 292 mg ai/kg bw. Based on sub-lethal effects (clinical signs of toxicity), the NOEL value is 14 mg ai/kg bw, e.i., the lowest concentration tested.

Results Synopsis

Test Organism Size/Age: Approximately 24-weeks old, 185-238 g (combined sexes)

LD_{50}: >292 mg a.i./kg bw
NOEL: 14 mg a.i./kg bw
LOEL: 23 mg a.i./kg bw
Endpoint(s) Affected: Clinical signs of toxicity

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The protocol followed procedures of the U.S. EPA Pesticide

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Assessment Guidelines, Subsection 71-1 (1982); and U.S. EPA Ecological Effects Test Guidelines (draft) No. 850-2100 (1996). The following deviations from §71-1 were noted:

1. Mortality observed during acclimation (if any) was not reported.
2. The photo-period (8 hours of light) was less than recommended (10 hours of light).
3. No mortality was observed up to the highest dose tested (292 mg a.i./kg), which was below the limit dose level of 2000 mg a.i./kg. Therefore, an accurate Toxicity Category could not be derived from data obtained in this study.
4. Statistical analyses should have been performed on body weight and feed consumption endpoints.

These deviations do not affect the validity of the study. This study was submitted as supplemental data to the primary acute toxicity study conducted with Northern Bobwhite quail (MRID 46235808). This study was designed to obtain a NOEL, since a NOEL was not established in the primary acute study. Alone, this study does not fulfill guideline requirements.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA, OECD, and Japan MAFF with the following exceptions: stability of the test substance under storage conditions at the test site has not been determined in accordance with GLP Standards, and verification of concentrations, stability, and homogeneity of the test substance in the diluent were not determined (p. 3).

A. MATERIALS:

1. Test Material XDE-750 Technical (aminopyralid)

Description: Pale yellow powder

Lot No./Batch No.: F0031-143 (TSN 102319)

Purity: 94.5%

Stability of Compound Under Test Conditions: N/A

Storage conditions of test chemicals: Ambient

OECD requires water solubility, stability in water and light, pK_a, P_{ow} and vapor pressure of the test compound. OECD requirements were not reported.

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2. **Test organism**:

Species:	Northern Bobwhite quail (*Colinus virginianus*)
Age at study initiation:	Approximately 24 weeks old
Weight at study initiation:	185-238 g (combined sexes)
Source:	K & L Quail, Oroville, CA

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The test dosages were established based upon available toxicity information, with particular consideration given to the previously-conducted acute oral toxicity test (MRID 462350808, Wildlife International Project No. 379-106; p. 9).

b. Definitive Study:

Table 1: Experimental Parameters.

Parameter	Details	Remarks *Criteria*
Acclimation period:	5 weeks	Beginning 2 days following arrival in the laboratory, test birds were given water soluble antibiotics in their drinking water for 7 consecutive days (p. 11).
Conditions (same as test or not):	Same as test	
Feeding:	Game bird ration (Wildlife International, Ltd., Appendix II, p. 22) and public water from the town of Easton were provided *ad libitum*, except during approximately 17 hours prior to testing.	*EPA recommends that birds be pre-conditioned to the test facilities for at least 15 days.*
Health (any mortality observed):	Birds exhibiting abnormal behavior or physical injury were not used; not otherwise specified.	*OECD recommends that birds be pre-conditioned to the test facilities for at least 7 days.*
Pen size and construction materials	Battery pens were 78 x 51 x 20/25 (sloping floors) cm, and were constructed with galvanized wire (ceilings and floors) and galvanized sheeting (side walls).	

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Parameter	Details	Remarks *Criteria*
		EPA requires: pens must conform to good husbandry practices and should not create crowding stress. *OECD lists no criteria for pen construction other than stating that pens should be suitable for the captive rearing of that species.*

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Parameter	Details	Remarks *Criteria*
Test duration	14 Days	*EPA requires a day for dosing and at least 14 days observation.*
Dose preparation Indicate method of confirmation of dose	Test substance was dispersed in deionized water using a magnetic stirrer (Appendix III, p. 23). Certificate of Analysis included	
Mode of dose administration	Orally intubated into the crop or proventriculus using a stainless steel 14 gauge cannula.	*Gavage or gelatin capsule.*
Dose levels nominal: measured:	0, 8, 14, 23, 38, 63, and 292 mg a.i./kg of body weight N/A	The dosages were adjusted to 100% a.i. (p. 11). *EPA requires a minimum of 5 treatment levels unless LD_{50} is demonstrated to be greater than 2250 mg ai/kg.*
Solvent/vehicle, if used type: amount/bw:	 Deionized water 0.4% (mL/g x 100)	The stock solutions were administered at a constant dosing volume of 4 mL/kg bw (or 0.4%; Appendix III, p. 23). *EPA recommends that the test material be administered without a vehicle if possible. Maximum vehicle should not exceed 0.1 to 1.0% of body weight.*
Number of birds per groups/treatment for negative control: for solvent/vehicle control: for treated:	 N/A 10 10/level	5 males and 5 females per treatment group. *EPA recommends 10 birds per treatment group and 10 birds for each control and vehicle group.*

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Parameter	Details	Remarks *Criteria*
No. of feed withholding days before dosing	Birds were fasted for at approximately 17 hours prior to dosing.	*EPA recommends that food should be withheld for at least 15 hours prior to dosing.*
Test conditions Temperature: Relative humidity: Photo-period:	 23.7 ± 0.6°C 14 ± 4% 8-hours light/16-hours dark.	The photo-period was less than recommended. The birds received an average 154 lux of illumination (p. 12). *EPA recommends that a 10 hr light/14 hr dark photo-period.*
Reference chemical, if used name: concentrations tested:	None used.	

2. Observations:

Table 2: Observations.

Parameter	Details	Remarks/Criteria
Parameters measured		
Parameters measured (mortality/individual body weight at test initiation and termination/ mean feed consumption/others)	- Mortality - Clinical signs of toxicity - Individual body weight - Avcrage feed consumption	*EPA recommends:* *Body weight measured at test initiation, on Day 14 and at end of the test if the test is extended beyond 14 days.* *Calculation of mortality. Mortality must NOT be more than 10% in controls.* *Feed consumption may be measured as average daily food consumption.*
Indicate if the test material was regurgitated	None reported.	*Regurgitation is an indication that the dose was rejected. The test may have to be repeated if the problem persists.*

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Parameter	Details	Remarks/Criteria
Groups on which necropsies were performed	None performed.	*EPA recommends that gross necropsies be performed with inspections of the GI tract, liver, kidneys, heart, and spleen.*
Observation intervals	Mortality and signs of toxicity: at least once daily. Body weight: Days 0 (prior to dosing), 3, 7, and 14. Feed consumption per pen: Days 0-3, 4-7, and 8-14.	
Were raw data included?	Yes, sufficient.	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality occurred in any control or treatment group during the 14-day study (Table 1, p. 17). The acute LD_{50} was >292 mg a.i./kg bw, the highest level tested.

Table 3: Effect of XDE-750 (aminopyralid) on mortality of *Colinus virginianus*.

Treatment (mg a.i./kg bw)	No. of birds	Cumulative mortality							
		day 0	day 2	day 4	day 6	day 8	day 10	day 12	day 14
Vehicle control	10	0	0	0	0	0	0	0	0
8	10	0	0	0	0	0	0	0	0
14	10	0	0	0	0	0	0	0	0
23	10	0	0	0	0	0	0	0	0
38	10	0	0	0	0	0	0	0	0
63	10	0	0	0	0	0	0	0	0
292	10	0	0	0	0	0	0	0	0
NOEL	292 mg a.i./kg bw								
LD_{50}	>292 mg a.i./kg bw								

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Treatment (mg a.i./kg bw)		No. of birds	Cumulative mortality							
			day 0	day 2	day 4	day 6	day 8	day 10	day 12	day 14
Reference chemical	mortality	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LD_{50}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	NOEL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. SUB-LETHAL TOXICITY ENDPOINTS:

Treatment-related clinical signs of toxicity were observed in birds from the ≥23 mg a.i./kg levels (p. 13). Effects included ruffled appearance, loss of coordination, reduced reaction to external stimuli (sound and movement), lethargy, neck curl, prostrate posture, and/or lower limb weakness. Effects subsided from the single affected bird at the 23 mg a.i./kg level within 5.5 hours of dosing, from the single affected bird at the 38 mg a.i./kg level within 2 hours of dosing, from the four affected birds at the 63 mg a.i/kg level by the morning of Day 1, and from the six affected birds at the 292 mg a.i./kg level by the morning of Day 2. Additional effects associated with injuries (toe lesions, associated lameness, and/or wing droop) were observed in one bird each at the 23, 38, and 63 mg a.i./kg treatment levels. No clinical signs of toxicity were observed at the control or 14 mg a.i./kg levels. At the 8 mg a.i./kg level, one male displayed a loss of coordination, a ruffled appearance, and was panting within 1.5 hours of dosing, but completely recovered by 2 hours. Due to the isolated nature of these effects, the immediate recovery, and lack of clinical signs noted at the 14 mg a.i./kg level, these effects were attributed to the stress from handling, and were not considered to be treatment-related. The NOEL for sub-lethal effects was 14 mg a.i/kg.

No treatment-related effects on body weight changes or feed consumption were observed (p. 14 and Tables 2 and 3, pp. 18-19). Statistical evaluations were not performed for either endpoint. The NOEL based on visual inspection of the data for both endpoints was 292 mg a.i./kg bw.

Table 4: Sub-lethal effects of XDE-750 (Aminopyralid) on *Colinus virginianus*.

Mean Body Weight (and Change[1]), g								
Treatment, mg a.i./kg bw	Males				Females			
	Day 0	Day 3	Day 7	Day 14	Day 0	Day 3	Day 7	Day 14
Vehicle control	204	209 (5)	207 (-2)	207 (0)	213	219 (5)	218 (-1)	220 (2)
8	204	209 (5)	208 (-2)	211 (4)	202	207 (5)	206 (-1)	207 (1)
14	209	215 (6)	214 (-1)	215 (1)	201	205 (4)	205 (1)	205 (0)
23	207	212 (5)	212 (0)	212 (0)	212	218 (6)	217 (-1)	217 (0)
38	212	218 (6)	218 (0)	219 (1)	203	208 (5)	208 (0)	209 (1)
63	200	203 (4)	203 (0)	205 (2)	207	213 (6)	212 (-1)	214 (1)

292		197	212 (5)	203 (1)	206 (3)	198	203 (5)	203 (0)	203 (0)
NOEL		292 mg a.i./kg				292 mg a.i./kg			
EC_{50}		Not determined				Not determined			
Reference chemical	effect: NOEL: LD_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[1] The mean change is calculated separately from the mean body weights using the individual changes in body weights.

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Mean Feed Consumption, g/bird/day							
Treatment, mg a.i./kg		**Males**			**Females**		
		Days 0-3	**Days 4-7**	**Days 8-14**	**Days 0-3**	**Days 4-7**	**Days 8-14**
Vehicle control		18	20	17	32	32	25
8		14	20	17	19	21	19
14		24	30	22	17	23	19
23		14	16	14	14	16	16
38		29	28	21	17	17	17
63		15	17	17	31	28	22
292		32	26	23	29	28	20
NOEL		292 mg a.i/kg			292 mg a.i./kg		
EC_{50}		Not determined			Not determined		
Reference chemical	effect NOEL LD_{50}	N/A			N/A		

C. REPORTED STATISTICS:

The LD_{50} and NOEL were visually determined based on mortality, body weight, and feed consumption data.

LD_{50}: >292 mg a.i./kg bw
NOEL: 14 mg a.i./kg bw
LOEL: 23 mg a.i./kg bw
Endpoint(s) Affected: Clinical signs of toxicity

D. VERIFICATION OF STATISTICAL RESULTS:

The LD_{50} and NOEL were visually determined based on mortality, body weight, and feed consumption data.

LD_{50}: >292 mg a.i./kg bw
NOEL: 14 mg a.i./kg bw
LOEL: 23 mg a.i./kg bw
Endpoint(s) Affected: Clinical signs of toxicity

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §71-1 that affected the validity of this study.

This study was submitted to provide supplemental data to the primary acute toxicity study conducted with Northern Bobwhite quail (MRID 462358-08). This study was designed to obtain a NOEL, since a NOEL was not established in the primary acute study. Alone, this study would not fulfill guideline requirements, as no mortality was observed up to the highest dose tested (292 mg a.i./kg), which was below the limit dose level of 2000 mg a.i./kg. However, this study is scientifically valid, and is classified as SUPPLEMENTAL.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors.

EAD Comments:
After review of the study data and the US EPA DER, the reviewer is in agreement with the conclusion reached by the US EPA.

A NOEL value could not be determined in a prior study (MRID 462358-08), because a sub-lethal effect was reported at all test levels; but a LD_{50} was reported. The NOEL value obtained from this supplemental study is 14 mg ai/kg bw and based on clinical signs of toxicity.

Values mentioned in the study are nominal concentrations. Doses of aminopyralid were not measured once mixed with the solvent (deionized water) or prior to administration by oral intubation to the birds. Also, homogeneity and stability of the mixture of aminopyralid with the solvent were not determined Thus, the aminopyralid dose given to the birds should be considered approximative.

G. CONCLUSIONS:

This toxicity study is scientifically sound. However, this study does not fulfill the guideline requirements for an acute toxicity study using the Bobwhite quail (§71-1) as the study was conducted at dosages well below the limit of 2000 mg a.i./kg. The 14-day acute oral toxicity LD_{50} was >292 mg a.i./kg bw (combined sexes); data obtained from this study could not be used to accurately define a Toxicity Category. Based on treatment-related effects on clinical signs of toxicity (the only endpoint affected), the NOEL was 14 mg a.i./kg bw.

LD_{50}: >292 mg a.i./kg bw
NOEL: 14 mg a.i./kg bw
LOEL: 23 mg a.i./kg bw
Endpoint(s) Affected: Clinical signs of toxicity

III. REFERENCES:

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Subsection 71-1.* Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.

U.S. Environmental Protection Agency. 1996. *Avian Acute Oral Toxicity Test.* Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.2100.

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Data Evaluation Report on the Acute Dietary Toxicity of XDE-750 (Aminopyralid) to Northern Bobwhite Quail (*Colinus virginianus*)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-10

Data Requirement:	PMRA DATA CODE	9.6.2.4
	EPA DP Barcode	D301682
	OECD Data Point	II A 8.1.2
	EPA MRID	462358-10
	EPA Guideline	§71-2a

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: Not reported
CAS name: 3,6-Dichloro-4-amino-2-pyridinecarboxylic acid
CAS No.: Not reported
Synonyms: XDE-750/XR-750

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature:
Date: 9/30/04

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/10/04

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB - IV
Signature: BDK 6/16/05
Date: 11/02/04

Secondary Reviewer(s): Brigitte Lavallée
PMRA (1595)
Signature:
Date: February 2, 2005

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

Date Evaluation Completed: 06/12/2005

CITATION: Gallagher, S.P., *et al.* 2001. XDE-750: A Dietary LC_{50} Study with the Northern Bobwhite. Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 379-107. Study sponsored by Dow AgroSciences, LLC, Indianapolis, IN. Study initiated June 27, 2001 and submitted October 5, 2001.

EXECUTIVE SUMMARY:

The acute dietary toxicity of XDE-750 (aminopyralid) to 10-day-old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 8 days. XDE-750 was administered to the birds in the diet at nominal concentrations of 0 (negative control), 178, 316, 562, 1000, 1780, 3160, and 5620 ppm. Mean-measured concentrations were <30.0 (<LOQ, control), 185, 309, 548, 979, 1720, 3053, and 5556 ppm a.i., respectively. Mean-measured values were not corrected for procedural recoveries, and represent 97-98% of nominal concentrations.

No mortality was observed during the study. The subsequent 8-day acute dietary LC_{50} was >5556 ppm a.i., which categorizes XDE-750 (aminopyralid) as practically non-toxic to Northern Bobwhite quail on an acute dietary basis. No clinical signs of toxicity or treatment-related effects on body weight or food consumption were observed.

This toxicity study is scientifically sound, fulfills the guideline requirements for an avian dietary study using the Northern Bobwhite quail (§71-2a), and is classified as **Acceptable.**

EAD Conclusion:
The EAD is in agreement with the conclusions reported by the US EPA reviewer. No mortality occurred during the study. Therefore, the 8-d acute oral LC_{50} for XDE-750 (aminopyralid) is > 5556 mg ai/kg dw of diet, which categorize aminopyralid as practically non-toxic to the bobwhite quail according to the US EPA classification scheme of avian acute dietary toxicity (US EPA, 1985). Due to absence of sub-lethal effects, the NOEC value is **5496 mg ai/kg dw of diet,** e.i., the highest concentration tested.

This toxicity study is classified as acceptable and satisfies the guideline requirement for an acute dietary toxicity study with the bobwhite quail.

Results Synopsis

Test Organism Size/Age: 10-days old; 17-25 g

LC_{50}: >5556 ppm a.i.
NOEC: 5556 ppm a.i.
LOEC: >5556 ppm a.i.
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The protocol followed procedures of the U.S. EPA Pesticide Assessment Guidelines, Subsection 71-2 (1982); OECD Guideline for Testing of Chemicals, No. 205 (1984); and ASTM Standard E857-87 (1987). The following deviations from §71-2 were noted:

1. Mortality observed during acclimation (if any) was not reported.
2. The average brooder temperature (39.2°C) exceeded recommendations (about 35°C).
3. Provisions for minimizing food spillage and prevention of air contamination were reported as unavoidable.

These deviations did not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA, OECD, and Japan MAFF with the following exceptions: stability of the test substance under storage conditions at the test site has not been determined in accordance with GLP Standards (p. 3).

A. MATERIALS:

1. **Test Material** XDE-750

Description: Cream-colored powder

Lot No./Batch No. : F0031-143 (TSN 102319)

Purity: 94.5%

Stability of Compound Under Test Conditions: Stability of the test material in avian diet was verified after 5 days of ambient storage under actual use conditions in treated feed prepared at the 178 (low) and 5620 ppm (high) test levels (Table 6 of Appendix IV, p. 30). Recoveries averaged 108 and 101% of initial measured concentrations, respectively.

Storage conditions of test chemicals: Ambient conditions

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. **Test organism**:

Species: Northern Bobwhite quail (*Colinus virginianus*)
Age at study initiation: 10 days
Weight at study initiation: 17 to 25 g
Source: Wildlife International Ltd. Production Flock

B. STUDY DESIGN:

1. **Experimental Conditions**

a. Range-finding Study: None reported. The dietary concentrations in the definitive study were established based upon known toxicity data and information supplied by the Sponsor (p. 9).

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	10 days Same as test Game bird ration (Wildlife International, Ltd., Appendix II, p. 22) and public water from the town of Easton were provided *ad libitum*. Birds exhibiting abnormal behavior or physical injury were not used; not otherwise specified.	No form of antibiotic medication was used during acclimation.
Pen size and construction materials	The pens were constructed of galvanized steel wire and sheeting; 72 x 90 cm floor space, 23 cm ceiling height	*EPA requires: about 35 x 100 x 24 cm*
Test duration	5 days with treated feed, and 3 days with"clean" feed.	*EPA requires: 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations nominal: measured:	0 (negative control), 178 316, 562, 1000, 1780, 3160, and 5620 ppm a.i. <30.0 (<LOQ, control), 185, 309, 548, 979, 1720, 3053, and 5556 ppm a.i.	Mean-measured concentrations were determined from the single batch of freshly prepared treated feed (Tables 4 and 5 of Appendix IV, pp. 28-29). Dietary test concentrations were corrected for purity of the test substance (p. 11), but were not adjusted for mean procedural recoveries from each sample set (p. 13). *Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC_{50} > 5000 ppm a.i..*

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Parameter	Details	Remarks *Criteria*
Solvent/vehicle, if used type: amount:	None used.	*EPA requires: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. Solvent not more than 2%.*
Diet preparation and feeding	The appropriate amount of test substance was quantitatively transferred to a Waring blender containing 100 g of basal diet (Appendix III, p. 23). The contents were blended for 1 minute, then quantitatively transferred to a Hobart mixer and mixed with the remaining basal diet for 10 minutes. Enough was made to last the 5-day treatment period, and the diet was presented at test initiation.	*EPA requires: Control group tested with diet containing the maximum amount of vehicle used in treated diets?*
Feed withholding period	None	
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes	
Number of birds per replicate/group for negative control: for vehicle control: for treated:	 30 N/A 10	*EPA requires: 10 (strongly recommended)*
Number of replicates/group (if used) for negative control: for vehicle control: for treated:	 6 N/A 2	

Parameter	Details	Remarks *Criteria*
Test conditions temperature:	Brooder: 39.2 ± 2.0°C Room: 28.40 ± 1.35°C	Light intensity averaged 140 lux (13 foot candles, p. 14).
relative humidity(%):	59 ± 11%	***Brooder temperature:*** *about 35°C (95°F)* ***Room temperature:*** *22-27°C (71-81°F)* ***Relative humidity:*** *30-80%* ***Photoperiod:*** *Minimum of 14 h of light.*
photo-period:	16 hours light/8 hours dark	
Reference chemical, if used	None used.	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured (mortality/body weight/ mean feed consumption/ others)	- Mortality - Clinical signs of toxicity - Mean feed consumption - Mean body weight	
Indicate the stability and homogeneity of test chemical in the diet	Stability: The 5-day ambient stability of the test material in avian diet was assessed under actual use conditions at the 178 (low) and 5620 ppm a.i. (high) levels (Table 6 of Appendix IV, p. 30). p. 58). Recoveries averaged 108 and 101% of initial measured concentrations, respectively. Homogeneity: Homogeneity was assessed in treated feed prepared at the 178 and 5620 ppm a.i. levels (Table 4 of Appendix IV, p. 28). Coefficients of variation were 2.67 and 1.63% respectively.	

Indicate if the test material was regurgitated	None reported	
Treatments on which necropsies were performed	None	
Observation intervals	Mortality and signs of toxicity were measured twice daily. Food consumption was recorded on Days 0-5 and 6-8. Body weights were determined on Days 0, 5, and 8.	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality occurred in any control or test group during the 8-day study (Table 1, p. 18). The 8-day LC_{50} was >5620 ppm a.i.

Table 3: Effect of XDE-750 (aminopyralid) on Mortality of *Colinus virginianus*.

Treatment, ppm a.i. mean-measured (and nominal)		No. of birds per treatment	Cumulative mortality								
			Days								
			0	1	2	3	4	5	6	7	8
Negative control		30	0	0	0	0	0	0	0	0	0
172 (178)		10	0	0	0	0	0	0	0	0	0
309 (316)		10	0	0	0	0	0	0	0	0	0
548 (562)		10	0	0	0	0	0	0	0	0	0
979 (1000)		10	0	0	0	0	0	0	0	0	0
1720 (1780)		10	0	0	0	0	0	0	0	0	0
3053 (3160)		10	0	0	0	0	0	0	0	0	0
5496 (5620)		10	0	0	0	0	0	0	0	0	0
NOEC		5620 ppm a.i. (nominal)									
LC_{50}		>5620 ppm a.i. (nominal)									
Reference chemical	mortality	N/A									
	LC_{50}	N/A									
	NOEC	N/A									

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B. SUB-LETHAL TOXICITY ENDPOINTS:

No clinical signs of toxicity were observed in the control or any test group during the study, and no treatment related effects on body weight changes or food consumption were observed (pp. 15-16, and Tables 2 and 3, pp. 19-20). Statistical analyses were not conducted on sub-lethal endpoints. The NOEL based on visual inspection of the data for sub-lethal endpoints was 5620 ppm a.i., the highest concentration tested.

Table 4: Sub-lethal effects of XDE-750 on *Colinus virginianus*.

Treatment, ppm a.i. Mean-measured (and nominal)		Observation				
		Mean body weight change (g)			Food consumption (g/bird/day)	
		Day			Day	
		0-5	5-8	0-8	0-5	6-8
Negative control		11	8	19	10	13
172 (178)		11	8	19	9	13
309 (316)		11	9	20	9	15
548 (562)		9	7	16	9	11
979 (1000)		11	8	19	7	11
1720 (1780)		10	8	18	8	14
3053 (3160)		9	8	17	8	10
5496 (5620)		11	9	20	7	12
NOEC		5620 ppm a.i. (nominal)			5620 ppm a.i. (nominal)	
EC_{50}		Not determined			Not determined	
Reference chemical	NOEC	N/A				
	EC_{50}	N/A				

C. REPORTED STATISTICS:

As there were no mortalities observed in this study, the LC_{50} value was determined to be greater than the highest concentration tested. Neither body weight or feed consumption data were statistically compared. The results are based on nominal concentrations.

LC_{50}: >5620 ppm a.i.
NOEC: 5620 ppm a.i.
LOEC: >5620 ppm a.i.
Endpoint(s) Affected: None

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D. VERIFICATION OF STATISTICAL RESULTS:

The LC_{50} could be determined visually, as there was no mortality in this study. Statistical analyses were not conducted to compare body weight and food consumption data, as results for these endpoints could also be verified visually.

LC_{50}: >5556 ppm a.i.
NOEC: 5556 ppm a.i.
LOEC: >5556 ppm a.i.
Endpoint(s) Affected: None

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §71-2 that affected the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to those of the study authors, except for the fact that the study authors based toxicity values on the nominal concentrations, while the reviewer based them on the measured concentrations. The reviewer's conclusions are reported in the Executive Summary and Conclusions sections.

To establish procedural recoveries, basal feed was fortified in the analytical laboratory with XDE-750 at 100, 1000, or 6000 ppm and the fortified samples were extracted and analyzed in the same manner used for the definitive test samples (p. 13). Mean recoveries were 91.7 and 92.8% of nominal concentrations on Days 0 and 5, respectively (Table 3 of Appendix IV, p. 27). Measured sample values were not corrected for the mean procedural recoveries based on sample set (p. 13).

EAD Comments:
After review of the study data and the US EPA DER, the reviewer is in agreement with the conclusion reached by the US EPA.

Both study authors and US EPA reviewer did not compared statistically the data for body weight and feed consumption, as they stated it could be assessed visually.

Measured sample values were not corrected by US EPA reviewer for the mean procedural recoveries based on sample set, representing 97% and 98% of nominal concentration for 172 and 5556 mg ai/kg dw of diet treatment levels. These values would then be 167 and 5445 mg a i/kg dw of diet. However, these new values would not have an impact on the risk assessment since the NOEC and LC_{50} are greater than the 5000 mg ai/kg dw of diet maximal concentration for testing the acute dietary toxicity to birds.

G. CONCLUSIONS:

This toxicity study is scientifically sound, fulfills the guideline requirements for an avian dietary LC_{50} study using the Northern Bobwhite quail (§71-2a), and is classified as CORE. No treatment-related effects on mortality, clinical signs of toxicity, body weight, or food consumption were observed at any test level. The LC_{50} exceeded the highest test concentration, 5496 ppm a.i., which categorizes XDE-750 (aminopyralid) as

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practically non-toxic to the Northern Bobwhite quail on an acute dietary basis.

LC_{50}: >5556 ppm a.i.
NOEC: 5556 ppm a.i.
LOEC: >5556 ppm a.i.
Endpoint(s) Affected: None

III. REFERENCES:

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms,* Subsection 71-2, Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.

Organization for Economic Cooperation and Development. 1984. *Avian Dietary Toxicity Test.* OECD Guideline for Testing of Chemicals. Guideline 205. Paris.

American Society for Testing and Materials. 1987. Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species. ASTM Standard E857-87. Annual Book of ASTM Standards, Vol. 11.04. Philadelphia, PA.

National Research Council. 1996. *Guide for the Care and Use of Laboratory Animals.* Washington, D.C. National Academy Press. 125 pp.

Stephan, C.E. 1977. Methods for Calculating an LC50. Pages 65-84 In *Aquatic Toxicology and Hazard Evaluations,* American Society for Testing and Materials. Pub. No. STP 634, Philadelphia, PA.

Finney, D.J. 1971. *Statistical Methods in Biological Assay,* Second edition, Griffin Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews.* Vol II, No. 2 (June): 115-145.

Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.

Data Evaluation Report on the Acute Dietary Toxicity of XDE-750 (Aminopyralid) to Mallard Duck (*Anas platyrhynchos*)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-11

Data Requirement:		
	PMRA DATA CODE	9.6.2.5
	EPA DP Barcode	D301682
	OECD Data Point	II A 8.1.2
	EPA MRID	462358-11
	EPA Guideline	§71-2b

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: Not reported
CAS name: 3,6-Dichloro-4-amino-2-pyridinecarboxylic acid
CAS No.: Not reported
Synonyms: XDE-750/XR-750

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature:
Date: 9/30/04

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/10/04

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB - IV
Signature: BDK 6/16/05
Date: 11/03/04

Secondary Reviewer(s): Brigitte Lavallée
PMRA (1595)
Signature:
Date: February 2, 2005

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

Date Evaluation Completed: 06/12/05

CITATION: Gallagher, S.P., *et al.* 2001. XDE-750: A Dietary LC_{50} Study with the Mallard. Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 379-108. Study submitted by Dow Chemical Company, Midland, MI for Dow AgroSciences LLC, Indianapolis, IN. Study initiated June 27, 2001 and submitted October 5, 2001.

EXECUTIVE SUMMARY:

The acute dietary toxicity of XDE-750 (aminopyralid) to 10-day-old mallard duck (*Anas platyrhynchos*) was assessed over 8 days. XDE-750 was administered to the birds in the diet at nominal concentrations of 0 (negative control), 178, 316, 562, 1000, 1780, 3160, and 5620 ppm. Mean-measured concentrations were <30.0 (<LOQ, control), 172, 309, 548, 979, 1720, 3053, and 5496 ppm a.i., respectively. Mean-measured values were not corrected for procedural recoveries, and represent 97-98% of nominal concentrations.

No mortality was observed during the study. The subsequent 8-day acute dietary LC_{50} was >5496 ppm a.i., which categorizes XDE-750 (aminopyralid) as practically non-toxic to mallard duck on an acute dietary basis. No clinical signs of toxicity or treatment-related effects on body weight or food consumption were observed.

This toxicity study is scientifically sound, fulfills the guideline requirements for an avian dietary study using the mallard duck (§71-2b), and is classified as Acceptable.

EAD Conclusion:
The EAD is in agreement with the conclusions reported by the US EPA reviewer. No mortality occurred during the study. Therefore, the 8-d acute oral LC_{50} for XDE-750 (aminopyralid) is > 5496 mg ai/kg dw of diet, which categorize aminopyralid as practically non-toxic to the mallard duck according to the US EPA classification scheme of avian acute dietary toxicity (US EPA, 1985). Due to absence of sub-lethal effects, the NOEC value is **5496 mg ai/kg dw of diet**, e.i., the highest concentration tested.

This toxicity study is classified as acceptable and satisfies the guideline requirement for an acute dietary toxicity study with the bobwhite quail.

Results Synopsis

Test Organism Size/Age: 10-days old; 150-209 g

LC_{50}: >5496 ppm a.i.
NOEC: 5496 ppm a.i.
LOEC: >5496 ppm a.i.
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The protocol followed procedures of the U.S. EPA Pesticide Assessment Guidelines, Subsection 71-2 (1982); OECD Guideline for Testing of Chemicals, No. 205 (1984); and ASTM Standard E857-87 (1987). The following deviations from §71-2 were noted:

1. Mortality observed during acclimation (if any) was not reported.
2. The average brooder temperature (30.1°C) was less than recommended (about 35°C).
3. Relative humidity ranged from 82-98%; guideline specifies no more than 80%.
4. Provisions for minimizing food spillage and prevention of air contamination were not reported.

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These deviations did not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA, OECD, and Japan MAFF with the following exception: stability of the test substance under storage conditions at the test site has not been determined in accordance with GLP Standards (p. 3).

A. MATERIALS:

1. Test Material XDE-750

Description: Cream-colored powder

Lot No./Batch No. : F0031-143 (TSN 102319)

Purity: 94.5%

Stability of Compound Under Test Conditions: Stability of the test material in avian diet was verified after 5 days of ambient storage under actual use conditions in treated feed prepared at the 178 (low) and 5620 ppm (high) test levels (Table 6 of Appendix IV, p. 29). Recoveries averaged 99 and 100% of initial measured concentrations, respectively.

Storage conditions of test chemicals: Ambient conditions

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. Test organism:

Species: Mallard duck (*Anas platyrhynchos*)

Age at study initiation: 10 days

Weight at study initiation: 150-209 g

Source: Whistling Wings, Inc., Hanover, IL

B. STUDY DESIGN:

1. Experimental Conditions

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a. Range-finding Study: None reported. The dietary concentrations in the definitive study were established based upon known toxicity data and information supplied by the Sponsor (p. 9).

b. Definitive Study:

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Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	10 days Same as test Game bird ration (Wildlife International, Ltd., Appendix II, p. 21) and public water from the town of Easton, MD were provided *ad libitum*. Birds exhibiting abnormal behavior or physical injury were not used; not otherwise specified.	No form of antibiotic medication was used during acclimation.
Pen size and construction materials	The pens were constructed of vinyl-coated wire grid; 62 x 92 cm floor space, 25.5 cm ceiling height	*EPA requires: about 70 x 100 x 24 cm*
Test duration	5 days with treated feed, and 3 days with untreated feed.	*EPA requires: 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations nominal: measured:	0 (negative control), 178 316, 562, 1000, 1780, 3160, and 5620 ppm a.i. <30.0 (<LOQ, control), 172, 309, 548, 979, 1720, 3053, and 5496 ppm a.i.	Mean-measured concentrations were determined from the single batch of freshly prepared treated feed (Tables 4 and 5 of Appendix IV, pp. 27-28). Dietary test concentrations were corrected for purity of the test substance (p. 11), but were not adjusted for mean procedural recoveries from each sample set (p. 13). *Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC_{50} > 5000 ppm a.i..*

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Parameter	Details	Remarks *Criteria*
Solvent/vehicle, if used type: amount:	None used.	*EPA requires: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. Solvent not more than 2%.*
Diet preparation and feeding	The appropriate amount of test substance was quantitatively transferred to a Waring blender containing 100 g of basal diet (Appendix III, p. 22). The contents were blended for 1 minute, then quantitatively transferred to a Hobart mixer and mixed with the remaining basal diet for 10 minutes. Enough was made to last the 5-day treatment period, and the diet was presented at test initiation.	*EPA requires: Control group tested with diet containing the maximum amount of vehicle used in treated diets?*
Feed withholding period	None	
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes	
Number of birds per replicate/group for negative control: for vehicle control: for treated:	 30 N/A 10	*EPA requires: 10 (strongly recommended)*
Number of replicates/group (if used) for negative control: for vehicle control: for treated:	 6 N/A 2	

Parameter	Details	Remarks *Criteria*
Test conditions temperature:	Brooder: 30.1 ± 1.3°C Room: 25.71 ± 1.01°C	Light intensity averaged 181 lux (p. 14).
relative humidity(%):	90 ± 8%	***Brooder temperature:*** *about 35°C (95°F)* ***Room temperature:*** *22-27°C (71-81°F)* ***Relative humidity:*** *30-80%* ***Photoperiod:*** *Minimum of 14 h of light.*
photo-period:	16 hours light/8 hours dark	
Reference chemical, if used	None used.	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured (mortality/body weight/ mean feed consumption/ others)	- Mortality - Clinical signs of toxicity - Mean feed consumption - Mean body weight	

Indicate the stability and homogeneity of test chemical in the diet	Stability: The 5-day ambient stability of the test material in avian diet was assessed under actual use conditions at the 178 (low) and 5620 ppm a.i. (high) levels (Table 6 of Appendix IV, p. 29). Recoveries averaged 99 and 100% of initial measured concentrations, respectively (representing 97 and 98% of the nominal concentrations, respectively).. Homogeneity: Homogeneity was assessed in treated feed prepared at the 178 and 5620 ppm a.i. levels (Table 4 of Appendix IV, p. 27). Coefficients of variation were 2.67 and 1.63% respectively.	
Indicate if the test material was regurgitated	None reported	
Treatments on which necropsies were performed	None	
Observation intervals	Mortality and signs of toxicity were measured twice daily. Food consumption was recorded on Days 0-5 and 6-8. Body weights were determined on Days 0, 5, and 8.	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality occurred in any control or test group during the 8-day study (Table 1, p. 17). The 8-day LC_{50} was >5620 ppm a.i.

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Table 3: Effect of XDE-750 (aminopyralid) on Mortality of *Anas platyrhynchos*.

Treatment, ppm a.i. mean-measured (and nominal)		No. of birds per treatment	Cumulative mortality								
			Days								
			0	1	2	3	4	5	6	7	8
Negative control		30	0	0	0	0	0	0	0	0	0
172 (178)		10	0	0	0	0	0	0	0	0	0
309 (316)		10	0	0	0	0	0	0	0	0	0
548 (562)		10	0	0	0	0	0	0	0	0	0
979 (1000)		10	0	0	0	0	0	0	0	0	0
1720 (1780)		10	0	0	0	0	0	0	0	0	0
3053 (3160)		10	0	0	0	0	0	0	0	0	0
5496 (5620)		10	0	0	0	0	0	0	0	0	0
NOEC		5620 ppm a.i. (nominal)									
LC_{50}		>5620 ppm a.i. (nominal)									
Reference chemical	mortality	N/A									
	LC_{50}	N/A									
	NOEC	N/A									

B. SUB-LETHAL TOXICITY ENDPOINTS:

No clinical signs of toxicity were observed in the control or any test group during the study, and no treatment related effects on body weight changes or food consumption were observed (p. 15, and Tables 2 and 3, pp. 18-19). Statistical analyses were not conducted on sub-lethal endpoints. The NOEL based on visual inspection of the data for sub-lethal endpoints was 5620 ppm a.i., the highest concentration tested.

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Table 4: Sub-lethal effects of XDE-750 on *Anas platyrhynchos*.

Treatment, ppm a.i. Mean-measured (and nominal)		Observation				
		Mean body weight change (g)			Food consumption (g/bird/day)	
		Day			Day	
		0-5	5-8	0-8	0-5	6-8
Negative control		150	83	232	107	135
172 (178)		146	83	229	98	140
309 (316)		146	78	223	102	149
548 (562)		144	84	228	106	158
979 (1000)		158	77	234	96	124
1720 (1780)		154	80	234	103	148
3053 (3160)		144	79	223	107	148
5496 (5620)		144	90	235	111	148
NOEC		5620 ppm a.i. (nominal)			5620 ppm a.i. (nominal)	
EC_{50}		Not determined			Not determined	
Reference chemical	NOEC	N/A				
	EC_{50}	N/A				

C. REPORTED STATISTICS:

As there were no mortalities observed in this study, the LC_{50} value was determined to be greater than the highest concentration tested. Neither body weight or feed consumption data were statistically compared. The results are based on nominal concentrations.

LC_{50}: >5620 ppm a.i.
NOEC: 5620 ppm a.i.
LOEC: >5620 ppm a.i.
Endpoint(s) Affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

The LC_{50} could be determined visually, as there was no mortality in this study. Statistical analyses were not conducted to compare body weight and food consumption data, as results for these endpoints could also be verified visually.

LC_{50}: >5496 ppm a.i.

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NOEC: 5496 ppm a.i.
LOEC: >5496 ppm a.i.
Endpoint(s) Affected: None

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §71-2 that affected the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to those of the study authors, except for the fact that the study authors based toxicity values on the nominal concentrations, while the reviewer based them on the measured concentrations. The reviewer's conclusions are reported in the Executive Summary and Conclusions sections.

To establish procedural recoveries, basal feed was fortified in the analytical laboratory with XDE-750 at 100, 1000, or 6000 ppm and the fortified samples were extracted and analyzed in the same manner used for the definitive test samples (p. 13). Mean recoveries were 91.7 and 92.8% of nominal concentrations on Days 0 and 5, respectively (Table 3 of Appendix IV, p. 26). Measured sample values were not corrected for the mean procedural recoveries based on sample set (p. 13).

EAD Comments:
After review of the study data and the US EPA DER, the reviewer is in agreement with the conclusion reached by the US EPA.

Both study authors and US EPA reviewer did not compared statistically the data for body weight and feed consumption, as they stated it could be assessed visually.

Measured sample values were not corrected by US EPA reviewer for the mean procedural recoveries based on sample set, representing 97% and 98% of nominal concentration for 172 and 5496 mg ai/kg dw of diet treatment levels. These values would then be 167 and 5386 mg a i/kg dw of diet. However, these new values would not have an impact on the risk assessment since the NOEC and LC_{50} are greater than the 5000 mg ai/kg dw of diet maximal concentration for testing the acute dietary toxicity to birds.

G. CONCLUSIONS:

This toxicity study is scientifically sound, fulfills the guideline requirements for an avian dietary LC_{50} study using the mallard duck (§71-2b), and is classified as Acceptable. No treatment-related effects on mortality, clinical signs of toxicity, body weight, or food consumption were observed at any test level. The LC_{50} exceeded the highest test concentration, 5496 ppm a.i., which categorizes XDE-750 (aminopyralid) as practically non-toxic to the mallard duck on an acute dietary basis.

LC_{50}: >5496 ppm a.i.
NOEC: 5496 ppm a.i.
LOEC: >5496 ppm a.i.
Endpoint(s) Affected: None

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III. REFERENCES:

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms,* Subsection 71-2, Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.

Organization for Economic Cooperation and Development. 1984. *Avian Dietary Toxicity Test.* OECD Guideline for Testing of Chemicals. Guideline 205. Paris.

American Society for Testing and Materials. 1987. Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species. ASTM Standard E857-87. Annual Book of ASTM Standards, Vol. 11.04. Philadelphia, PA.

National Research Council. 1996. *Guide for the Care and Use of Laboratory Animals.* Washington, D.C. National Academy Press. 125 pp.

Stephan, C.E. 1977. Methods for Calculating an LC50. Pages 65-84 In *Aquatic Toxicology and Hazard Evaluations*, American Society for Testing and Materials. Pub. No. STP 634, Philadelphia, PA.

Finney, D.J. 1971. *Statistical Methods in Biological Assay*, Second edition, Griffin Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews.* Vol II, No. 2 (June): 115-145.

Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.

Data Evaluation Report on the Reproductive Effects of XDE-750 (Aminopyralid) on Avian Species *Anas platyrhynchos* (Mallard Duck)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-13

Data Requirement:	PMRA DATA CODE	9.6.3.2
	EPA DP Barcode	D301682
	OECD Data Point	II A 8.1.4
	EPA MRID	46235813
	EPA Guideline	§71-4b

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: Not reported
CAS name: 3,6-Dichloro-4-amino-2-pyridinecarboxylic acid
CAS No.: Not reported
Synonyms: XDE-750/XR-750

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/05/04

QC Reviewer: Teri S. Myers, PhD
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/11/04

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB - IV
Signature:
Date: 11/16/2004 6/16/05

Secondary Reviewer(s): Brigitte Lavallée
PMRA (1595)
Signature:
Date: February 3, 2005

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

CITATION: Mach, J.J. 2003. Avian Reproduction Study with XDE-750 in Mallards (*Anas platyrhynchos*). Unpublished study performed by Genesis Laboratories, Inc., Wellington, CO. Laboratory Study No. 02002. Study submitted by Dow Chemical Company, Midland, MI for Dow AgroSciences LLC, Indianapolis, IN. Study initiated May 14, 2002 and submitted February 25, 2003.

EXECUTIVE SUMMARY:

The one-generation reproductive toxicity of XDE-750 (aminopyralid) to groups (13 pens/level) of 1 male and 1 female of 18-week-old Mallard duck was assessed over approximately 20 weeks. XDE-750 was administered to the birds in the diet at nominal concentrations of 0 (solvent control; concentration not specified), 675, 1350, and 2700 ppm. Mean-measured concentrations were <1.00 (<LOD, control), 642, 1287, and 2623 ppm a.i., representing 95-97% of nominal concentrations.

There were no significant treatment-related effects on any adult or offspring parameter. The NOEC and LOEC levels were 2623 and >2623 ppm a.i. diet, respectively.

This toxicity study is scientifically sound and fulfills the guideline requirement for an avian reproduction toxicity study using Mallard duck (§71-4b) and is classified as Acceptable. Deviations include: only 13 pairs were used per replicate, a LOEC was not established, and the quantity and fate of the acetone used in test diet preparation was not specified.

EAD Conclusion:
This toxicity study is classified as acceptable and satisfies the guideline requirement for a mallard duck reproductive toxicity study. **The NOEC of cyazofamid to the mallard duck based on the reproductive parameters is 2623 mg ai/kg dw of diet, the highest tested concentration.**

This toxicity study is classified **as acceptable and satisfies the** guideline requirement for a mallard duck reproductive toxicity study.

Results Synopsis

Test Organism Size/Age: Approximately 18 weeks old at test initiation (860-1386 g)

NOEC: 2623 ppm a.i.
LOEC: >2623 ppm a.i.
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures of the U.S. EPA Pesticide Assessment Guidelines, Series 71-4 (1988); and OECD Guidelines for Testing of Chemicals, No. 206 (1984). Deviations from §71-4 are:

1. The highest concentration tested did not elicit an adverse effect; therefore, a LOEC was not established.
2. The concentration of acetone used in preparation of the tests diets was not specified. Also, it was not specified if the acetone was allowed to completely evaporate off the treated feed prior to offering.
3. Only 13 pens (each containing 1 pair) were maintained for each group, whereas at least 16 pens/level are strongly recommended when birds are pair-housed.

These deviations did not affect the scientific validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with United States and OECD standards with the following exception: portions of the sub-batches were not correctly weighed. For each sub-batch, two smaller quantities of feed (≤20 kg) must be weighed to total the sub-batch size. These smaller weights were not recorded, only the total weight of the sub-batch. This will not affect the integrity of the study, as the total weights of the feed were recorded (p. 3).

A. MATERIALS:

1. Test Material XDE-750 (aminopyralid)

Description: White powder

Lot No./Batch No.: F0031-143 (TSN102319)

Purity: 94.5%

Stability of Compound Under Test Conditions: The stability of XDE-750 in avian feed was not assessed.

Storage conditions of test chemical: Ambient

OECD requires water solubility, stability in water and light, pK_a, P_{ow} and vapor pressure of the test compound. OECD requirements were not reported.

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2. **Test organism**: Mallard duck

Table 1: Test organism.

Parameter	Details	Remarks *Criteria*
Species (common and scientific names):	Mallard duck (*Anas platyrhynchos*)	*EPA requires: a wild waterfowl species, preferably the mallard, Anas platyrhynchos, or an upland game species, preferably the northern bobwhite, Colinus virginianus.*
Age at Study Initiation:	Approximately 18 weeks	It was stated that birds were approaching their first breeding season. *EPA requires birds should be approaching their first breeding season.*
Body Weight: (mean and range)	Males: Overall range (n=52) 1.002-1.386 kg, with group means of 1.187 to 1.221 kg. Females: Overall range (n=52) 0.860-1.304 kg, with group means of 1.003 to 1.069 kg.	Individual body weights were recorded at Weeks 0, 2, 4, 6, 8, and 20 (test termination). *EPA requires that body weights should be recorded at test initiation and at biweekly intervals up to week eight or up to the onset of egg laying and at termination.*
Source:	Whistling Wings, Inc. Hanover, IL	Birds were from the same hatch, and were phenotypically indistinguishable from wild birds. *EPA requires that all birds should be from the same source.*

B. STUDY DESIGN:

1. **Experimental Conditions**

a. Range-finding Study - None reported.

b. Definitive Study

Table 2: Experimental Parameters.

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	20 days Same as test Dry non-medicated Ranchway 16% Poultry Layer Complete (Ranch-Way, Fort Collins, CO) and municipal water from the Northern Colorado Water Association were provided *ad libitum*. All birds were normal and active (p. 19). No disease or abnormalities were observed and no medication was provided.	Birds were observed once daily for general physical condition, disease, and abnormalities. Birds were also examined by a veterinarian to assess their general physical condition and suitability for testing. *EPA recommends a 2-3 week health observation period prior to selection of birds for treatment. Birds must be generally healthy without excess mortality. Feeding should be ad libitum, and sickness, injuries or mortality be noted.*
Test duration pre-laying exposure: egg-laying exposure: withdrawal period, if used:	Approximately 10 weeks Approximately 10 weeks None	*EPA requires* ***Pre-laying exposure duration*** *At least 10 weeks prior to the onset of egg-laying.* ***Exposure duration with egg-laying*** *At least 10 weeks.* ***Withdrawal period*** *If reduced reproduction is evident, a withdrawal period of up to 3 weeks should be added to the test phase.*

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Parameter	Details	Remarks *Criteria*
Pen (for parental and offspring) size:	Parents (one pair) were housed in cages measuring 61 x 76 x 46 cm (floor surface of 4636 cm^2). Offspring (by set and group) were housed in 90 x 70 x 23 cm and 90 x 80 x 25 cm poultry brooders (floor surface of 6300 or 7200 cm^2, respectively).	*Pens* *Adequate room and arranged to prevent cross contamination* *Materials* *Nontoxic material and nonbinding material, such as galvanized steel.* *Number* *At least 5 replicate pens are required for mallards housed in groups of 7. For other arrangements, at least 12 pens are required, but considerably more may be needed if birds are kept in pairs. Chicks are to be housed according to parental grouping.*
construction materials:	Parental pens were constructed of perfluorocarbon-coated steel. Offspring pens were described as box-type (not further specified).	
number:	13 parental pens (replicates) for each level.	
Number of birds per pen (male:female)	2 birds/pen (1 male:1 female)	*EPA requires one male and 1 female per pen. For quail, 1 male and 2 females is acceptable. For ducks, 2 males and 5 females is acceptable.*
Number of pens per group/treatment negative control: solvent control: treated:	 N/A 13 pens 13 pens/treatment	*EPA requires at least 12 pens, but considerably more if birds are kept in pairs. At least 16 is strongly recommended.*

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Parameter	Details	Remarks *Criteria*
Test concentrations (ppm diet) nominal: measured:	0 (solvent control), 675, 1350, and 2700 ppm diet <1.00 (<LOD, control), 642, 1287, and 2623 ppm a.i.	Mean-measured concentrations were determined from freshly-prepared treated feed collected from Batches 1, 2, and 10 (Table 1, p. 24). Concentrations were corrected for the purity of the test substance (p. 14). *EPA requires at least two concentrations other than the control are required; three or more are recommended.*
Maximum labeled field residue anticipated and source of information:	Not specified	Ancillary information(label) shows highest test concentration is above maximum EEC. *EPA requires that the highest test concentrations should show a significant effect or be at or above the actual or expected field residue level. The source [i.e., maximum label rate (in lb ai/A & ppm), label registration no., label date, and site should be cited]*
Solvent/vehicle, if used type: amount:	Acetone Not specified	 *EPA requires corn oil or other appropriate vehicle not more than 2% of diet by weight*
Was detailed description and nutrient analysis of the basal diet provided? (Yes/No)	Yes. Basal diets contained 16.0% protein, 3.5% fat, 7.0% fiber, and 3.0-4.0% calcium (Appendix D1, p. 108).	Offspring received Ranch-Way Turkey & Game Bird Starter without the addition of test substance (Appendix D2, p. 109). *EPA requires a commercial breeder feed (or its equivalent) that is appropriate for the test species.*

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Parameter	Details	Remarks *Criteria*
Preparation of test diet	The appropriate amount of test material was suspended in acetone, then combined with basal ration and mixed for 15 minutes (p. 14). To facilitate mixing, each test group was split into sub-batches and pooled together after the mix to form a single batch. Treated diets were prepared bi-weekly, and were stored at approximately -17°C until needed.	The final acetone concentration was not reported, and it was not specified if the acetone was allowed to completely evaporate prior to offering. *A premixed containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it must be completely evaporated prior to feeding.*
Indicate whether stability and homogencity of test material in diet determined (Yes/No)	homogeneity, yes	Ancillary information strongly suggests stability in feed.
Were concentrations in diet verified by chemical analysis?	Yes	Samples were analyzed from feed collected from Batches 1, 2, and 10 (Table 1, p. 24).
Did chemical analysis confirm that diet was stable? and homogeneous?	Stability was not assessed.	
Feeding and husbandry	Feeding and husbandry conditions appeared to be adequate, given guideline recommendations.	
Test conditions (pre-laying) temperature: relative humidity: photo-period:	20-27°C, with a mean range of 22-23°C. 31-85%, with a mean range of 50-71% 7 hours light/day up through Week 8, then increased 2 hours/day for 5 days to 17 hours light/day thereafter.	An average light intensity of 14.1 foot-candles was maintained at bird level. *EPA Requires* *Temperature:* *About 21 °C (70 F)* *Relative humidity:* *About 55%* *Lighting* *First 8 weeks: 7 h per day.* *Thereafter: 16-17 h per day.* *At least 6 foot candles at bird level.*

Parameter	Details	Remarks *Criteria*
Egg Collection and Incubation		
Egg collection and storage collection interval: storage temperature: storage humidity:	Daily 13-19°C, with a mean range of 15-17°C 44-92%, with a mean range of 52-69%	*EPA requires eggs to be collected daily; egg storage temperature approximately 16°C (61°F); humidity approximately 65%.*
Were eggs candled for cracks prior to setting for incubation?	Yes	*EPA requires eggs to be candled on day 0*
Were eggs set weekly?	Yes	
Incubation conditions temperature: humidity:	83-100°F, with a mean range of 89-100°F 49-96%, with a mean of 66%	Incubation and hatching occurred in the same incubator, in different compartments.
When candling was done for fertility?	Day 14 for fertility and Day 21 for viability.	*EPA requires:* *Quail: approx. day 11* *Ducks: approx. day 14*
When the eggs were transferred to the hatcher?	Day 24	*EPA requires:* *Bobwhite: day 21* *Mallard: day 23*
Hatching conditions temperature: humidity: photo-period:	83-100°F, with a mean range of 89-100°F 49-96%, with a mean of 66% 14 hours light/day (hatchlings)	Incubation and hatching occurred in the same incubator, in different compartments. *EPA requires:* *temperature of 39°C (102°F)* *humidity of 70%*
Day the hatched eggs were removed and counted	Day 27	*EPA requires Bobwhite: day 24* *Mallard: day 27*

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Parameter	Details	Remarks *Criteria*
Were egg shells washed and dried for at least 48 hrs before measuring?	Yes	
Egg shell thickness no. of eggs used: intervals: mode of measurement:	All eggs laid on one day Day 2 of Weeks 12, 14, 16, 18, and 20. Three points around the equatorial circumference were measured to the nearest 0.001 mm.	*EPA requires newly hatched eggs be collected at least once every two weeks. Thickness of the shell plus membrane should be measured to the nearest 0.01 mm; 3 - 4 measurements per shell.*
Reference chemical, if used	None used	

2. Observations:

Table 3: Observations.

Parameter	Details	Remarks/Criteria
Parameters measured		
Parental: (mortality, body weight, mean feed consumption)	- mortality - signs of toxicity, injury, or illness - body weight - food consumption - necropsy	At necropsy, specific examination was made on the gastro-intestinal tract, liver, kidneys, bile duct, heart, spleen, and reproductive organs. Other observations were recorded as necessary.
Egg collection and subsequent development: (no. of eggs laid, no. of eggs cracked, shell thickness, no. of eggs set, no. of viable embryos, no. of live 3 week embryos, no. hatched, no. of 14-day survivors, average weight of 14-day-old survivors, mortality, gross pathology, others)	- eggs laid - eggs broken, cracked, small, and soft shelled, etc. - egg shell thickness - eggs set - viable embryos - live 3-week embryos - number of hatchlings - signs of toxicity and physical defects of hatchlings - number of 14-day-old survivors - 14-day-old survivor body weight	*EPA requires:* • *Eggs laid/pen* • *Eggs cracked/pen* • *Eggs set/pen* • *Viable embryos/pen* • *Live 3-week embryos/pen* • *Normal hatchlings/pen* • *14-day-old survivors/pen* • *14-day-old survivors/pen* • *Weights of 14-day-old survivors (mean per pen)* • *Egg shell thickness* • *Food consumption (mean per pen)* • *Initial and final body weight (mean per pen)*

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Parameter	Details	Remarks/Criteria
Indicate if the test material was regurgitated	No indications of dietary regurgitation.	
Observation intervals (for various parameters)	Mortality and signs of toxicity were observed daily for adults and hatchlings. Parental body weights were recorded at Weeks 0, 2, 4, 6, 8, and 20 (test termination), and food consumption was determined weekly.	*Body weights and food consumption must be measured at least biweekly.*
Were raw data included?	Yes	Raw data pertaining to hatchling weights were not provided.

I. RESULTS AND DISCUSSION:

A. MORTALITY:

No treatment-related mortality was observed during the study. However, 1 male from the 1350 ppm group was found with his bill caught in the mesh of the cage during Week 11 (p. 19 and Table II, p. 25). The bird was severely injured (bleeding from nares, and feather loss of the head and breast) and was subsequently euthanized. No other mortality occurred during the study. Only summarized data were provided regarding mortality, clinical effects, and necropsy findings.

Table 4: Effect of XDE-750 (aminopyralid) on Mortality of *Anas platyrhynchos*.

Treatment, ppm a.i. measured (and nominal) concentrations	Observation Period					
	Week 7		Week 14		Week 20	
	No. Dead		No. Dead		No. Dead	
	Male	Female	Male	Female	Male	Female
Solvent control	0	0	0	0	0	0
642 (675)	0	0	0	0	0	0
1287 (1350)	0	0	1	0	1	0
2623 (2700)	0	0	0	0	0	0

B. REPRODUCTIVE AND OTHER ENDPOINTS:

Abnormal Effects/Behavior: No treatment-related signs of toxicity were apparent. Incidental effects observed at all test levels included injuries (foot/leg), feather loss (head/breast), and a swollen eye (Table II, p. 25). Raw clinical effects data were not provided.

Food Consumption: No treatment-related effects on food consumption were observed (p. 20 and Table III, p. 26). Overall feed consumption averaged 113-122 g/bird/day for all treatment and control groups.

Body Weight: No treatment-related effects on the differences in body weights were observed (p. 20, and Table IV, p. 27).

Necropsy: No treatment-related findings were observed at necropsy (p. 20, and Tables V and VI, pp. 28-29). Feather loss was the predominant observation in all groups.

Reproductive Effects: No treatment-related effects on egg production or quality, fertility, embryonic development, hatchability, or chick survival were observed at any test level (Tables VII-XVIII, pp. 30-41). In addition, none of the chicks showed any test substance-related toxicological symptoms during the 14-day maintenance period, and no treatment-related effects on 14-day old chick body weights were observed (p. 23 and Tables XIX and XX, pp. 42-43).

Table 5: Reproductive and other parameters (nominal concentrations).

Parameter	Control	675 ppm	1350 ppm	2700 ppm	NOEC/ LOEC
Eggs laid	634	630	571	668	N/A
Eggs laid/hen	48.8	48.5	47.6	51.4	2700 ppm >2700 ppm
Eggs laid/hen/week	4.9	4.8	4.8	5.1	2700 ppm >2700 ppm
Eggs candled	580	578	518	609	N/A
Eggs soft shelled, broken, or damaged	5	1	11	8	N/A
Eggs cracked	0	0	0	0	N/A
Eggs cracked/eggs candled (%)	0	0	0	0	2700 ppm >2700 ppm
Shell thickness (mm)	0.341	0.334	0.342	0.329	2700 ppm >2700 ppm
Eggs set	580	578	518	609	N/A
Viable 14-day old embryos	503	553	470	565	N/A
Viable embryos/eggs set (%)	86.7	95.7	90.7	92.8	2700 ppm >2700 ppm
Live 21-day old embryos	500	547	462	553	N/A
Live 21-day old embryos/viable embryos (%)	99.4	98.9	98.3	97.9	2700 ppm >2700 ppm
No. of total hatchlings	385	399	286	395	N/A

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Parameter	Control	675 ppm	1350 ppm	2700 ppm	NOEC/ LOEC
Total hatchlings/viable embryos (%)	76.5	72.2	60.9	69.9	2700 ppm >2700 ppm
No. of normal hatchlings	385	399	286	394	N/A
Normal hatchlings/total hatchlings (%)	100	100	100	99.7	2700 ppm >2700 ppm
No. of normal 14-day old survivors	366	363	272	366	N/A
No. of 14-day old survivors/No. of normal hatchlings (%)	95.1	91.0	95.1	92.9	2700 ppm >2700 ppm
No. of 14-day old survivors/eggs laid (%)	57.7	57.6	47.6	54.8	2700 ppm >2700 ppm
14-day old survivors weight (g)	83	77	80	82	2700 ppm >2700 ppm
Mean adult food consumption (g/pen/day)	113	122	117	116	2700 ppm >2700 ppm
Weight of adult males, kg at start of treatment: at Week 8: at Week 20 (study termination):	 1.132 1.173 1.158	 1.221 1.239 1.226	 1.186 1.283 1.256	 1.187 1.193 1.208	 2700 ppm >2700 ppm
Weight of adult females, kg at start of treatment: at Week 8: at Week 20 (study termination):	 1.003 1.041 1.159	 1.029 1.068 1.174	 1.069 1.102 1.204	 1.068 1.101 1.224	 2700 ppm >2700 ppm
Gross pathology (proportion of birds with pathological incidents)	No treatment-related abnormalities observed.				

N/A = Not statistically-analyzed.

C. REPORTED STATISTICS:

The following variables were statistically analyzed: adult body weight at each determined interval, weekly mean feed consumption, eggs laid/hen, egg shell thickness, percentage of no. eggs cracked/ no. eggs candled, percentage of no. viable 14-day embryos/no. eggs set, percentage of no. live 21-day embryos/no. viable 14-day embryos, percentage of no. of total hatchlings/no. viable 14-day embryos, percentage of no. normal hatchlings/no. total hatchlings, percentage of no. normal 14-day survivors/no. normal hatchlings, percentage of no. 14-day survivors/no. eggs laid, and 14-day old hatchling body weights (Table XXI, p. 44).

Data were assessed for normality using the Chi-square test and for homogeneity of variance using Bartlett's test. If the data set passed the tests for normality and homogeneity, an analysis of variance (ANOVA) was

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performed to determine statistically-significant differences between groups. If necessary, Dunnett's test (equal replicates) or Bonferroni's test (not equal replicates) was then used to compare the treatment means with the control group mean. If the data set did not pass the tests for normality and homogeneity, they were transformed and re-analyzed. If an appropriate transformation did not succeed in normalizing the distribution, or if the variance was not homogeneous, the original untransformed data were analyzed by Kruskal-Wallis's non-parametric test (H-statistic). Dunn's multiple comparison procedure was used to compare each treatment group with the control. Proportional (percentage) data were arc sine transformed prior to analysis.

All variables were analyzed using TOXSTAT Version 3.4. Sample units were the individual pens within each experimental group, except adult body weights, where the sample unit was the individual bird. Nominal concentrations were used for all estimations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Analysis was conducted using "chicks.sas" (Ver. 3; March 2002), a SAS program used by EFED/OPP/USEPA. Data for all endpoints were examined graphically using box plots to determine if they exhibited a dose-dependent response, which was ultimately used to select the multiple comparison test to detect LOEC and NOEC. Data for each endpoint were tested to determine if their distributions were normal and if their variances were homogeneous using Shapiro-Wilk's or Levene's tests, respectively. Data that satisfied these assumptions were subjected to Dunnett's and William's tests and data that did not satisfy these assumptions were subjected to the non-parametric Mann-Whitney-U (with a Bonferroni adjustment) or Jonckheere's tests. Data for dead birds were excluded from the analyses. See Appendix I for output of reviewer's statistical verification.

Table 6. Reproductive and other parameters (mean-measured concentrations; reviewer-reported).

Parameter	Control	642 ppm	1287pm	2623 ppm	NOEC/ LOEC
Eggs laid/pen	48.8	48.5	47.6	51.4	2623 ppm >2623 ppm
Eggs cracked/pen	0	0	0	0	2623 ppm >2623 ppm
Eggs not cracked/eggs laid (%)	NA	NA	NA	NA	2623 ppm >2623 ppm
Eggs set/pen	44.6	44.5	43.2	46.9	2623 ppm >2623 ppm
Shell thickness	0.34	0.33	0.34	0.32	2623 ppm >2623 ppm
Eggs set/eggs laid (%)	91.6	91.6	90.3	90.8	2623 ppm >2623 ppm
Viable embryo/pen	38.7	42.3	39.2	43.5	2623 ppm >2623 ppm
Viable embryos/eggs set (%)	86.1	94.7	90.8	92.3	2623 ppm >2623 ppm

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Parameter	Control	642 ppm	1287pm	2623 ppm	NOEC/ LOEC
Live embryos/pen	38.7	41.9	38.5	42.5	2623 ppm >2623 ppm
Live embryo/viable embryo (%)	100.0	98.8	98.1	97.8	2623 ppm >2623 ppm
No. of hatchlings/pen	29.6	30.7	23.8	30.3	2623 ppm >2623 ppm
No. of hatchlings/eggs laid (%)	58.5	61.7	50.1	58.4	2623 ppm >2623 ppm
No. of hatchlings/eggs set (%)	64.0	67.1	55.8	64.2	2623 ppm >2623 ppm
No. of hatchlings/live embryos (%)	73.2	71.3	61.7	70.6	2623 ppm >2623 ppm
Hatchling survival/pen	28.2	27.9	22.7	28.2	2623 ppm >2623 ppm
Hatchling survival/eggs set (%)	60.7	61.4	52.8	60.0	2623 ppm >2623 ppm
Hatchling survival/no. of hatchlings (%)	91.9	89.5	92.8	92.1	2623 ppm >2623 ppm
Hatchling weight (g)	NA	NA	NA	NA	NA
Survivor weight (mg)	82.5	76.9	79.7	81.6	2623 ppm >2623 ppm
Mean food consumption (g/bird/day)	112.6	121.6	117.7	116.0	2623 ppm >2623 ppm
Male weight gain (mg)	26.0	5.1	76.2	20.9	2623 ppm >2623 ppm
Female weight gain (mg)	153.3	144.8	136.2	155.5	2623 ppm >2623 ppm

NA=not analyzed; data not provided

E. STUDY DEFICIENCIES:

This study is considered scientifically valid with few deviations from §71-4 guidance. However the volume of acetone used in test diet preparation was not reported, nor was it specified if the acetone was allowed to completely evaporate prior to offering.

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F. REVIEWER'S COMMENTS:

Results of the reviewer's statistical analyses were nearly identical to those of the study author. The discrepancies between the reviewer's conclusions and the study author's conclusions were due to the fact that the reviewer based NOEC and LOEC values on mean-measured concentrations, whereas the study author used nominal values. Mean-measured concentrations are reported in the Conclusions and Executive Summary sections.

In the analytical report, it was reported that the sensitivity and reproducibility (of the analytical method) were determined by injecting the 2.46 ppm analytical standard six times (p. 114 of Appendix F). The mean, standard deviation, and coefficient of variation were calculated. The standard deviation for the six replicates was multiplied by three in order to determine the limit of detection (LOD) and multiplied by ten in order to determine the limit of quantitation (LOQ). It was then reported that the LOD for the method was 0.050 µg/mL (1.00 ppm) and the LOQ was 0.084 µg/mL (1.68 ppm).

The recovery of the analytical method, determined from analysis of six fortified matrix blanks, averaged 93.7 ± 1.4% (CV = 1.49%; pp. 114-115 of Appendix F). It was not reported if sample results were corrected for the mean procedural recovery.

EAD Comments:
After review of the study data and the US EPA DER, the reviewer is in agreement with the conclusion reached by the US EPA.

US EPA reviewer classified this study as acceptable and core while the equivalent study with the bobwhite quail was classified as supplemental due to a greater number of mortalities in the parental birds. In both studies, raw data submission was deficient, and usage of acetone in the diet preparation was an issue. The main difference was the lower parental mortality for mallard duck.

Stability of aminopyralid mixed with acetone was not assessed. Study author did not give a rationale for using a solvent in the preparation of the diet. In previous acute oral and dietary toxicity studies, aminopyralid was mixed with diet preparation without solvent (dietary studies, MRID 4622358-10 and 462358-11) or diluted with water (oral studies, MRID 462358-08, 462358-09). However, results from certain fate studies with aminopyralid suggest that the compound is stable.

Based on the results of acute oral and acute toxicity studies for bobwhite quail and mallard duck (MRID 462358-08 to 462358-11), aminopyralid is not expected to have an effect on mallard duck at the tested levels (642, 1287, and 2623 mg ai/kg of diet).

G. CONCLUSIONS:

This study is scientifically sound and fulfills guideline requirements for an avian reproduction study using the Mallard duck (§71-4b) and is classified as Acceptable.

NOEC: 2623 ppm a.i.
LOEC: >2623 ppm a.i.
Endpoint(s) Affected: None

III. REFERENCES:

U.S. Environmental Protection Agency. 1988. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Series 71-4: Avian Reproduction Test. pp. 48-57.

Organization for Economic Cooperation and Development. 1984. OECD Guidelines for Testing of Chemicals, 206, Avian Reproduction Test. 10 pp.

Stromberg, J. 1975. A guide to better hatchling. Stomberg Publishing Company. Pine River, Minnesota. 100 pp.

APPENDIX L OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Mallard repro, Aminopyralid, MRID 4625813
PRINTOUT OF RAW DATA

Obs	TRT	EL	EC	ENC_EL	ES	ES_EL	VE	VE_ES	LE	LE_VE	NH	NH_EL	NH_ES
1	Ctrl	52	0	100.00	48	92.31	48	100.00	48	100.00	41	78.85	85.42
2	Ctrl	55	0	100.00	50	90.91	47	94.00	47	100.00	43	78.18	86.00
3	Ctrl	42	0	100.00	39	92.86	35	89.74	35	100.00	29	69.05	74.36
4	Ctrl	61	0	100.00	55	90.16	50	90.91	50	100.00	46	75.41	83.64
5	Ctrl	47	0	100.00	43	91.49	42	97.67	42	100.00	32	68.09	74.42
6	Ctrl	44	0	100.00	41	93.18	13	31.71	13	100.00	9	20.45	21.95
7	Ctrl	57	0	100.00	51	89.47	51	100.00	51	100.00	49	85.96	96.08
8	Ctrl	48	0	100.00	44	91.67	18	40.91	18	100.00	12	25.00	27.27
9	Ctrl	40	0	100.00	37	92.50	36	97.30	36	100.00	11	27.50	29.73
10	Ctrl	40	0	100.00	36	90.00	30	83.33	30	100.00	6	15.00	16.67
11	Ctrl	50	0	100.00	46	92.00	46	100.00	46	100.00	38	76.00	82.61
12	Ctrl	50	0	100.00	47	94.00	46	97.87	46	100.00	35	70.00	74.47
13	Ctrl	48	0	100.00	43	89.58	41	95.35	41	100.00	34	70.83	79.07
14	Dose1	56	0	100.00	51	91.07	51	100.00	51	100.00	27	48.21	52.94
15	Dose1	31	0	100.00	27	87.10	23	85.19	22	95.65	6	19.35	22.22
16	Dose1	53	0	100.00	50	94.34	46	92.00	46	100.00	37	69.81	74.00
17	Dose1	51	0	100.00	46	90.20	43	93.48	41	95.35	25	49.02	54.35
18	Dose1	62	0	100.00	58	93.55	57	98.28	57	100.00	54	87.10	93.10
19	Dose1	66	0	100.00	61	92.42	58	95.08	58	100.00	46	69.70	75.41
20	Dose1	44	0	100.00	40	90.91	39	97.50	37	94.87	16	36.36	40.00
21	Dose1	57	0	100.00	53	92.98	51	96.23	50	98.04	37	64.91	69.81
22	Dose1	55	0	100.00	51	92.73	50	98.04	50	100.00	39	70.91	76.47
23	Dose1	29	0	100.00	27	93.10	26	96.30	26	100.00	18	62.07	66.67
24	Dose1	55	0	100.00	49	89.09	47	95.92	47	100.00	41	74.55	83.67
25	Dose1	43	0	100.00	39	90.70	34	87.18	34	100.00	31	72.09	79.49
26	Dose1	28	0	100.00	26	92.86	25	96.15	25	100.00	22	78.57	84.62
27	Dose2	35	0	100.00	33	94.29	33	100.00	33	100.00	24	68.57	72.73
28	Dose2	46	0	100.00	40	86.96	37	92.50	37	100.00	35	76.09	87.50
29	Dose2	52	0	100.00	50	96.15	49	98.00	49	100.00	11	21.15	22.00
30	Dose2	.	.	.	.	.	.	.	.	.	.	.	.
31	Dose2	38	0	100.00	34	89.47	26	76.47	25	96.15	10	26.32	29.41
32	Dose2	48	0	100.00	40	83.33	33	82.50	32	96.97	12	25.00	30.00
33	Dose2	66	0	100.00	62	93.94	50	80.65	50	100.00	34	51.52	54.84
34	Dose2	52	0	100.00	48	92.31	46	95.83	46	100.00	16	30.77	33.33
35	Dose2	50	0	100.00	47	94.00	46	97.87	42	91.30	33	66.00	70.21
36	Dose2	33	0	100.00	26	78.79	26	100.00	26	100.00	22	66.67	84.62
37	Dose2	61	0	100.00	56	91.80	55	98.21	55	100.00	45	73.77	80.36
38	Dose2	41	0	100.00	37	90.24	29	78.38	27	93.10	13	31.71	35.14
39	Dose2	49	0	100.00	45	91.84	40	88.89	40	100.00	31	63.27	68.89
40	Dose3	46	0	100.00	43	93.48	43	100.00	42	97.67	37	80.43	86.05
41	Dose3	45	0	100.00	39	86.67	36	92.31	34	94.44	32	71.11	82.05
42	Dose3	25	0	100.00	21	84.00	17	80.95	17	100.00	10	40.00	47.62
43	Dose3	58	0	100.00	54	93.10	53	98.15	52	98.11	22	37.93	40.74
44	Dose3	56	0	100.00	51	91.07	46	90.20	46	100.00	34	60.71	66.67
45	Dose3	53	0	100.00	47	88.68	46	97.87	46	100.00	42	79.25	89.36
46	Dose3	55	0	100.00	50	90.91	46	92.00	44	95.65	8	14.55	16.00
47	Dose3	62	0	100.00	58	93.55	55	94.83	55	100.00	45	72.58	77.59
48	Dose3	49	0	100.00	46	93.88	35	76.09	34	97.14	25	51.02	54.35
49	Dose3	43	0	100.00	40	93.02	40	100.00	40	100.00	34	79.07	85.00
50	Dose3	61	0	100.00	56	91.80	48	85.71	48	100.00	41	67.21	73.21
51	Dose3	50	0	100.00	45	90.00	42	93.33	37	88.10	15	30.00	33.33
52	Dose3	65	0	100.00	59	90.77	58	98.31	58	100.00	49	75.38	83.05

Mallard repro, Aminopyralid, MRID 4625813
PRINTOUT OF RAW DATA (continued)

Obs	TRT	NH_LE	HS	HS_ES	HS_NH	THICK	HATWT	SURVWT	FOOD	WTGAINM	WTGAINF
1	Ctrl	85.42	41	85.42	100.00	0.35	.	101	148	46	230
2	Ctrl	91.49	42	84.00	97.67	0.34	.	67	115	154	56

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3	Ctrl	82.86	29	74.36	100.00	0.34	.	78	109	-16	240
4	Ctrl	92.00	45	81.82	97.83	0.33	.	79	110	-84	214
5	Ctrl	76.19	31	72.09	96.88	0.34	.	78	128	28	58
6	Ctrl	69.23	9	21.95	100.00	0.35	.	96	98	82	-126
7	Ctrl	96.08	48	94.12	97.96	0.33	.	80	111	24	244
8	Ctrl	66.67	11	25.00	91.67	0.36	.	90	91	68	156
9	Ctrl	30.56	10	27.03	90.91	0.31	.	88	104	-22	286
10	Ctrl	20.00	3	8.33	50.00	0.35	.	71	103	48	84
11	Ctrl	82.61	37	80.43	97.37	0.35	.	72	120	-152	234
12	Ctrl	76.09	26	55.32	74.29	0.35	.	83	125	42	254
13	Ctrl	82.93	34	79.07	100.00	0.37	.	90	102	120	102
14	Dose1	52.94	24	47.06	88.89	0.33	.	80	133	256	226
15	Dose1	27.27	3	11.11	50.00	0.31	.	83	141	-54	-4
16	Dose1	80.43	36	72.00	97.30	0.35	.	79	137	-18	314
17	Dose1	60.98	24	52.17	96.00	0.33	.	62	124	166	240
18	Dose1	94.74	45	77.59	83.33	0.33	.	87	120	-34	52
19	Dose1	79.31	39	63.93	84.78	0.32	.	68	103	-166	146
20	Dose1	43.24	15	37.50	93.75	0.34	.	73	156	188	132
21	Dose1	74.00	37	69.81	100.00	0.34	.	88	111	10	176
22	Dose1	78.00	32	62.75	82.05	0.33	.	65	116	-96	180
23	Dose1	69.23	18	66.67	100.00	0.38	.	71	102	-114	6
24	Dose1	87.23	39	79.59	95.12	0.34	.	84	110	2	140
25	Dose1	91.18	30	76.92	96.77	0.33	.	78	128	-130	230
26	Dose1	88.00	21	80.77	95.45	0.32	.	81	100	56	44
27	Dose2	72.73	20	60.61	83.33	0.34	.	61	138	148	26
28	Dose2	94.59	35	87.50	100.00	0.31	.	85	120	-4	84
29	Dose2	22.45	8	16.00	72.73	0.35	.	75	109	106	114
30	Dose2	.	.	.	.	.	.	.	.	.	.
31	Dose2	40.00	9	26.47	90.00	0.35	.	87	112	52	138
32	Dose2	37.50	12	30.00	100.00	0.34	.	73	109	-50	138
33	Dose2	68.00	33	53.23	97.06	0.33	.	95	117	134	172
34	Dose2	34.78	16	33.33	100.00	0.37	.	68	108	44	94
35	Dose2	78.57	32	68.09	96.97	0.36	.	82	107	-64	244
36	Dose2	84.62	22	84.62	100.00	0.34	.	79	132	18	146
37	Dose2	81.82	45	80.36	100.00	0.34	.	86	121	74	190
38	Dose2	48.15	10	27.03	76.92	0.34	.	79	125	176	218
39	Dose2	77.50	30	66.67	96.77	0.34	.	86	114	280	70
40	Dose3	88.10	36	83.72	97.30	0.30	.	75	121	-2	56
41	Dose3	94.12	30	76.92	93.75	0.30	.	77	120	94	198
42	Dose3	58.82	10	47.62	100.00	0.30	.	106	121	22	112
43	Dose3	42.31	20	37.04	90.91	0.30	.	96	121	-28	338
44	Dose3	73.91	32	62.75	94.12	0.29	.	76	125	32	166
45	Dose3	91.30	41	87.23	97.62	0.33	.	90	122	210	318
46	Dose3	18.18	7	14.00	87.50	0.33	.	77	109	-150	76
47	Dose3	81.82	40	68.97	88.89	0.35	.	74	104	22	116
48	Dose3	73.53	23	50.00	92.00	0.35	.	88	111	62	104
49	Dose3	85.00	33	82.50	97.06	0.34	.	76	105	36	218
50	Dose3	85.42	38	67.86	92.68	0.36	.	75	127	44	140
51	Dose3	40.54	11	24.44	73.33	0.33	.	68	101	-73	-26
52	Dose3	84.48	45	76.27	91.84	0.30	.	83	121	2	206

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE EL (Eggs Laid)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.978	0.468	1.837	0.153	USE PARAMETRIC TESTS

****
BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	13	48.77	6.43	1.78	13.19	44.88, 52.66
Dose1	13	48.46	12.53	3.48	25.86	40.89, 56.04
Dose2	12	47.58	9.89	2.85	20.77	41.30, 53.86
Dose3	13	51.38	10.47	2.90	20.37	45.06, 57.71

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	48.00	40.00	61.00	.	.
Dose1	53.00	28.00	66.00	99.37	0.63
Dose2	48.50	33.00	66.00	97.57	2.43
Dose3	53.00	25.00	65.00	105.36	-5.36

****
PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	0.34	0.800

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	48.77	.	49.08	.	1.000	0.991	0.911	.	.
Dose1	48.46	0.723	49.08	0.616	.	0.996	0.881	.	.
Dose2	47.58	0.635	49.08	0.651	.	.	0.782	.	.
Dose3	51.38	0.924	49.08	0.670	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

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Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE NEG_EC (Eggs Cracked)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
.	.	.	.	NO DATA FOR TEST

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	13	0.00	0.00	0.00	.	. , .
Dose1	13	0.00	0.00	0.00	.	. , .
Dose2	12	0.00	0.00	0.00	.	. , .
Dose3	13	0.00	0.00	0.00	.	. , .

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	0.00	0.00	0.00	.	.
Dose1	0.00	0.00	0.00	.	.
Dose2	0.00	0.00	0.00	.	.
Dose3	0.00	0.00	0.00	.	.

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE ENC_EL ((EL-EC)/EL (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
.	.	.	.	NO DATA FOR TEST

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	13	100.00	0.00	0.00	0.00	. , .
Dose1	13	100.00	0.00	0.00	0.00	. , .
Dose2	12	100.00	0.00	0.00	0.00	. , .
Dose3	13	100.00	0.00	0.00	0.00	. , .

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	100.00	100.00	100.00	.	.
Dose1	100.00	100.00	100.00	100.00	0.00
Dose2	100.00	100.00	100.00	100.00	0.00
Dose3	100.00	100.00	100.00	100.00	0.00

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE ES (Eggs Set)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.978	0.445	2.028	0.123	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.	Interval
Ctrl	13	44.62	5.62	1.56	12.60	41.22,	48.01
Dose1	13	44.46	11.79	3.27	26.53	37.33,	51.59
Dose2	12	43.17	10.21	2.95	23.66	36.68,	49.66
Dose3	13	46.85	10.12	2.81	21.61	40.73,	52.96

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	44.00	36.00	55.00	.	.
Dose1	49.00	26.00	61.00	99.66	0.34
Dose2	42.50	26.00	62.00	96.75	3.25
Dose3	47.00	21.00	59.00	105.00	-5.00

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	0.31	0.816

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	44.62	.	44.80	.	1.000	0.982	0.936	.	.
Dose1	44.46	0.737	44.80	0.604	.	0.987	0.923	.	.
Dose2	43.17	0.601	44.80	0.639	.	.	0.780	.	.
Dose3	46.85	0.911	44.80	0.658	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE ES_EL (EggsSet/EggsLaid (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.907	<.001	4.181	0.011	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	13	91.55	1.44	0.40	1.58	90.68,	92.42
Dose1	13	91.62	2.02	0.56	2.21	90.40,	92.84
Dose2	12	90.26	5.03	1.45	5.58	87.06,	93.46
Dose3	13	90.84	2.94	0.82	3.24	89.06,	92.62

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	91.67	89.47	94.00	.	.
Dose1	92.42	87.10	94.34	100.08	-0.08
Dose2	91.82	78.79	96.15	98.59	1.41
Dose3	91.07	84.00	93.88	99.23	0.77

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	0.27	0.965

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	91.67	.	.
Dose1	92.42	1.000	0.687
Dose2	91.82	1.000	0.532
Dose3	91.07	1.000	0.410

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE VE (Viable Embryo(d14))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.947	0.023	0.425	0.736	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	13	38.69	12.05	3.34	31.15	31.41, 45.97
Dose1	13	42.31	12.00	3.33	28.35	35.06, 49.56
Dose2	12	39.17	9.95	2.87	25.41	32.84, 45.49
Dose3	13	43.46	10.48	2.91	24.11	37.13, 49.79

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	42.00	13.00	51.00	.	.
Dose1	46.00	23.00	58.00	109.34	-9.34
Dose2	38.50	26.00	55.00	101.23	-1.23
Dose3	46.00	17.00	58.00	112.33	-12.33

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	0.56	0.643

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	38.69	.	40.94	.	0.843	1.000	0.699	.	.
Dose1	42.31	0.947	40.94	0.780	.	0.896	0.994	.	.
Dose2	39.17	0.789	40.94	0.809	.	.	0.773	.	.
Dose3	43.46	0.972	40.94	0.829	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE VE_ES (ViableEmbryo/EggsSet (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.755	<.001	5.196	0.004	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	13	86.06	22.68	6.29	26.35	72.36,	99.76
Dose1	13	94.72	4.32	1.20	4.57	92.11,	97.33
Dose2	12	90.78	8.99	2.60	9.91	85.06,	96.49
Dose3	13	92.29	7.45	2.07	8.08	87.78,	96.79

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	95.35	31.71	100.00	.	.
Dose1	96.15	85.19	100.00	110.06	-10.06
Dose2	94.17	76.47	100.00	105.48	-5.48
Dose3	93.33	76.09	100.00	107.24	-7.24

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	0.43	0.934

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	95.35	.	.
Dose1	96.15	1.000	0.601
Dose2	94.17	1.000	0.399
Dose3	93.33	1.000	0.394

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE LE (Live Embryo(d21))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.953	0.044	0.338	0.798	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	13	38.69	12.05	3.34	31.15	31.41, 45.97
Dose1	13	41.85	12.13	3.36	28.99	34.52, 49.18
Dose2	12	38.50	10.14	2.93	26.34	32.06, 44.94
Dose3	13	42.54	10.71	2.97	25.17	36.07, 49.01

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	42.00	13.00	51.00	.	.
Dose1	46.00	22.00	58.00	108.15	-8.15
Dose2	38.50	25.00	55.00	99.50	0.50
Dose3	44.00	17.00	58.00	109.94	-9.94

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	0.44	0.727

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	38.69	.	40.43	.	0.892	1.000	0.822	.	.
Dose1	41.85	0.932	40.43	0.739	.	0.881	0.999	.	.
Dose2	38.50	0.736	40.43	0.770	.	.	0.809	.	.
Dose3	42.54	0.952	40.43	0.791	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE LE_VE (LiveEmbryo/ViableEmbryo (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.786	<.001	7.457	<.001	USE NON-PARAMETRIC TESTS

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BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.Interval
Ctrl	13	100.00	0.00	0.00	0.00	. , .
Dose1	13	98.76	2.06	0.57	2.08	97.52, 100.00
Dose2	12	98.13	3.09	0.89	3.15	96.16, 100.00
Dose3	13	97.78	3.47	0.96	3.55	95.68, 99.88

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	100.00	100.00	100.00	.	.
Dose1	100.00	94.87	100.00	98.76	1.24
Dose2	100.00	91.30	100.00	98.13	1.87
Dose3	100.00	88.10	100.00	97.78	2.22

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	6.93	0.074

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	100.00	.	.
Dose1	100.00	1.000	0.017
Dose2	100.00	1.000	0.018
Dose3	100.00	1.000	0.008

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	<lowest dose	Dose1

80

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE NH (Number Hatched)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.962	0.101	0.319	0.811	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	13	29.62	15.05	4.17	50.81	20.52, 38.71
Dose1	13	30.69	13.36	3.70	43.52	22.62, 38.76
Dose2	12	23.83	11.64	3.36	48.83	16.44, 31.23
Dose3	13	30.31	13.33	3.70	43.99	22.25, 38.36

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	34.00	6.00	49.00	.	.
Dose1	31.00	6.00	54.00	103.64	-3.64
Dose2	23.00	10.00	45.00	80.48	19.52
Dose3	34.00	8.00	49.00	102.34	-2.34

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	0.70	0.555

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	29.62	.	30.15	.	0.997	0.706	0.999	.	.
Dose1	30.69	0.820	30.15	0.626	.	0.583	1.000	.	.
Dose2	23.83	0.297	27.20	0.417	.	.	0.627	.	.
Dose3	30.31	0.797	27.20	0.427	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

81

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE NH_EL (NumberHatched/EggsLaid (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.885	<.001	1.359	0.267	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.	Interval
Ctrl	13	58.49	25.91	7.19	44.31	42.83,	74.15
Dose1	13	61.74	18.72	5.19	30.32	50.43,	73.06
Dose2	12	50.07	21.37	6.17	42.69	36.49,	63.65
Dose3	13	58.40	21.65	6.00	37.06	45.32,	71.49

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	70.00	15.00	85.96	.	.
Dose1	69.70	19.35	87.10	105.57	-5.57
Dose2	57.39	21.15	76.09	85.61	14.39
Dose3	67.21	14.55	80.43	99.86	0.14

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	2.46	0.483

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	70.00	.	.
Dose1	69.70	1.000	0.429
Dose2	57.39	0.334	0.082
Dose3	67.21	1.000	0.307

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

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Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE NH_ES (NumberHatched/EggsSet (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.902	<.001	1.540	0.217	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.Interval	
Ctrl	13	63.98	28.56	7.92	44.64	46.72,	81.23
Dose1	13	67.13	19.86	5.51	29.59	55.13,	79.14
Dose2	12	55.75	24.39	7.04	43.74	40.26,	71.25
Dose3	13	64.23	23.60	6.54	36.74	49.97,	78.49

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	74.47	16.67	96.08	.	.
Dose1	74.00	22.22	93.10	104.94	-4.94
Dose2	61.86	22.00	87.50	87.15	12.85
Dose3	73.21	16.00	89.36	100.40	-0.40

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	1.40	0.707

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	74.47	.	.
Dose1	74.00	1.000	0.409
Dose2	61.86	0.657	0.148
Dose3	73.21	1.000	0.346

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

83

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE NH_LE (NumberHatched/LiveEmbryo (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.893	<.001	0.443	0.723	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.	Interval
Ctrl	13	73.24	23.02	6.39	31.44	59.33,	87.15
Dose1	13	71.27	20.10	5.58	28.21	59.12,	83.42
Dose2	12	61.73	23.77	6.86	38.50	46.63,	76.83
Dose3	13	70.58	23.53	6.53	33.34	56.36,	84.80

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	82.61	20.00	96.08	.	.
Dose1	78.00	27.27	94.74	97.32	2.68
Dose2	70.36	22.45	94.59	84.28	15.72
Dose3	81.82	18.18	94.12	96.37	3.63

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	2.39	0.495

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	82.61	.	.
Dose1	78.00	0.945	0.304
Dose2	70.36	0.349	0.074
Dose3	81.82	1.000	0.284

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

84

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE HS (Hatching Survival(d14))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.958	0.071	0.488	0.692	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.	Interval
Ctrl	13	28.15	15.23	4.22	54.10	18.95,	37.36
Dose1	13	27.92	11.80	3.27	42.26	20.79,	35.05
Dose2	12	22.67	12.16	3.51	53.65	14.94,	30.39
Dose3	13	28.15	12.76	3.54	45.32	20.44,	35.86

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	31.00	3.00	48.00	.	.
Dose1	30.00	3.00	45.00	99.18	0.82
Dose2	21.00	8.00	45.00	80.51	19.49
Dose3	32.00	7.00	45.00	100.00	0.00

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	0.52	0.667

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	28.15	.	28.15	.	1.000	0.722	1.000	.	.
Dose1	27.92	0.735	27.92	0.564	.	0.748	1.000	.	.
Dose2	22.67	0.308	25.52	0.393	.	.	0.722	.	.
Dose3	28.15	0.752	25.52	0.402	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

85

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE HS_ES (HatchingSurvival/EggsSet (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.924	0.003	1.700	0.180	USE NON-PARAMETRIC TESTS

****
BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.Interval
Ctrl	13	60.69	29.50	8.18	48.60	42.86, 78.51
Dose1	13	61.37	20.04	5.56	32.65	49.26, 73.48
Dose2	12	52.82	25.36	7.32	48.00	36.71, 68.94
Dose3	13	59.95	23.58	6.54	39.33	45.70, 74.20

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	74.36	8.33	94.12	.	.
Dose1	66.67	11.11	80.77	101.13	-1.13
Dose2	56.92	16.00	87.50	87.04	12.96
Dose3	67.86	14.00	87.23	98.78	1.22

****
NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	0.99	0.805

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	74.36	.	.
Dose1	66.67	0.673	0.213
Dose2	56.92	0.780	0.133
Dose3	67.86	1.000	0.259

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

86

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE HS_NH (HatchingSurvival/NumberHatched (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.733	<.001	1.075	0.369	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.Interval
Ctrl	13	91.89	14.40	3.99	15.67	83.19, 100.00
Dose1	13	89.50	13.35	3.70	14.91	81.43, 97.56
Dose2	12	92.82	9.83	2.84	10.59	86.57, 99.06
Dose3	13	92.08	6.69	1.85	7.26	88.03, 96.12

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	97.67	50.00	100.00	.	.
Dose1	95.12	50.00	100.00	97.40	2.60
Dose2	97.01	72.73	100.00	101.01	-1.01
Dose3	92.68	73.33	100.00	100.20	-0.20

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	3.47	0.325

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	97.67	.	.
Dose1	95.12	0.231	0.067
Dose2	97.01	1.000	0.393
Dose3	92.68	0.212	0.167

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

87

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE THICK (Eggshell thickness)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.985	0.765	3.619	0.020	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.Interval	
Ctrl	13	0.34	0.01	0.00	4.18	0.33,	0.35
Dose1	13	0.33	0.02	0.00	5.17	0.32,	0.34
Dose2	12	0.34	0.02	0.00	4.50	0.33,	0.35
Dose3	13	0.32	0.02	0.01	7.40	0.31,	0.34

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	0.35	0.31	0.37	.	.
Dose1	0.33	0.31	0.38	97.84	2.16
Dose2	0.34	0.31	0.37	100.28	-0.28
Dose3	0.33	0.29	0.36	94:14	5.86

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	9.36	0.025

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	0.35	.	.
Dose1	0.33	0.133	0.036
Dose2	0.34	1.000	0.516
Dose3	0.33	0.052	0.041

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose2	Dose3

88

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE HATWT (Hatchling Weight)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
.	.	.	.	NO DATA FOR TEST

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.Interval
Ctrl	0	.	.	.	.	. , .
Dose1	0	.	.	.	.	. , .
Dose2	0	.	.	.	.	. , .
Dose3	0	.	.	.	.	. , .

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	.	.	.	.	.
Dose1	.	.	.	.	.
Dose2	.	.	.	.	.
Dose3	.	.	.	.	.

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE SURVWT (Survivor Wt (d14))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.984	0.739	0.239	0.869	USE PARAMETRIC TESTS

****
BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	13	82.54	10.04	2.78	12.16	76.47,	88.60
Dose1	13	76.85	8.36	2.32	10.87	71.80,	81.90
Dose2	12	79.67	9.32	2.69	11.69	73.75,	85.59
Dose3	13	81.62	10.61	2.94	13.00	75.20,	88.03

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	80.00	67.00	101.00	.	.
Dose1	79.00	62.00	88.00	93.10	6.90
Dose2	80.50	61.00	95.00	96.52	3.48
Dose3	77.00	68.00	106.00	98.88	1.12

****
PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	0.89	0.454

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	82.54	.	82.54	.	0.441	0.878	0.995	.	.
Dose1	76.85	0.158	79.37	0.242	.	0.884	0.590	.	.
Dose2	79.67	0.434	79.37	0.265	.	.	0.957	.	.
Dose3	81.62	0.656	79.37	0.268	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE FOOD (Food Consumption)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.963	0.107	1.977	0.130	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	13	112.62	14.96	4.15	13.28	103.58, 121.65
Dose1	13	121.62	16.96	4.70	13.94	111.37, 131.86
Dose2	12	117.67	9.97	2.88	8.47	111.33, 124.00
Dose3	13	116.00	8.75	2.43	7.54	110.71, 121.29

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	110.00	91.00	148.00	.	.
Dose1	120.00	100.00	156.00	107.99	-7.99
Dose2	115.50	107.00	138.00	104.49	-4.49
Dose3	121.00	101.00	127.00	103.01	-3.01

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	1.05	0.380

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	112.62	.	117.29	.	0.314	0.773	0.913	.	.
Dose1	121.62	0.996	117.29	0.885	.	0.877	0.699	.	.
Dose2	117.67	0.962	117.29	0.906	.	.	0.989	.	.
Dose3	116.00	0.923	116.00	0.869	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

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Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE WTGAINM (Male wt gain)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.972	0.256	1.395	0.256	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.Interval
Ctrl	13	26.00	81.20	22.52	312.31	-23.07, 75.07
Dose1	13	5.08	129.87	36.02	2558.11	-73.40, 83.56
Dose2	12	76.17	99.04	28.59	130.04	13.24, 139.10
Dose3	13	20.85	84.15	23.34	403.67	-30.01, 71.70

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	42.00	-152.00	154.00	.	.
Dose1	-18.00	-166.00	256.00	19.53	80.47
Dose2	63.00	-64.00	280.00	292.95	-192.95
Dose3	22.00	-150.00	210.00	80.18	19.82

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	1.15	0.338

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	26.00	.	34.68	.	0.951	0.600	0.999	.	.
Dose1	5.08	0.531	34.68	0.674	.	0.302	0.978	.	.
Dose2	76.17	0.982	34.68	0.708	.	.	0.521	.	.
Dose3	20.85	0.702	20.85	0.578	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

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Mallard repro, Aminopyralid, MRID 4625813
ANALYSIS RESULTS FOR VARIABLE WTGAINF (Female wt gain)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.981	0.563	1.381	0.260	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.	Interval
Ctrl	13	156.31	116.71	32.37	74.67	85.78,	226.83
Dose1	13	144.77	97.52	27.05	67.36	85.84,	203.70
Dose2	12	136.17	63.33	18.28	46.51	95.93,	176.40
Dose3	13	155.54	101.49	28.15	65.25	94.21,	216.87

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	214.00	-126.00	286.00	.	.
Dose1	146.00	-4.00	314.00	92.62	7.38
Dose2	138.00	26.00	244.00	87.11	12.89
Dose3	140.00	-26.00	338.00	99.51	0.49

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	47	0.12	0.947

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	156.31	.	156.31	..	0.990	0.955	1.000	.	.
Dose1	144.77	0.632	145.74	0.463	.	0.996	0.992	.	.
Dose2	136.17	0.537	145.74	0.496	.	.	0.959	.	.
Dose3	155.54	0.745	145.74	0.510	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

Data Evaluation Report on the acute toxicity of XDE-750 (Aminopyralid) to Rainbow Trout (*Onchorhynchus mykiss*)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-14

Data Requirement:	PMRA DATA CODE	9.5.2.1
	EPA DP Barcode	D301682
	OECD Data Point	
	EPA MRID	462358-14
	EPA Guideline	72-1(c)

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: 2-pyridinecarboxylic acid, 4-amino-3,6-dichloro
CAS name: Not reported
CAS No.: 150114-71-9
Synonyms: XR-750

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation

Signature:
Date:7/27/2004

QC Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation

Date: 8/4/2004

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB IV

Signature:
Date: 11/22/2004 6/6/05

Secondary Reviewer(s): 1610
EAD, PMRA

Date: N/A

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

Date Evaluation Completed:

CITATION: Marino, T.A, McClaymont, E.L. *et al.* 2001. XDE-750 Herbicide: An Acute Toxicity Study with the Rainbow Trout (*Onchorhynchus mykiss*). Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland Michigan. Laboratory Project No. 011078. Study sponsored by Dow AgroSciences LLC, Indianapolis, Indiana. Study completed November 19, 2001.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow Trout (*Onchorhynchus mykiss*) were exposed to XDE-750 (aminopyralid) at nominal treatment concentrations of 0 (negative control), and 100 ppm a.i. under static conditions. Mean-measured treatment concentrations were <5.9 (<LOQ; negative control) and 100 ppm a.i.

By 96-hours, no mortalities were observed in either the control or 100 ppm a.i. treatment group. Two (7%) fish exhibited partial loss of equilibrium following the 96-hour exposure period. The LC_{50} was >100 ppm a.i., which categorizes XDE-750 as practically non-toxic to juvenile Rainbow Trout (*Onchorhynchus mykiss*) on an acute toxicity basis. The NOEC and LOEC based on mortality and sub-lethal effects were <100 and >100 ppm a.i., respectivcly, as there was an observed partial loss of equilibrium in 7% of the fish at 96 hours.

This study is scientifically sound, fulfills U.S. EPA guideline §72-1c, and is classified as **Acceptable.**

EAD Conclusion:
The EAD is in agreement with the conclusion reported by the study author and the EPA reviewer. Based on mortality and sublethal effects, the NOEC was < 100 ppm a.i and the LC_{50} was > 100 ppm a.i. This study is classified as acceptable for use in a risk assessment.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): 1.14 ± 0.2 g (post-exposure), 49 ± 3 mm
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour
LC_{50}: >100 ppm a.i. 95% C.I.: N/A
Probit slope: N/A
NOEC: <100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in U.S. EPA- FIFRA Standard Evaluation Procedure 540/9-85-006: Pesticide Assessment Guidelines Subdivision E, Hazard Evaluation, Guideline 72-1: OECD Guidelines for Testing of Chemicals Number 203, "Fish, Acute Toxicity Test": Official Journal of the European Communities; Method C.1. Acute Toxicity for Fish. Deviation from §72-1a included:

1. Test vessel size (12L) and fill volume (10L) were smaller than EPA recommended size (19L) and fill volume (15-30L), however, six replicates were used with only five fish/replicate, which reduced the loading rate to acceptable levels.

2. The reported dilution water hardness (58 mg/L as CaCO3) was higher than recommended (40-48 mg/L as CaCO3). The pH range (5.6-7.1) was lower than recommended (7.2-7.6).

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3. Replicate test vessels were aerated at a rate of approxiamately 100 bubbles/minute. However, chemical analysis of test solution was conducted on days-0 and -4 with recoveries of ~100% of nominal treatment concentrations.

The above deviations were considered minor and did not affect the validity or acceptability of this study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160-FIFRA), OECD ENV/MC/CHEM(98)17 (1997), and EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

A. MATERIALS:

1. Test Material XDE-750

Description: Solid

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound Under Test Conditions: The stability of the test substance in dilution water was demonstrated by analytical determination on day 0 (97.8% of nominal) and day 4 (103% of nominal) which resulted in a mean-measured concentration of 100%.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

Storage conditions of test chemicals: Stored under refrigeration (temperature not reported).

2. Test organism:

Species: Rainbow Trout (*Onchorhynchus mykiss*)

Age at test initiation: Juvenile

Weight at study initiation: 1.14 ± 0.2 g (post-exposure)
EPA requires: mean 0.5 - 5 g
Length at study initiation: 49 ± 3 mm (post-exposure)
EPA requires: Longest not > 2x shortest; OECD requires 2.0 ± 1.0 cm for bluegill and 5.0 ± 1.0 cm for rainbow trout

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Source: Thomas Fish Company, Anderson, California

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: Fish were exposed to nominal concentrations of 0 (negative control), 0.781, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, and 100 ppm a.i. No mortality or sub-lethal effects were observed at any of the selected treatment levels following 96-hours of exposure. Therefore, the definitive study was conducted as a limit test.

b) Definitive Study: The definitive nominal test concentration of 100 ppm a.i. was conducted as a limit test due to the lack of any treatment related effects following a 96-hour range-finding test at treatment level ≤100 ppm a.i.

Table 1. Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	All fish were acclimated for at least 14 days.	
Conditions: (same as test or not)	Same as test	
Feeding:	Aquatic Diet Number 1 Lot #992236, Harlan-Teklad, Madison, Wisconsin, was provided daily except during the 48 hours prior to and during testing.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	During acclimation, fish showed no signs of disease, stress, or mortality.	
Duration of the test	96-hour	*EPA/OECD requires: 96 hour*

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Parameter	Details	Remarks Criteria
Test condition static/flow through	 Static	
Type of dilution system- for flow through method. Renewal rate for static renewal	N/A N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Acration, if any	~100 bubbles/minute	Replicate test vessels were aerated at a rate of approx. 100 bubble/minute, chemical analysis of test solution was conducted on days-0 and -4 with recoveries of ~100% of nominal treatment concentrations. *EPA requires: no aeration; OECD permits aeration*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass beakers 12 L 10 L	Test vessel size (12L) and fill volume (10L) were smaller than EPA recommended size (19L) and fill volume (15-30L) however, six replicates were used with only five fish/replicate, which greatly reduced the loading rate to acceptable levels. *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	Untreated Saginaw Bay of Lake Huron water supplied by the City of Midland Water Treatment Plant that was limed and flocculated with ferric chloride. Before use in the lab, water was sand-filtered, pH-adjusted with gaseous CO_2, carbon filtered, and UV-irradiated.	 *EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

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Parameter	Details	Remarks Criteria
Water parameters: Hardness	58 mg $CaCO_3$ /L	The reported dilution water hardness (58 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH range (5.6-7.1) was lower than recommended (7.2-7.6). Alkalinity and Conductivity were 36 mg $CaCO_3$ /L and 53.3 µmho/cm, respectively.
pH	5.6-7.1	
Dissolved oxygen	8.5-10.3 mg/L (≥81% saturation)	
Total Organic Carbon	<1000 ng/mL	
Particulate Matter	Total Suspended Solids (TSS) were <1000 ng/mL	
Metals	Not detected	
Pesticides	Not detected	
Chlorine	Chloride was 14,000 ± 1000 ng/ml	
Temperature	11.9-12.7°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	The temperature, DO and pH were measured at test initiation and every 24 hrs thereafter. Temperature was measured continuously in one test vessel throughout the study. Hardness, alkalinity, and conductivity were measured in dilution water at test initiation.	

Parameter	Details	Remarks Criteria
		Hardness and pH *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.* ***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Concentration of test material: nominal: measured:	0 (negative control), and 100 ppm a.i. <5.9 (<LOQ; negative control), and 100 ppm a.i.	The definitive test was performed as a limit test. *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	none	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*

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Parameter	Details	Remarks Criteria
Number of fish/replicates: negative control: solvent control: treated:	30 fish total, 6replicates/level, 5 fish/replicate NA 30 fish total, 6replicates/level, 5 fish/replicate	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.569 g fish/L (p. 15)	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark transitional photoperiod	*EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Not fed during testing.	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	100% of nominal 5.9 ppm a.i. Not reported.	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

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2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	0, 24, 48, 72, and 96 hrs	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

By 96-hours, no mortalities were observed in either the control or the mean-measured 100 ppm a.i. treatment group. The NOEC and LC_{50} values based on mortality were 100 and > 100 ppm a.i.

Table 3: Effect of XDE-750 on Mortality of Rainbow Trout (*Onchorhynchus* mykiss).

Treatment, ppm a.i., Mean-Measured and (Nominal) Concentration	No. of Fish at Start of Study	0-24 Hours		48-72 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative control	30	0	0	0	0	0	0
100 (100)	30	0	0	0	0	0	0
NOEC (mortality)	100 ppm a.i.						
LC_{50} (95% C.I.)	>100 ppm a.i.						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

N/A = Not Applicable

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B. NON-LETHAL TOXICITY ENDPOINTS:

Sub-lethal effects included partial loss of equilibrium in only two fish at the 100 ppm a.i. treatment group by 96 hrs. These sub-lethal effects were not considered to be significantly different from the control group. The NOEC based on sub-lethal effects was 100 ppm a.i.

Table 4. Sub-Lethal Effect of XDE-750 on Rainbow Trout (*Onchorhynchus* mykiss).

Treatment, ppm a.i., Mean-Measured and (Nominal) Concentration	Observation Period			
	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected[1]	% Affected	% Affected	% Affected
Negative control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
100 (100)	No abnormalities detected	No abnormalities detected	No abnormalities detected	7.0%-Partial loss of equilibrium
NOEC (sub-lethal)	<100 ppm a.i.			
LOEC (sub-lethal)	>100 ppm a.i.			
EC_{50}	>100 ppm a.i.			
Positive control, if used % sub-lethal effect: EC_{50}:	N/A	N/A	N/A	N/A

[1] % Affected is the number of fish exhibiting symptoms/number of surviving fish x 100.
N/A = Not Applicable

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C. REPORTED STATISTICS:

Statistical Method: Because no mortality was observed during any observation period in any of the groups, no statistical analyses were performed.

96-Hour
LC_{50}: >100 ppm a.i. 95% C.I.: N/A
Probit slope: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50}, NOEC and LOEC values were determined visually due to a lack of mortality and less than 10% sub-lethal effects in the treatment group.

96-Hour
LC_{50}: >100 ppm a.i. 95% C.I.: N/A
Probit slope: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

E. STUDY DEFICIENCIES:

All deficiencies were considered minor and did not effect the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors.

Terminal mean fish weight and length from the 100 ppm a.i treatment group (1199 mg ± 198 and 50 ± 3 mm, respectively) was higher than that of the control group (1076 mg ± 248 and 48 ± 4 mm, respectively).

In a previous range-finding study fish were exposed to nominal concentrations of 0 (negative control), 0.781, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, and 100 ppm a.i. No mortality or sub-lethal effects were observed at any of the selected treatment levels following 96-hours of exposure. The definitive test was performed as a limit test consequently, only one treatment level (100 ppm a.i.) was tested and compared to the a negative control.

EAD comments:

After review of the study data and the US EPA DER, the EAD reviewer is in agreement with the conclusion reached by the US EPA. Deficiencies mentioned above are not considered to have impact on the results of this study.

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No amendments to the DER are recommended.

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-1c, and is classified as CORE. Based on the results of this study, XDE-750 is categorized as practically non-toxic to juvenile Rainbow Trout (*Onchorhynchus mykiss*) on an acute toxicity basis. The 96-hour NOEC based on mortality and sub-lethal effects was <100 ppm a.i. and the LC_{50} was >100 ppm .ai.

96-Hour
LC_{50}: >100 ppm a.i. 95% C.I.: N/A
Probit slope: N/A
NOEC: <100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

III. REFERENCES:

EPA-FIFRA. Environmental Protection Agency. Hazard Evaluation Division, Standard Evaluation Procedure: Acute Toxicity Test For Fish. EPA-540/9-85-006. June 1985.

U.S. Environmental Protection Agency. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Guideline 72-1, Acute Toxicity Test For Freshwater Fish. EPA-540/9-87-198. December 1986.

OECD. OECD Guidelines for Testing of Chemicals, Method 203, "Fish, Acute Toxicity Test", ISBN 92-64-12221-4. Adopted July, 1992.

Official Journal of the European Communities. European Economic Community (EEC) Method C.1. Acute Toxicity for Fish. ISSN 0378-6978. December 1992.

Environmental Protection Agency- FIFRA GLPS; Title 40 CFR Part 160- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Dow AgroSciences LLC, Test Substance Distribution Certificate. TSN102319, Dow AgroSciences LLC, Indianapolis, Indiana. 23 October 2000.

Certificate of Analysis for Test Substance, TSN102319. Lab Report Number DECO GL-AL MD-2000-005682, Analytical Sciences Laboratory, The Dow Chemical Company. 25 October 2000.

Data Evaluation Report on the acute toxicity of XR-750 (Aminopyralid) to Bluegill Sunfish (*Lepomis macrochirus*)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-15

Data Requirement:	PMRA DATA CODE	9.5.2.2
	EPA DP Barcode	D301682
	OECD Data Point	
	EPA MRID	462358-15
	EPA Guideline	72-1(a)

Test material: XR-750 (p. 10) **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC:4-amino-3,6-dichloro-picolinic acid (picolinic acid synonymous with 2-carboxylic acid)
CAS name: Not reported
CAS No.: 150114-71-9
Synonyms: XDE-750

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date:7/27/2004

QC Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/4/2004

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB-IV
Signature: BDK 6/16/05
Date: 11/23/2004

Secondary Reviewer(s): 1610
PMRA
BvSignature:
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

Date Evaluation Completed:

CITATION: Machado, M.W. 2003. XDE-750- Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Static Conditions. Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Project No. 12550.6162. Study sponsored by The Dow Chemical Company, Midland, Michigan. Study initiated October 10, 2001 and completed amended final report on October 10, 2003.

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Bluegill Sunfish (*Lepomis macrochirus*) were exposed to XR-750 (synonyms XDE-750; aminopyralid) at nominal treatment concentrations of 0 (negative and solvent controls), and 100 ppm a.i. under static conditions. Mean measured treatment concentrations of <6.8 (<LOQ; control) and 100 ppm a.i.

By 96-hours, no mortalities were observed in either the control group or the 100 ppm a.i. treatment group. The LC_{50} was >100 ppm a.i., which categorizes XR-750 as practically non-toxic to juvenile Bluegill Sunfish (*Lepomis macrochirus*) on an acute toxicity basis. The NOEC and LOEC values based on the lack of mortality and sub-lethal effects were 100 and >100 ppm a.i., respectively.

This study is scientifically sound but does not satisfy the guideline requirements for an acute toxicity study with Bluegill Sunfish [§72-1(a)] because test fish wet-weight range (0.18-0.92 g) was lower than recommended (0.5-5 g). Consequently, this study is classified as SUPPLEMENTAL. The study provides information that may be useful for future risk assessment purposes.

EAD Conclusion:

The EAD is in agreement with the conclusion reported by the study author and the EPA reviewer. The LC_{50} for XR-750 (Aminopyralid) was >100 ppm a.i. The NOECand LOEC values based on the mortality and sub-lethal effects were 100 and > 100 ppm a.i., respectively. This study is classified as acceptable for use in a risk assessment.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	wet-weight: mean 0.54 (0.18-0.92) g, length: mean 36 (23-44) mm based on a representative sample (n = 30) of the test population.
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour
LC_{50}:>100 ppm a.i. 95% C.I.: N/A
Probit Slope: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: N

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the US EPA Pesticide Assessment Guidelines Subdivision E, Series 72-1 (1982), the Standard Evaluation Procedure issued by the hazard Evaluation Division of EPA's Office of Pesticide Programs (1985), and the OECD Guideline for Testing of Chemicals #203, Fish, Acute Toxicity Test (1992). Deviations from §72-1a included:

1. The hardness (52 to 54 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$) and the

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pH was lower (5.6-7.0) than the US EPA recommended range (7.2-7.6).

2. Test fish wet-weight ranged (0.18-0.92 g) lower than recommended (0.5-5 g).

The use of smaller than recommended fish in the definitive test affected the acceptability of this study. All other deviation were considered minor and did not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160), and OECD.

A. MATERIALS:

1. Test Material XR-750 (Synonym: XDE-750; p. 10)

Description: Not Reported

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was verified by analytical determination at 0 hour (97% of nominal), and 96 hours (100% of nominal). QC samples spiked at 80.0, 100, and 110 ppm a.i. and analyzed concurrently with test samples had recoveries of 93.6-102% of nominal.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

Storage conditions of test chemicals: Stored in dark, ambient conditions (temperature not reported).

2. Test organism:

Species: Bluegill Sunfish (*Lepomis macrochirus*)

Age at test initiation: Juvenile

Weight at study initiation: 0.54 g (range of 0.18 to 0.92 g) based on a representative sample (n = 30) of the test population.

Length at study initiation: 36 mm (range of 23 to 44 mm) based on a representative sample (n = 30) of the test population.

Source: Osage Catfisheries, Osage Beach, Missouri

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B. STUDY DESIGN:

1. Experimental Conditions

a) Preliminary Study: Range finding test not reported.

b) Definitive Study: The definitive nominal test concentration of 100 mg a.i./L was selected by the Study Sponsor as a limit test.

Table 1. Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	All fish were acclimated for at least 14 days.	
Conditions: (same as test or not)	Same as test	
Feeding:	Commercially-prepared diet (Prostar) was provided *ad libitum* daily except during the 48 hours prior to and during testing.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	During acclimation, fish showed no signs of disease, stress, or mortality.	
Duration of the test	96-hour	*EPA/OECD requires: 96 hour*
Test condition static/flow through	Static	
Type of dilution system- for flow through method.	N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Renewal rate for static renewal	N/A	
Aeration, if any	None reported	*EPA requires: no aeration; OECD permits aeration*

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Parameter	Details	Remarks Criteria
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass aquaria 19.5 L 15 L	 *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	The dilution water was drawn from a 100 meter deep bedrock well into a reservoir, aerated and then supplemented with well water supplied by the Town of Wareham, Massachusetts.	*EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

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Parameter	Details	Remarks Criteria
Water parameters: Hardness	52 to 54 mg $CaCO_3$ /L	The hardness (52 to 54 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$) and the pH was lower than recommended.
pH	5.6-7.0	
Dissolved oxygen	6.6-9.7 mg/L (≥74% saturation)	
Total Organic Carbon	0.63 mg/L (p.14)	
Particulate Matter	Not reported	
Metals	<LOD	
Pesticides	<LOD	
Chlorine	Not reported	
Temperature	21-23°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	The DO and pH were measured at test initiation and every 24 hrs thereafter. Temperature was measured in each replicate daily throughout the test. Hardness was measured in dilution water at test initiation.	

Parameter	Details	Remarks Criteria
		Hardness and pH *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.* ***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Concentration of test material: nominal:	0 (negative and solvent control) and 100 ppm of a.i.	Definitive test was performed as a limit test.
measured:	<6.8 (<LOQ; negative and solvent control) and 100 ppm a.i.	*EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	dimethylformamide (0.1 ppm)	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*

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Parameter	Details	Remarks Criteria
Number of fish/replicates: negative control: solvent control: treated:	 30 fish total, 10 fish/replicate 30 fish total, 10 fish/replicate 30 fish total, 10 fish/replicate	three replicates/treatment *EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.36 g fish/L	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark	Light intensity of 60-80 foot candles at test solution surface. Abrupt changes were avoided. *EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	97% of nominal @ 0 hrs 100% of nominal @ 96 hrs 0.50-6.8 ppm a.i. Not reported.	Based on QC matrix fortifications analyzed concurrently with the test samples (Table 2, p. 21).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

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2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	0, 24, 48, 72, and 96 hrs	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

By 96-hours, no mortalities were observed in either the control or the mean-measured 100 ppm a.i. treatment group. The NOEC and LC_{50} values based on mortality were 100 and >100 ppm a.i.

Table 3: Effect of XR-750 on Mortality of Bluegill Sunfish (*Lepomis macrochirus*).

Treatment, ppm a.i., measured and (nominal conc.)	No. of fish at start of study	24 Hours		48-72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	30	0	0	0	0	0	0
Solvent control	30	0	0	0	0	0	0
100 (100)	30	0	0	0	0	0	0
NOEC (mortality)	100 ppm a.i.						
LC_{50} (95% C.I.)	>100 ppm a.i.						
Positive control, if used mortality: LC_{50}:	N/A*	N/A	N/A	N/A	N/A	N/A	N/A

* N/A = Not Applicable

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B. NON-LETHAL TOXICITY ENDPOINTS:

By 96-hours, no sub-lethal effects were observed in either the control or the mean-measured 100 ppm a.i. treatment group.

Table 4. Sub-lethal Effect of XR-750 on Bluegill Sunfish (*Lepomis macrochirus*).

Treatment, ppm a.i., Mean-Measured and (Nominal) Concentration	Observation Period			
	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected[1]	% Affected	% Affected	% Affected
Negative control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
Solvent control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
100 (100)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
NOEC (sub-lethal)	100 ppm a.i.			
LOEC (sub-lethal)	>100 ppm a.i.			
EC_{50}	>100 ppm a.i.			
Positive control, if used % sub-lethal effect: EC_{50}:	N/A*	N/A	N/A	N/A

[1] % Affected is the number of fish exhibiting symptoms/number of surviving fish x 100.
* N/A = Not Applicable

C. REPORTED STATISTICS:

Statistical Method: Because no mortality was observed during any observation period in any of the groups, no statistical analysis was performed.

96-Hour
LC_{50}: >100 ppm a.i. 95% C.I.: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

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D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50}, NOEC and LOEC values were determined visually due to a lack of mortality and no observed sub-lethal effects in the controls and treatment group.

96-Hour
LC_{50}: >100 ppm a.i. 95% C.I.: N/A
Probit slope: N/A 95% C.I.: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

E. STUDY DEFICIENCIES:

Test fish wet-weight ranged (0.18-0.92 g) lower than recommended (0.5-5 g). The use of smaller than recommended fish in the definitive test affected the acceptability of this study. All other deviations were considered minor and did not affect the validity or acceptability of the study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors.

EAD comments:

After review of the study data and the US EPA DER, the EAD reviewer is in agreement with the conclusion reached by the US EPA. Deficiences mentioned above are not considered to have impact on the results of this study.

No amendments to the DER are recommended.

G. CONCLUSIONS:

This study is scientifically sound but does not satisfy the guideline requirements for an acute toxicity study with Bluegill Sunfish [§72-1(a)] because test fish wet-weight ranged (0.18-0.92 g) lower than recommended (0.5-5 g). Consequently, this study is classified as SUPPLEMENTAL. The study provides information that may be useful for future risk assessment purposes. Based on the results of this study, XR-750 is categorized as practically non-toxic to juvenile Bluegill Sunfish (*Lepomis macrochirus*) on an acute toxicity basis.

96-Hour
LC_{50}:>100 ppm a.i. 95% C.I.: N/A
Probit Slope: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

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III. REFERENCES:

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OECD. 1992. Guideline for Testing of Chemicals. Fish Acute Toxicity Test. Guideline #203. Adopted 17 July 1992.

OECD. 1997. Good Laboratory Practice in the Testing of Chemicals. Paris, France.

U.S. EPA. 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009). 61pp.

U.S. EPA. 1982. Office of Pesticide Programs. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-85-024. October 1982. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1985. Office of Pesticide Programs. Standard Evaluation Procedure for Acute Toxicity Test for Freshwater Fish. EPA-540/9-85-006. June 1985. U.S. Environmental Protection Agency, Washington, D.C.

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Data Evaluation Report on the Acute Toxicity of XDE-750 (Aminopyralid) to Larvae of the Northern Leopard Frogs (*Rana pipiens*)
PMRA Submission Number {.....} EPA MRID Number 462358-16

Data Requirement:	PMRA DATA CODE	9.5.2.3
	EPA DP Barcode	D301682
	OECD Data Point	II A 8.16.1
	EPA MRID	462358-16
	EPA Guideline	Non-guideline; Protocol based on §72-1a

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: 3,6-dichloro-4-amino-2-pyridinecarboxylic acid
CAS name: Not reported
CAS No.: Not reported
Synonyms: XR-750, X660750

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date:8/16/2004

QC Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/5/2004

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB-IV
Signature:
Date: 12/02/2004 6/16/05

Secondary Reviewer(s): Barb Martinovic
PMRA
Signature:
Date: 01/27/2005

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

Date Evaluation Completed: 06/12/2005

CITATION: Henry, K.S., McClaymont, E.L., *et al.* 2003. XDE-750: 96-h Acute Toxicity to Larval Amphibians Using the Northern Leopard Frog, *Rana pipiens*, as a Biological Model. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland Michigan. Laboratory Project No. 031030 Study sponsored by Dow AgroSciences LLC, Indianapolis, Indiana. Study completed June 30, 2003.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, larvae of the Northern Leopard Frogs (*Rana pipiens*) were exposed to XDE-750 (aminopyralid) at nominal concentrations of 0 (negative control) and 100 ppm a.i. under static conditions. Mean-measured concentrations were <2.16 (<LOQ; negative control) and 95.2 ppm a.i.

After 96 hours of exposure, no mortalities or sub-lethal effects were observed in the control or treatment group. The LC_{50} was >95.2 ppm a.i., which categorizes XDE-750 (Aminopyralid) as practically non-toxic to larvae of Northern Leopard Frog (*Rana pipiens*) on an acute toxicity basis. The NOEC and LOEC values based on mortality and sub-lethal effects were 95.2 and >95.2 ppm a.i., respectively.

This study is scientifically sound but does not fulfill U.S. EPA guideline §72-1(a) because it was performed using a non-guideline species. Consequently, the study is classified as SUPPLEMENTAL. This study provides information that is useful for risk assessment purposes.

The PMRA categorizes the acute toxicity study as ACCEPTABLE. PMRA would prefer a study longer in length which examines endpoints such as growth, weight and deformities. However, this is acceptable for acute mortality testing. The test species, Leopard frog, has the same qualities (ubiquitous, small, easy to collect, sensitive toxicological species) as bullfrogs for testing of chemicals, thus, this species is acceptable.

It should be noted that the toxicity criteria is based on fish as there is no criteria for frog species.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): 7 days post-hatch
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour
LC_{50}:>95.2 ppm a.i. 95% C.I.: N/A
NOEC: 95.2 ppm a.i.
LOEC: >95.2 ppm a.i.
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in U.S. EPA-FIFRA Standard Evaluation Procedure 540/9-85-006 (1985); U.S. EPA Pesticide Assessment Guidelines Subdivision E, Hazard Evaluation, Guideline 72-1 (1986); OECD Guidelines for Testing of Chemicals Number 203, "Fish, Acute Toxicity Test" (1992); Official Journal of the European Communities, Directive 92-69 EEC. C.1. Acute Toxicity for Fish (1992); and ASTM Standard Guide for Conducting Acute Toxicity Tests on Test materials with Fishes, Macroinvertebrates and Amphibeans, E729-96 (1996). Deviation from §72-1a included:

1. Test vessels (4 L with 3.5 L fill volume) is smaller than EPA recommended size (19 L with a fill volume of 15L).

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2. The biomass loading rate was not reported.

3. The test material storage conditions were not reported.

4. The definitive test was performed using a non-guideline species.

5. The sublethal endpoints were not defined.

All deviations were considered minor and did not affect the validity of the definitive test. However, this study is classified as SUPPLEMENTAL because the test was performed using a non-guideline species.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160), and OECD ENV/MC/CHEM (98) 17.

A. MATERIALS:

1. Test Material XDE-750 (aminopyralid)

Description: Solid

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound Under Test Conditions: The stability of the test substance in dilution water was verified by analytical determination on day 0 (94.9% of nominal) and day 4 (95.5% of nominal) which resulted in a mean-measured concentration of 95.2%. Analytical standards ranged 2.16 to 174 ppm a.i., actual recovered concentrations were not reported (p. 13, Table 4, p. 22). The LOQ was 2.16 ppm a.i.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

Storage conditions of test chemicals: Not Reported.

2. Test organism:

Species: Northern Leopard Frogs (*Rana pipiens*)

Age at test initiation: larvae (7 days post-hatch)

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Weight at study initiation: N/A

Length at study initiation: N/A

Source: Nasco, Inc., Ft. Atkinson, WI

B. STUDY DESIGN:

1. Experimental Conditions

a) Preliminary Study: 96-hour probe study with one control vessel and one test vessel (100 ppm a.i.). At test termination, no mortality or sub-lethal effects were observed.

b) Definitive Study: The definitive nominal test concentration of 100 mg a.i./L was used as a limit test and results were compared to a negative control.

Table 1. Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	All tadpoles were acclimated for 7 days (pg 11).	Reported acclimation period (7 days), is shorter than EPA recommended time of 14 days based on fish criteria. All tadpoles perfomed well in control and treated, therefore this did not impact the study
Conditions: (same as test or not)	Same as test	
Feeding:	Test organisms were fed Frog Brittle, prior to test, and were not fed during test.	*EPA recommends/requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	During acclimation, tadpoles showed no signs of disease, stress, or mortality.	
Duration of the test	96-hour	*EPA/OECD require: 96 hour*

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Parameter	Details	Remarks Criteria
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Static N/A N/A	 *EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	100 bubbles/minute	Chemical analysis of test solution was conducted on day 0 and day 4; mean-measured recovery of 95.2% of nominal indicating the test material was stable under test conditions. *EPA requires: no aeration; OECD permits aeration*
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	 Glass aquaria 4 L 3.5 L	Test vessels (4 L with 3.5 L fill volume) is smaller than EPA recommended size (19 L with a fill volume of 15L) based on fish criteria. *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	Lab dilution water supplied by City of Midland Water Treatment Plant (pre-municipal treatment), obtained from the upper Saginaw Bay of Lake Huron off of Whitestone Point and is limed and flocculated with with ferric chloride. Before use, water is aerated, filtered and pH adjusted.	Ammonia and chlorine should be removed. *EPA 1975; Soft reconstituted water or water from a natural source, not dechlorinated tap water;* *OECD permits dechlorinated tap water.*

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Parameter	Details	Remarks Criteria
Water parameters: Hardness	67-70 mg $CaCO_3$ /L	[Hardness - EPA: 40-48 mg/L as $CaCO_3$; OECD: 10-250 mg/L as $CaCO_3$
pH	6.7-7.6	pH - EPA: 7.2-7.6, OECD: 6-8.5]
Dissolved oxygen	7.3-8.9 mg/L (≥86% saturation)	20-24°C is ideal for R pipiens
Total Organic Carbon	1774 µg/l	Note: High OC results in less toxicity for some chemicals due to bioavability.
Particulate Matter	TSS: <LOD	
Metals	<LOD	
Pesticides	<LOD	
Chlorine	<LOD	
Temperature	21.4-21.8°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	The temperature, DO and pH were measured at test initiation and every 24 hrs thereafter. Temperature was measured continuously in one test vessel throughout the study. Hardness was measured in dilution water at test initiation.	

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Parameter	Details	Remarks Criteria
Concentration of test material: nominal:	0 (negative control), and 100 ppm of XDE-750	test was performed as a limit test based on the results of a preliminary range-finding study.
measured:	<2.16 (LOQ in negative control), and 95.2 ppm a.i.	*EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)		*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*
Number of fish/replicates: negative control:	30 tadpoles, divided into three replicates containing 10 fish each	
solvent control:	NA	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
treated:	30 tadpoles, divided into three replicates containing 10 fish each	

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Parameter	Details	Remarks Criteria
Biomass loading rate		Biomass loading rate not reported. *Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark	Light intensity was not reported. *EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	 *EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	95.2% of nominal 2.16 ppm a.i. Not reported.	Based on mean-measured test sample recovery (Table 3, p. 21).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	0, 24, 48, 72, and 96 hrs	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, mortality was 0% in control and mean-measured 95.2 ppm a.i. treatment group. The NOEC and LC_{50} values based on mortality were 95.2 and >95.2 ppm a.i., respectively.

Table 3: Effect of XDE-750 (Aminopyralid) on Mortality of Northern Leopard Frog Larvae (*Rana pipiens*).

Treatment, ppm a.i., 96-Hour Mean-Measured and (Nominal Conc.)	No. of Fish at Start of Study	24 Hours		48-72 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control	30	0	0	0	0	0	0
95.2 (100)	30	0	0	0	0	0	0
NOEC (mortality)	95.2 ppm a.i.						
LC_{50} (95% C.I.)	>95.2 ppm a.i.						
Positive control, if used mortality: LC_{50}:	N/A*	N/A	N/A	N/A	N/A	N/A	N/A

* N/A = Not Applicable

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B. NON-LETHAL TOXICITY ENDPOINTS:

After 96 hours of exposure, sub-lethal effects were 0% in control and mean-measured 95.2 ppm a.i. treatment group. The NOEC value based on sub-lethal effects was 95.2 ppm a.i.

Table 4. Sub-Lethal Effect of XDE-750 (Aminopyralid) on Northern Leopard Frog Larvae (*Rana pipiens*).

Treatment, ppm a.i., 96-Hour Mean-Measured and (Nominal Conc.)	Observation Period			
	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected[1]	% Affected	% Affected	% Affected
Negative control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
95.2 (100)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
NOEC (sub-lethal)	95.2 ppm a.i.			
LOEC (sub-lethal)	>95.2 ppm a.i.			
EC_{50}	>95.2 ppm a.i.			
Positive control, if used % sub-lethal effect: EC_{50}:	N/A*	N/A	N/A	N/A

[1] % Affected is the number of larvae exhibiting symptoms/number of surviving larvae x 100.
--- 100% mortality
* N/A = Not Applicable

C. REPORTED STATISTICS:

Statistical Method: All statistical analyses were performed visually because no mortality or sub-lethal effects were observed during any observation period in the control or treatment group.

96-Hour
LC_{50}: >100 ppm a.i. 95% C.I.: N/A
Probit Slope: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

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D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The 96-hour LC50, NOEC and LOEC were visually determined due to a lack of mortality or sub-lethal effects in the control or treatment group.

96-Hour
LC_{50}: >95.2 ppm a.i. 95% C.I.: N/A
Probit slope: N/A
NOEC: 95.2 ppm a.i.
LOEC: >95.2 ppm a.i.
Endpoints affected: None

E. STUDY DEFICIENCIES:

This study was conducted as a limit test using one nominal treatment level (100 ppm a.i.) using a US EPA non-guideline species, Northern Leopard Frog (*Rana pipiens*). The test protocol was based on US EPA guideline §72-1a and was in compliance with the Good Laboratory Standards outlined by the US EPA (40 CFR Part 160). All deficiencies/deviations from §72-1a were considered to be minor and did not affect the validity or acceptability of this study. However, because the study was performed using a non-guideline species, the study is classified as SUPPLEMENTAL.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors.

G. CONCLUSIONS:

This study is scientifically sound but does not fulfill U.S. EPA guideline §72-1(a) because it was performed using a non-guideline species. Consequently, the study is classified as SUPPLEMENTAL. This study provides information that maybe useful for future risk assessment purposes. Based on the results of this study, XDE-750 (Aminopyralid) is categorized as practically non-toxic to larvae of the Northern Leopard Frogs (*Rana pipiens*) on an acute toxicity basis.

96-Hour
LC_{50}:>95.2 ppm a.i. 95% C.I.: N/A
Probit Slope: N/A
NOEC: 95.2 ppm a.i.
LOEC: >95.2 ppm a.i.
Endpoints affected: None

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III. REFERENCES:

ASTM. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. Designation: E 729-96. Approved 10 May, 1996.

EPA-FIFRA. Environmental Protection Agency. Hazard Evaluation Division, Standard Evaluation Procedure: Acute Toxicity Test For Fish. EPA-540/9-85-006. June 1985.

U.S. Environmental Protection Agency. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Guideline 72-1, Acute Toxicity Test For Freshwater Fish. EPA-540/9-87-198. December 1986.

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Environmental Protection Agency- FIFRA GLPS; Title 40 CFR Part 160- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

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EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Dow AgroSciences LLC, Test Substance Assay Certificate. TSN102319, Lot Number F0031-143. 26 March 2003.

Certificate of Analysis . FA & PC Number 023243. TSN102319. 6 November 2002.

Data Evaluation Report on the Acute Toxicity of XDE-750 (Aminopyralid) to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 462358-17

Data Requirement:		
	PMRA DATA CODE	{.....}
	EPA DP Barcode	D301682
	OECD Data Point	
	EPA MRID	462358-17
	EPA Guideline	72-2a

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: 2-pyridinecarboxylic acid, 4-amino-3,6-dichloro
CAS name: Not reported
CAS No.: Not reported
Synonyms: XR-750, X660750

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/4/2004

QC Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/4/2004

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB-IV
Signature: BDK 6/16/05
Date: 11/30/2004

Secondary Reviewer(s):
PMRA
Signature:
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

Date Evaluation Completed:

CITATION: Marino, T.A., Hales, C.A., McClymont, E.L., and Yaroch, A.M. 2001. XDE-750 Herbicide: An Acute Toxicity Study with the Daphnid, *Daphnia magna* Straus. Unpublished study performed by The Dow Chemical Company, Toxicology & Environmental Research and Consulting, Midland, Michigan. Laboratory Project ID No. 011079. Study submitted by Dow AgroSciences LLC, Indianapolis, Indiana. Study initiated June 19, 2001 and completed November 6, 2001.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of XDE-750 (aminopyralid) to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control) and 100 ppm a.i.; mean-measured concentrations were <6 (LOQ, negative control) and 98.6 ppm a.i.

After 48 hours, no immobilization or sub-lethal effects were observed in the control or mean-measured 98.6 ppm a.i. treatment group. The 48-hour LC_{50}/EC50 was >98.6 ppm a.i., which categorizes XDE-750 (aminopyralid) as practically non-toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. The 48-hour NOEC and LOEC levels were 98.6 and >98.6 ppm a.i., respectively.

This study is scientifically sound and fulfills U.S. EPA guideline §72-2a for an acute toxicity study with freshwater invertebrates. This study is classified as Acceptable.

Results Synopsis

Test Organism Age (eg. 1st instar): <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour
LC_{50}/EC_{50}: >98.6 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 98.6 ppm a.i.
LOEC: >98.6 ppm a.i.
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in U.S. EPA-FIFRA Standard Evaluation Procedure 540/9-85-005 Pesticide Assessment Guidelines Subdivision E, Hazard Evaluation: Guideline 72-2; OECD guideline No. 202 Daphnia sp., Acute Immobilization Test, Part 1; EC Directive 91/414 Annex I 8.2.5; and Official Journal of the European Communities. Method C.2. Acute Toxicity for Daphnia. Deviations from §72-2a included:

1. The storage conditions of the test material were not reported.
2. It was not reported whether or not the test vessels were aerated during the exposure period.
3. The hardness (150 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$).

These deviations did not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. This study was conducted in compliance with the U.S. EPA-FIFRA GLPs, Title 40 CFR Part 160, OECD Principles of GLP (1997), and the EC Commission Directive 99/11/EC (1999).

A. MATERIALS:

1. **Test Material** XDE-750 (Aminopyralid)

Description: Solid

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound Under Test Conditions: The stability of the test substance under test conditions was verified by analytical determination at 0 and 48 hours. Recoveries were 98.2% of nominal concentrations at 0 hours and 98.5-99.4% at 48 hours (Table 3, p. 22).

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The OECD requirements were not reported..

Storage conditions of test chemicals: Not reported.

2. **Test organism**:

Species: *Daphnia magna* (Straus)

Age at test initiation: Neonates, <24 hours old

Source: In-house laboratory cultures.

B. STUDY DESIGN:

1. **Experimental Conditions**

a) Range-finding Study: Definitive test concentrations were based upon results of a range-finding test. The 48-hour range-finding test concentrations were 25.0, 50.0, and 100 ppm a.i with a dilution water control. No effects were observed at any of the dose levels.

b) Definitive Study: Limit test.

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Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous laboratory cultures were maintained. Same as test *Daphnia* cultures were fed 4 times/week with a mixture of *Ankistrodesmus convolutus* (algae) and YCT trout chow (yeast-ceraphyll trout). Not specified	Daphnids were not fed during the test. *EPA requires 7 day minimum acclimation period. No feeding during study.*
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through Type of dilution system (for flow through method) Renewal rate (for static renewal)	Static N/A N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	It was not reported whether or not the test vessels were aerated during the exposure period.	
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	 Glass jars 250 mL 200 mL	*EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water was city water (prior to municipal treatment) from Lake Huron. The water was limed and flocculated with ferric chloride, filtered (sand and carbon), pH-adjusted, and UV-irradiated.	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*

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Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 150 mg/L as $CaCO_3$ 6.2-7.6 8.6-8.7 mg/L (>97%) 19.9-20.6°C <1000 ng/mL <LOD (total suspended solids) <LOD (See Table 1, p. 20) <LOD (See Table 2, p. 21) <LOD	The hardness (150 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH (6.2-7.6) ranged lower than recommended (7.2-7.6). *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*
Number of replicates Solvent control: Negative control: Treatments:	 N/A 3 3	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 N/A 10/replicate, 3reps./level 10/replicate, 3reps./level	The biomass loading rate was not specified. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*

Parameter	Details	Remarks *Criteria*
Treatment concentrations nominal: measured:	0 (negative control) and 100 ppm a.i. <6 (LOQ, negative control) and 98.6 ppm a.i.	*EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	N/A	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark	The light intensity ranged from 1860-1970 lux. *EPA requires 16 hours light, 8 hours dark.*
Stability of chemical in the test system	Stable, based on mean analytical recoveries from 0 and 48 hours.	Analyzed concentrations were 98.2% of nominal concentrations at 0 hours and 98.5-99.4% at 48 hours.
Recovery of chemical Level of Quantitation Level of Detection	98.2-99.4% of nominal 6 ppm a.i. Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

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2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Immobility and sub-lethal effects	
Observation intervals	After 24 and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A: MORTALITY:

After 48-hours of exposure, mortality was 0% in the negative control and the mean-measured 98.6 ppm a.i. treatment group. The 48-hour EC_{50} was >98.6 ppm a.i. and the NOEC for mortality/immobility was 98.6 ppm a.i.

Table 3: Effects of XDE-750 (Aminopyralid) on Mortality/Immobilization of *Daphnia magna,*

Treatment, ppm a.i. 48-Hour Mean-Measured and (Nominal) Concn.	Observation Period			
	24 Hours		48 Hours	
	No. Dead	% Affected	No. Dead	% Affected
Dilution Water Control	0	0	0	0
98.6 (100)	0	0	0	0
NOEC, ppm a.i.	98.6			
LOEC, ppm a.i.	>98.6			
EC_{50} (with 95% C.I.), ppm a.i.	>98.6			

B. SUB-LETHAL TOXICITY ENDPOINTS:

After 48-hours of exposure, all surviving daphnids were reported to be normal in the negative control and mean-measured 98.6 ppm a.i. treatment group.

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C. REPORTED STATISTICS:

Statistical Method: The EC_{50}, NOEC, and LOEC values were determined visually due to the lack of any treatment-related effects.

48-Hour
LC_{50}/EC_{50}: >98.6 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 98.6 ppm a.i.
LOEC: >98.6 ppm a.i.
Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The EC_{50}, NOEC, and LOEC values were determined visually due to the lack of any treatment-related effects.

48-Hour
LC_{50}/EC_{50}: >98.6 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 98.6 ppm a.i.
LOEC: >98.6 ppm a.i.
Endpoints affected: None

E. STUDY DEFICIENCIES:

All deviations from U.S. EPA guideline §72-2a were considered minor and did not affect validity or acceptability this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors'.

This definitive test was performed as a limit-test, nominal 100 ppm a.i., based on the results of a preliminary range-finding study.

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G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-2a, and is classified as Acceptable. Based on the results of this study, XDE-750 (aminopyralid) is categorized as practically non-toxic to the water flea, *Daphnia magna*, on an acute toxicity basis.

48-Hour
LC_{50}/EC_{50}: >98.6 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 98.6 ppm a.i.
LOEC: >98.6 ppm a.i.
Endpoints affected: None

III. REFERENCES:

EPA-FIFRA. Environmental Protection Agency. Hazard Evaluation Division, Standard Evaluation Procedure: Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005.

Environmental Protection Agency. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Guideline 72-2, Acute Toxicity Test For Freshwater Aquatic Invertebrates. EPA-540/09-87-198.

Organization for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals. Method 202, Daphnia sp., Acute Immobilization Test, Part 1. ISBN 92-64-12221-4.

European Community (EC) Directive 91/414 Annex I 8.2.5.

Official Journal of the European Communities. (EEC) Method C.1. Acute Toxicity Test for Daphnia. ISSN 0378-6978. 29 December 1992.

Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

OECD Series on Principles on Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM (98)17.

EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Dow AgroSciences LLC, Test Substance Distribution Certificate. TSN 102319, Dow AgroSciences LLC, Indianapolis, Indiana. 23 October 2000.

Certificate of Analysis for Test Substance, TSN 102319. Lab Report Number DECO GL-AL MD-2000-005682, Analytical Services Laboratory, The Dow Chemical Company. 25 October 2000.

Product Technology Information Platform (PTIP) Database. Dow AgroSciences LLC, Indianapolis, Indiana.

DATA EVALUATION RECORD
ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK
SHELL DEPOSITION STUDY
§72-3(B) PMRA DACO: 9.4.4

1. **CHEMICAL:** Aminopyralid PC Code No.: 005100

2. **TEST MATERIAL:** XR-750 (XDE-750) Technical Purity: 94.5%

3. **CITATION:**

Author: Cafarella, M.

Title: XDE-750 Technical - Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions.

Study Completion Date: April 23, 2002

Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, Massachusetts 02571-1075

Sponsor: The Dow Chemical Company
for Dow AgroSciences LLC
1803 Building
Midland, Michigan 48674

Laboratory Report ID: 12550.6189

MRID No.: 462358-18 PMRA Submission #: 2004-0789

DP Barcode: D301682

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation **Date:** 8/3/2004

APPROVED BY: Gregory Hess, Staff Scientist, Dynamac Corporation **Date:** 10/4/2004

5. **APPROVED BY:** Brian D. Kiernan, Biologist, OPP/EFED/ERBIV **Date:** 12/01/2004

Signature: 6/16/05

213, EAD, PMRA **Date:** January 26, 2005

Signature:

2030239

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Crassostrea virginica*

Age or Size of Test Organism: Mean valve height: 39 ± 4 mm (similar age)

Definitive Test Duration: 96 hours

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

In this 96-hour, flow-through acute EC_{50} test with an estuarine/marine mollusk, the Eastern oyster (*Crassostrea virginica*) was exposed to XR-750 Technical (Synonym: XDE-750; aminopyralid) at nominal treatment concentrations of 0 (negative and solvent controls), 13, 22, 36, 60, and 100 ppm a.i. Mean-measured treatment concentrations were <1.2 (<LOQ; negative and solvent controls),12, 21, 31, 50, and 89 ppm a.i. with recoveries of 84-97% of nominal.

No mortalities or sublethal effects were observed during the test. Shell growth was inhibited 12% in the 89 ppm a.i. treatment group compared to the pooled control. Mean shell growth in the 12, 21, 31, and 50 ppm a.i. treatment groups were slightly higher than the pooled control. No statistically-significant reductions in shell growth compared to the pooled control were identified. The **NOEC is 89 ppm a.i.** and the 96-hour **EC_{50} is >89 ppm a.i.** Because the mean measured concentration was only 89% of nominal at the 100 ppm level XR-750 Technical (aminopyralid) is classified as **slightly toxic** to the Eastern oyster (*Crassostrea virginica*) on an acute toxicity basis.

This study is scientifically valid and fulfills the requirements of an acute toxicity test with an estuarine/marine mollusk [§72-3(b)]. This study is classified as **Acceptable**.

EAD Conclusion:

This study is scientifically sound and is classified as **acceptable**. The 96-hour EC_{50} and NOEC of aminopyralid to the Eastern oyster were > 89 ppm a.i. and 89 ppm a.i., respectively.

Results Synopsis

EC_{50}: >89 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 89 ppm a.i.

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LOEC: >89 ppm a.i.

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: The guideline deviation was considered to be minor and did not impact the acceptability or validity of the study. Missing information should be provided to U.S. EPA EFED.

C. Repairability: N/A

9. BACKGROUND:

10. GUIDELINE DEVIATIONS:

1. The total organic carbon in the dilution water was not reported.
2. The mean measured concentration at the highest nominal concentration was only 89%.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of XR-750 Technical (Aminopyralid) to an estuarine/marine mollusk for the purpose of chemical registration.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	39 ± 4 mm
Supplier	Circle C Oysters Ridge, Maryland
Are all oysters from same source?	Yes

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Guideline Criteria	Reported Information
Are all oysters from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	13 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	3-5 mm
Feeding during the acclimation Must be fed to avoid stress.	Supplementary algal diets of *Tetraselmus maculata*.
Pretest Mortality <3% mortality 48 hours prior to testing	<1% mortality during the 7 days prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural unfiltered seawater collected directly from the Cape Cod Canal, Bourne, Massachusetts.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) salinity, weekly range: <6 ‰	32-33‰

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Guideline Criteria	Reported Information
Water Temperature 15-30°C, consistent in all test vessels	20-21°C
pH	7.2-8.0
Dissolved Oxygen ≥60% throughout	5.6-7.8 mg/L (>60% saturation)
Total Organic Carbon	Not reported
Test Aquaria Should be constructed of glass or stainless steel.	Glass, 49.5 x 25.5 x 29 cm, 18-L fill volume
Type of Dilution System Must provide reproducible supply of toxicant	Constant-flow serial diluter
Flow rate Consistent flow rate	6.0 turnovers/aquarium/day, or 5.25 L/oyster/hr.
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes; study authors reported that oysters were spaced equidistant from one another with valve inflow openings facing toward the flow of water.
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark with a transition period
Solvents Not to exceed 0.5 mL/L	Dimethylformamide, 0.5 mL/L

D. Test Design

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Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 or more oysters, then no definitive test is required.	A flow-through 96-hour range-finding study was performed at 0 (dilution water control), 2.6, 6.4, 16, 40, and 100 ppm a.i. By 96 hours, the reduction in shell growth was 16, 14, 13, 13, and 42% in the 2.6, 6.4, 16, 40, and 100 ppm a.i. treatment groups, respectively, compared to the control.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; conc. should be in a geo-metric series	0 (negative and solvent controls), 13, 22, 36, 60, and 100 ppm a.i.
Number of Test Organisms Minimum 20 individual per test level and in each control	40 oysters/level, divided into two replicates with 20 oysters each
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1. Measured daily in each aquarium and continuously in one 100 ppm a.i. replicate vessel. 2. Measured daily in each aquarium.

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Guideline Criteria	Reported Information
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

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13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	No mortality occurred.
Control Shell Deposition Must be at least 2 mm.	Negative Control: 2.7 ± 1.3 mm (mean ± SD); Solvent Control: 2.6 ± 1.1 mm (mean ± SD).
Recovery of Chemical	Based on QC samples prepared at each sampling interval at fortification levels of 10.0, 40.0, and 100 ppm a.i. and analyzed concurrently with the test samples, recoveries ranged from 90.4 to 120% of nominal (Table 2, p. 24).
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

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Shell Growth

Concentration (ppm a.i.)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Negative (dilution water) Control	---	40	0	2.7 ± 1.3	---
Solvent Control	---	40	0	2.6 ± 1.1	---
Pooled Control	---	40	0	2.7 ± 0.16	---
13	12	40	0	2.9 ± 1.1	0
22	21	40	0	2.9 ± 1.2	0
36	31	40	0	3.1 ± 0.91	0
60	50	40	0	2.8 ± 1.1	0
100	89	40	0	2.4 ± 1.0	12

Limit of quantitation = 1.1-1.2 mg a.i./L

No mortalities or sub-lethal effects were observed during the test. Shell growth was reduced 11% in the 89 ppm a.i. treatment group compared to the pooled control, but not statistically significant. The shell growth in the 12, 21, 31, and 50 ppm a.i. treatment groups were similar to the controls. No significant reductions in shell growth compared to the pooled control were identified.

B. Statistical Results

The EC_{50} was estimated based on a visual inspection of the terminal growth data. The NOEC was determined using the Williams' test. All toxicity values were determined in terms of the mean-measured concentrations.

EC_{50}: >89 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 89 ppm a.i.
LOEC: >89 ppm a.i.

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14. VERIFICATION OF STATISTICAL RESULTS:

Shell deposition data satisfied the assumptions of normality and homogeneity of variances. William's test revealed no significant differences between treatment and pooled control. Statistical analyses were performed using TOXSTAT software. Reductions in shell deposition did approach 50%, so the EC_{50} value was visually determined to be greater than the highest treatment concentration. All toxicity values were determined in terms of the mean-measured concentrations.

EC_{50}: >89 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 89 ppm a.i.
LOEC: >89 ppm a.i.

15. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors. The EC_{50} was >89 ppm a.i., which categorizes XR-750 Technical (Aminopyralid) as slightly toxic to the Eastern oyster [72-3(b)] on an acute toxicity basis.

The oysters in each test aquarium were fed supplemental feedings of algae (*Tetraselmus maculata*) at a rate of 10^7 cells/mL three times daily (p. 13).

This study was conducted in accordance with U.S. EPA Good Laboratory Practice Regulations with the exception of the routine water screening analyses (p. 3). Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided.

EAD comments:

This study is scientifically sound and is classified as **acceptable**. This study was done using US EPA Guideline § 72-3(B) with minor deviations which did not impact the acceptibility or validity of the study. The EPA reviewer classified this study to be acceptable and core, and it fulfills OPP guideline requirement.

No amendments to the DER are required.

16. REFERENCES:

ASTM. 2000. Standard practice for conducting acute toxicity tests with fishes, microinvertebrates, and amphibians. Standard E-729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshoken, PA 19428.

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Benoit, D.A., *et al.* 1982. A continuous flow mini-diluter system for toxicity testing. *Water Research.* 16:457-464.

Rand, G.M. and S.R. Petrocelli. 1985. Fundamentals of Aquatic Toxicology. Hemisphere Publishing Co., New York.

Sokal, R.R., and F.J. Rohlf. 1981. *Biometry.* 2nd Edition. W.H. Freeman and Company, New York. 859 pp.

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U.S. EPA. 1985. Standard evaluation procedures for acute toxicity test for estuarine and marine organisms (Mollusc 96-hour flow-through shell deposition study). EPA-540/9-85-011. June 1985. Emended August 1990.

U.S. EPA. 1985. Office of Pesticide Programs. Pesticide Assessment Procedure for Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-hour flow-through shell deposition study). EPA-540/9-85-011. June 1985. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1989. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1025. Oyster Acute Toxicity Test (Shell deposition). "Public Draft". EPA 712-C-96-115. April 1996. U.S. Environmental Protection Agency, Washington, D.C.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared to a zero dose control. *Biometrics* 27:103-117.

Williams, D.A. 1972. A comparison of several dose levels with a zero control. *Biometrics* 28:519-531.

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17. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Shell Deposition (mm; 96 hours)
File: 5818gd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.779	0.156	3.391
Within (Error)	8	0.370	0.046	
Total	13	1.149		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Shell Deposition (mm; 96 hours)
File: 5818gd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	2.650	2.650		
2	12	2.950	2.950	-1.615	
3	21	2.850	2.850	-1.077	
4	31	3.150	3.150	-2.692	
5	50	2.800	2.800	-0.808	
6	89	2.350	2.350	1.615	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

Shell Deposition (mm; 96 hours)
File: 5818gd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	12	2	0.538	20.3	-0.300
3	21	2	0.538	20.3	-0.200
4	31	2	0.538	20.3	-0.500
5	50	2	0.538	20.3	-0.150
6	89	2	0.538	20.3	0.300

Shell Deposition (mm; 96 hours)
File: 5818gd Transform: NO TRANSFORMATION

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	2.650	2.650	2.850
2	12	2	2.950	2.950	2.850
3	21	2	2.850	2.850	2.850
4	31	2	3.150	3.150	2.850
5	50	2	2.800	2.800	2.800
6	89	2	2.350	2.350	2.350

Shell Deposition (mm; 96 hours)
File: 5818gd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	2.850				
12	2.850	1.074		1.86	k= 1, v= 8
21	2.850	1.074		1.96	k= 2, v= 8
31	2.850	1.074		2.00	k= 3, v= 8
50	2.800	0.805		2.01	k= 4, v= 8
89	2.350	1.611		2.02	k= 5, v= 8

s = 0.215
Note: df used for table values are approximate when v > 20.

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DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE ORGANISM
§72-3(C) - SHRIMP PMRA DACO: 9.4.2

1. **CHEMICAL:** Aminopyralid PC Code No.: 005100

2. **TEST MATERIAL:** XR-750 Technical (Syn. XDE-750 Tech.) Purity: 94.5%

3. **CITATION:**

Author: Machado, M.W.

Title: XDE-750-Acute Toxicity to Mysids *(Americamysis bahia)* Under Static Conditions

Study Completion Date: April 4, 2002

Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, Massachusetts 02571-1075

Sponsor: The Dow Chemical Company
for Dow AgroSciences LLC
1803 Building
Midland, Michigan 48674

Laboratory Report ID: 12550.6190

MRID No.: 462358-19 PMRA Submission# : 2004-0789

DP Barcode: D301682

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation **Date:** 8/6/2004

APPROVED BY: Gregory Hess, Staff Scientist, Dynamac Corporation **Date:** 10/5/2004

5. **APPROVED BY:** Brian D. Kiernan, Biologist, OPP/EFED/ERB-IV **Date:** 11/30/2004

Signature: 6/16/05

213, PMRA-EAD **Date:** January 24, 2005

Signature:

2030240

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Americamysis bahia*

Age or Size of Test Organism: <24 hours old

Definitive Test Duration: 96 hours

Study Method: Static

Type of Concentration: Mean-measured

7. CONCLUSIONS:

The 96-hour acute toxicity of XR-750 (Synonym: XDE-750; Aminopyralid) to the saltwater mysid, *Americamysis bahia*, was studied under static conditions. Mysids were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 13, 22, 36, 60, and 100 ppm a.i.; mean measured concentrations were <1.2 (<LOQ; controls), 14, 22, 36, 59, and 100 ppm a.i.. During the 96-hour test, no mortalities or sub-lethal effects were observed in the controls or treatment groups. The **96-hour LC_{50} value was > 100 ppm a.i.**, which categorizes XR-750 (Aminopyralid) as **practically non-toxic** to the saltwater mysid, *Americamysis bahia*, on an acute toxicity basis. Based on mortality and sublethal effects, the **NOEC and LOEC values were 100 and > 100 ppm a.i.**, respectively.

This study is scientifically valid and fulfills the requirements of an acute LC_{50} test with an estuarine/marine organism (Subdivision E, §72-3(C) [mysid]). This study is classified as **Acceptable**.

EAD Conclusion:

This study is scientifically sound and is classified as **acceptable**. The 96-hour LC_{50} value was > 100 ppm a.i. Based on mortality and sublethal effects, the NOEC and LOEC values were 100 and > 100 ppm a.i., respectively.

Results Synopsis
96-Hour:
LC_{50}: > 100 ppm a.i. 95% C.I.: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

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8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: The guideline deviations were considered to be minor and did not impact the acceptability or validity of this study.

C. Repairability: N/A

9. BACKGROUND:

10. GUIDELINE DEVIATIONS:

1. The pretest mortality/health of the mysids was not reported.
2. It was not reported if all test mysids were from the same year class.
3. The test vessel overall and fill volumes (1L and 900 ml, respectively) were smaller than recommended (3.9L and 2-3L, respectively) for the test species.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of XR-750 (aminopyralid) to mysids for the purpose of chemical registration.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Americamysis bahia, Penaeus setiferus, P. duorarun, P. aztecus* and *Palaemonetes sp.*	*Americamysis bahia*
Age Juvenile (≤ 24 hours old) mysids should be used	<24 hours old

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Guideline Criteria	Reported Information
Supplier	Juveniles were collected from in-house laboratory cultures. The original brood stock was obtained from Aquatic BioSystems, Inc., Ft. Collins, Colorado.
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	Continuous
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	None reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Live brine shrimp (*Artemia salina* nauplii) was provided twice daily during acclimation and once daily during testing.
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported

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C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Natural seawater collected directly from the Cape Cod Canal, Bourne, Massachusetts, filtered (20- and 5-micron), and adjusted for salinity by addition of laboratory well-water. .
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range <6 ‰	21‰
Water Temperature Approx. 22 ± 1 °C	24-25°C
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	6.8-7.9
Dissolved Oxygen Between 60 and 105% saturation. If needed, aerate prior to introduction of chemical.	6.2-8.1 mg/L (>60%)
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	<2.0 mg/L (February 2002)

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Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	Glass beakers (1 L) filled with approximately 900 mL of test water.
Type of Dilution System Must provide reproducible supply of toxicant	Static
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day (N/A for mysids)	N/A for mysids
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark, sudden transitions from light to dark were avoided.
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Dimethylformamide, 0.10 mL/L

D. Test Design

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Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	The 96-hour range finding studies included two static studies each with a different age class (<24 hours old and 5-6 days old; 10 mysids/level and control). The XDE-750 test concentrations were 0 (negative control), 0.10, 1.0, 10, and 100 ppm a.i.. By 96 hours, no mortalities or adverse effects were observed in the treatment groups and controls of both tests.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0 (negative and solvent controls), 13, 22, 36, 60, and 100 ppm a.i.
Number of Test Organisms Minimum 20/level, may be divided among containers	20 mysids/level, divided into two replicates of 10 mysids each.
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary >1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Measured daily in each aquarium and continuously in one negative control replicate. 2. Measured daily in each test vessel.

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Guideline Criteria	Reported Information
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Analytical determination of test substance was performed on samples collected from each test vessel at the beginning and end of the test.

13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	98-110% of nominal based on mean-measured recoveries from the test vessels; 93.5-102% of nominal, based on quality control samples run concurrently with the test samples.
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0% mortality was observed in the negative and solvent controls.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

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Mortality

Concentration (ppm a.i.)		Number of Shrimp	Mean cumulative mortality (%)			
			Hours of Study			
Nominal	Mean Measured		24	48	72	96
Negative Control	ND	20	0	0	0	0
Solvent Control	ND	20	0	0	0	0
13	14	20	0	0	0	0
22	22	20	0	0	0	0
36	36	20	0	0	0	0
60	59	20	0	0	0	0
100	100	20	0	0	0	0

ND=Not detected; LOQ = 1.2 ppm a.i.

During the 96-hour test, no mortalities or sub-lethal effects were observed in the treatment or control groups.

B. Statistical Results

Statistical Method: The 96-hour LC_{50}, NOEC, and LOEC were estimated by visual interpretation of the mortality and clinical observation data.

96-Hour:
LC_{50}: > 100 ppm a.i. 95% C.I.: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The 96-hour LC_{50}, NOEC, and LOEC were visually determined due to a lack of mortality and sub-lethal effects in the controls and treatment groups.

96-Hour:
LC_{50}: > 100 ppm a.i. 95% C.I.: N/A
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.
Endpoints affected: None

15. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study author.

Based on the LC_{50} value (>100 ppm a.i.), XR-750 (Syn. XDE-750; Aminopyralid) is categorized as practically non-toxic to saltwater mysids *(Americamysis bahia)* on an acute toxicity basis.

This study was conducted in accordance with USEPA (40 CFR Part 160) Good Laboratory Practice Regulations. Quality Assurance and No Data Confidentiality Statements were included.

EAD comments:

This study is scientifically sound and is classified as **acceptable**. This study was done using US EPA Guideline § 72-3(C) with minor deviations which are not considered to affect validity of the study. The EPA reviewer classified this study to be acceptable and core, and it fulfills OPP guideline requirement.

16. REFERENCES

ASTM. 2000. Standard practice for conducting acute toxicity tests with fishes, microinvertebrates, and amphibians. Standard E729-96. American Society for Testing and Substances, Barr Harbor Drive, West Conshocken, PA. 19428.

APHA, AWWA, WPCF. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edition, Washington, DC.

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U.S. EPA. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency,

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U.S. EPA. 1985. Office of Pesticide Programs. Standard Evaluation Procedure for Acute Toxicity Test for Estuarine and Marine Organisms. EPA-540/9-85-010. June 1985. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1035. Mysid Acute Toxicity Test. "Public Draft". EPA 712-C-96-136. April 1996. U.S. Environmental Protection Agency, Washington, D.C.

Data Evaluation Report on the acute toxicity of XR-750 Technical (Aminopyralid) to Sheepshead Minnow (*Cyprinodon variegatus*)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-20

Data Requirement:		
	PMRA DATA CODE	9.5.2.4
	EPA DP Barcode	D301682
	OECD Data Point	{.....}
	EPA MRID	462358-20
	EPA Guideline	72-3a

Test material: XR-750 Technical **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: 2-pyridinecarboxylic acid, 4-amino-3,6-dichloro
CAS name: Not reported
CAS No.: Not reported
Synonyms: XDE-750

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/4/04

QC Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/5/04

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB-IV
Signature: BDK 6/16/05
Date: 11/22/2004

Secondary Reviewer(s): 1610
PMRA
Signature:
Date: N/A

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

Date Evaluation Completed:

CITATION: Machado, M.W. 2002. XDE-750-Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Static Acute Conditions. Unpublished study performed by Springborn Smithers Laboratories, Inc., Wareham, Massachusetts. Laboratory Study No. 12550.6191. Study submitted by The Dow Chemical Company, Midland, Michigan. Experimental start date February 14, 2002 and experimental termination date February 18, 2002. The final report issued April 23, 2002.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, the Sheepshead Minnow (*Cyprinodon variegatus*) was exposed to XR-750 Technical (Synonym XDE-750 Tech.; aminopyralid) at nominal treatment concentrations of 0 (negative and solvent controls) 13, 22, 36, 60, and 100 ppm a.i. under static conditions. Mean-measured concentrations were <1.2 (<LOQ; negative and solvent controls), 12, 21, 34, 64, and 120 ppm a.i.

By 96-hours, there were no mortalities or sub-lethal effects observed in either control group or at any treatment level. The LC_{50} was determined to be >120 ppm a.i., which categorizes XR-750 (Aminopyralid) as practically non-toxic to the Sheepshead minnow (*Cyprinus variegatus*) on an acute toxicity basis. The NOEC and LOEC values were determined to be 120 and >120 ppm a.i., respectively.

This study is scientifically sound and fulfills U.S. EPA Guideline §72-3a and is categorized as **Acceptable**. However, it was conducted using marine salinity. If salinity were to be found to affect the activity of aminopyralid, a study reflecting estuarine salinity would be necessary to address the salinity difference between estuarine and marine habitats.

EAD Conclusion:

The EAD is in agreement with the conclusion reported by the study author and the EPA reviewer. The 96-hour LC_{50} and LOEC were >120 mg a.i./L and the NOEC was 120 mg a.i./L based on lack of mortality and sublethal effects. There were no sublethal effects in this study. This study is scientifically sound and fulfills US EPA Guideline [§72-3a]. Based on the results of 96-hours acute toxicity test, XR-750 (Aminopyralid) is classified as acutely non toxic to Sheepshead minnow.

This study is classified as acceptable and fulfills guideline requirements for an acute toxicity study with the Sheepshead minnow (*Cyprinodon variegatus*) [§72-3(a)].

Results Synopsis

Test Organism Size/Age (mean Weight or Length): A representative sample of fish from the test population (n = 30) had a mean wet weight of 0.38 g (0.20-0.58 g) and mean length of 28 mm (23-32 mm).
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour
LC_{50}: >120 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 120 ppm a.i.
LOEC: >120 ppm a.i.
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA FIFRA Guideline 72-3 and OPPTS Draft Guideline 850.1075. Deviations from U.S. EPA FIFRA Guideline §72-3a included:

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1. pH of the treatment tanks was well below recommended for the first 24 hrs (48 hrs for the highest treatment level). All treatment levels were below the minimum of 8.0 for marine studies. The salinity used in this study was for marine systems.

2. The concentrations of chlorine and particulate matter within the dilution water were not reported.

3. Test fish had a mean wet weight of 0.38 g (0.20-0.58 g), which was lower than the EPA recommended weight range of 0.5-5.0 g.

4. The range finding study determined the LD50 to be greater than 100 ppm, thus the definitive study was not required.

The deviations were not considered to affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided.

A. MATERIALS:

1. Test Material XR-750 Technical (Synonym XDE-750; Aminopyralid)

Description: Not Reported

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water was verified by analytical determination at 0- and 96-hours. Recoveries from mean-measured treatment concentrations were from 95-120% of nominal. Concurrent quality control samples fortified at 10.0, 40.0, and 100 ppm a.i. at test initiation (0-hours) and termination (96-hours) had recoveries of 93.7-102% of nominal.

OECD requires water solubility, stability in water and light, pK_a, P_{ow} and vapor pressure of the test compound.

Water solubility: Not reported

Storage conditions of test chemicals: Room temperature in the dark.

2. Test organism:

Species: Sheepshead minnow (*Cyprinodon variegatus*)

Age at test initiation: Not reported

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Weight at test initiation: mean 0.38 g (average of 30 fish); range of 0.20-0.58 g

Length at test initiation: mean 28 mm (average of 30 fish); range of 23-32 mm

Source: Aquatic BioSystems, Ft. Collins, Colorado

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A preliminary range-finding test was performed at nominal XDE-750 concentrations of 0.0 (negative control) 0.10, 1.0, 10, and 100 ppm a.i. under static conditions (10 fish per control and treatment group). By 96 hours, no mortalities or sub-lethal effects were observed in the treament groups or the control.

b) Definitive Study: Based on the results from the range-finding test, a 96-hour acute toxicity test was conducted under static conditions with nominal XDE-750 concentrations of 13, 22, 36, 60, and 100 ppm a.i.

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	At least 14 days prior to testing.	
Conditions: (same as test or not)	Same as test	
Feeding:	Dry commercial flaked food provided, *ad libitum*, daily except 48 hours prior to and during testing.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	No mortality was observed 48-hours prior to testing.	
Duration of the test	96 hours	*EPA/OECD requires: 96 hours*

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Parameter	Details	Remarks Criteria
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Static N/A N/A	 *EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	None reported	 *EPA requires: no aeration; OECD permits aeration*
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	 Glass 19.5 L (39 x 20 x 25 cm) 15 L	 *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	The dilution water was natural filtered seawater from Cape Cod Canal, Bourne, Massachusetts and was filtered at 20- and 5-microns.	 *EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

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Parameter	Details	Remarks Criteria
Water parameters: Hardness	Not reported	The dilution water hardness was not measured.
pH	6.8-7.9	
Dissolved oxygen	5.0-8.2 mg/L (>60%)	
Total Organic Carbon	<2.0 mg/L (February 2002)	***Hardness and pH*** *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.* ***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Particulate Matter	Not reported	
Metals	Not detected	
Pesticides	Not detected	
Chlorine	Not reported	
Temperature	21-23°C	
{Salinity for marine or estuarine species}	33-35‰	
Intervals of water quality measurement	DO, pH, salinity, and temperature were determined daily. Temperature was also continuously measured in the solvent control.	

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Parameter	Details	Remarks Criteria
Concentration of test material: Nominal: Measured:	0 (negative and solvent controls) 13, 22, 36, 60, and 100 ppm a.i. <1.2 (<LOQ; negative and solvent controls) 12, 21, 34, 64, and 120 ppm a.i.	The 96-hour mean-measured concentration recoveries ranged from 95 to 120% of the nominal *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	Dimethylformamide; 0.50 ppm	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*
Number of fish/replicates: Negative control: Solvent Control: Treated:	10 fish 10 fish 10 fish/treatment	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	Not reported	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark, sudden transitions from light to dark were avoided	*EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*

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Parameter	Details	Remarks Criteria
Recovery of chemical	93.7-103% of nominal	Based on QC matrix fortifications analyzed concurrently with the test samples (Table 2, p. 22).
Level of Quantitation	1.2 ppm a.i.	
Level of Detection	Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. **Observations:**

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	0, 6, 24, 48, 72 and 96 hours of exposure	*EPA/OECD requires: minimally every 24 hours*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

By 96-hours, there were no mortalities in the control or the treatment groups. The LC_{50}, LOEC, and NOEC values based on mortality were reported to be >120, >120, and 120 ppm a.i., respectively.

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Table 3: Effect of XR-750 (Aminopyralid) on Mortality of Sheepshead minnow (*Cyprinodon variegatus*).

Treatment, ppm a.i., 96 Hour Mean-Measured and (Nominal Conc.)	No. of Fish at Start of Study	Observation Period					
		0-24 Hours		48-72 Hours		96 Hours	
		No. Dead	% Mortality	No. Dead	% Mortality	No. Dead	% Mortality
Negative control	10	0	0	0	0	0	0
Solvent control	10	0	0	0	0	0	0
12 (13)	10	0	0	0	0	0	0
21 (22)	10	0	0	0	0	0	0
34 (36)	10	0	0	0	0	0	0
64 (60)	10	0	0	0	0	0	0
120 (100)	10	0	0	0	0	0	0
NOEC (mortality)	120 ppm a.i.						
LC_{50} (95% C.I.)	>120 ppm a.i.						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. NON-LETHAL TOXICITY ENDPOINTS:

By 96-hours, no sub-lethal effects were observed in the control or the treatment groups. The NOEC and LOEC values based on sub-lethal effects were 120 and >120 ppm a.i., respectively.

Table 4. Sub-Lethal Effects of XR-750 (Aminopyralid) on Sheepshead minnow (*Cyprinodon variegatus*).

Treatment, ppm a.i., 96 Hour Mean-Measured and (Nominal Conc.)	Observation Period			
	Endpoint at 0-24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected	% Affected	% Affected	% Affected
Negative control	AN	AN	AN	AN
Solvent control	AN	AN	AN	AN

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Treatment, ppm a.i., 96 Hour Mean-Measured and (Nominal Conc.)	Observation Period			
	Endpoint at 0-24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected	% Affected	% Affected	% Affected
12 (13)	AN	AN	AN	AN
21 (22)	AN	AN	AN	AN
34 (36)	AN	AN	AN	AN
61 (60)	AN	AN	AN	AN
120 (100)	AN	AN	AN	AN
NOEC (sublethal)	100 ppm a.i.			
LOEC (sublethal)	>100 ppm a.i.			
EC_{50}	>100 ppm a.i.			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

AN = Appeared Normal

C. REPORTED STATISTICS:

Statistical Method: The 96-hour LC_{50}, NOEC, and LOEC were visually determined, due to the lack of treatment-related mortality or sub-lethal effects at any treatment level.

96-Hour
LC_{50}: >120 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 120 ppm a.i.
LOEC: >120 ppm a.i.
Endpoints affected: None

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D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} based on mortality and the NOEC and LOEC values based on mortality and sub-lethal effects were determined visually due to a lack of treatment related effects at any level during the definitive exposure period.

96-Hour
LC_{50}: >120 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 120 ppm a.i.
LOEC: >120 ppm a.i.
Endpoints affected: None

E. STUDY DEFICIENCIES:

A representative sample of fish from the test population (n = 30) had a mean terminal wet weight of 0.38 g (0.20-0.58 g), which was lower than the EPA recommended weight range of 0.5-5.0 g. Additionally, the pH of the treatment tanks was well below recommended for the first 24 hrs (48 hrs for the highest treatment level). All treatment levels were below the minimum of 8.0 for marine studies. The salinity used in this study was for marine systems.

All of the deficiencies were considered to be minor and did not effect the validity or acceptability of the definitive test.

F. REVIEWER'S COMMENTS:

Results of the reviewer's statistical verification were identical to those of the study authors.

This study was conducted under marine conditions. If salinity were to be found to affect the activity of aminopyralid, a study reflecting estuarine salinity would be necessary to address the salinity difference between estuarine and marine habitats.

EAD comments:
After review of the study data and the US EPA DER, the EAD reviewer is in agreement with the conclusion reached by the US EPA. Deficiencies mentioned above were considered minor and did not impact the results of the study.

No amendments to the DER are recommended.

G. CONCLUSIONS:

This study is scientifically sound and fulfills U.S. EPA Guidelines [§72-3a]; therefore it is categorized as CORE. Based on the results of the 96-hour acute toxicity test, XDE-750 (Aminopyralid) is categorized as practically non-toxic to the Sheepshead minnow (*Cyprinus variegatus*) on an acute toxicity basis. The 96-hour LC_{50} and LOEC value was >120 ppm a.i. and the NOEC was 120 ppm a.i. based on a lack of mortality and sub-lethal effects.

96-Hour
LC_{50}: >120 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 120 ppm a.i.
LOEC: >120 ppm a.i.
Endpoints affected: None

III. REFERENCES:

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U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act; Good Laboratory Practice Standards; Final Rule. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1982. Office of Pesticide Programs. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-85-024. October 1982. U.S. Environmental Protection Agency, Washington, D.C.

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U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1075. Fish Acute Toxicity Test, Freshwater and Marine. "Public Draft". EPA 712-C-96-118. April 1996. U.S. Environmental Protection Agency, Washington, D.C.

Data Evaluation Report on the Toxicity of XDE-750 (Aminopyralid) to the Early Life Stage of Fathead Minnow (*Pimphales promelas*)
PMRA Submission Number 2004-0789 EPA MRID Number 462358-21

Data Requirement:		
	PMRA DATA CODE	9.5.3.1
	EPA DP Barcode	D301682
	OECD Data Point	
	EPA MRID	462358-21
	EPA Guideline	§72-4a

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: 4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name: 2-pyridinecarboxylic acid, 4-amino-3,6-dichloro
CAS No.: 150114-71-9
Synonyms: Aminopyralid, XR-750, X660750

Primary Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 9/30/04

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/11/04

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB-IV
Signature: BDK 6/16/05
Date: 11/24/2004

Secondary Reviewer(s): 1610
EAD, PMRA
Signature:
Date: N/A

Company Code
Active Code
EPA PC Code 005100

Date Evaluation Completed:

CITATION: Marino, T.A., E.L. McClymont, A.M. Yaroch, C.A. Hales, and L.G. McFadden. 2002. XDE-750: Toxicity to the Early Life Stages of the Fathead Minnow, *Pimephales promelas* Rafinesque. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland Michigan, and sponsored by Dow AgroSciences, LLC, Indianapolis, Indiana Laboratory Project No: 021029. Study initiated February 14, 2002 and completed September 9, 2002, revised October 21, 2003.

EXECUTIVE SUMMARY:

The chronic toxicity of XDE-750 (Aminopyralid) to the early life-stage of Fathead Minnow (*Pimphales promelas*) was evaluated under flow-through conditions for 36 days (4-day hatch period and 32-day post-hatch period). Fertilized eggs/embryos (100 embryos/treatment), approximately 17-24 hours old, were exposed to XDE-750 (Aminopyralid) at nominal concentrations of 0 (negative and solvent controls), 0.780, 1.30, 2.16, 3.60, 6.00, and 10.0 ppm a.i.. Mean-measured concentrations were <0.09 (<LOQ, controls), 0.708, 1.36, 2.44, 3.89, 6.71, and 11.4 ppm a.i., respectively (equivalent to 90.5-114% of nominal concentrations).

Embryos began hatching between Days 2 and 5, and ≥88% hatch occurred in the control (93% pooled control) and all treatment groups by Day 4 (day 0 post-hatch; Appendix F, p. 46). Hatching was verified to be complete on Day 5 in all control and treatment groups. Day-to-mean hatch was 3.3 and 3.0 days in the negative and solvent controls, respectively, and 3.3, 3.0, 3.3, 3.0, 2.5, and 2.8 days for the mean-measured 0.706, 1.36, 2.44, 3.89, 6.71, and 11.4 ppm a.i. treatment groups, respectively. Based on study authors' statistical analysis of the days-to-mean-hatch treatment data compared to the pooled control, the NOEC for time-to-hatch was 11.4 ppm a.i. Hatching success was not statistically-reduced at any treatment level compared to the pooled control (Table 7, p. 33). Hatching success by Day 5 averaged 91 and 99% for the negative and solvent control groups, respectively, and 96, 99, 93, 97, 92, and 89% for the mean-measured 0.706, 1.36, 2.44, 3.89, 6.71, and 11.4 ppm a.i. treatment groups, respectively. The NOEC for hatching success was 11.4 ppm a.i.

Day 36 survival of minnow larvae was statistically-reduced at the 2.44 ppm a.i. treatment levels compared to the pooled control (Table 7, p. 33). At Day 36 (study termination), survival was 85.1 and 87.8% in negative and solvent control groups, respectively, and 90.6, 81.7, 58.2, 16.1, 0.0, and 0.0% in the 0.706, 1.36, 2.44, 3.89, 6.71, and 11.4 ppm a.i. treatment groups, respectively. All hatched larvae died at the 6.71 and 11.4 ppm a.i. treatment levels by Days 28 and 14 (24 and 10 days post-hatch), respectively. The NOEC for larval survival was 1.36 ppm a.i.

Statistically-significant treatment-related sub-lethal signs of toxicity were reported at the mean-measured 2.44 through 11.4 ppm a.i. treatment levels (based on % normal compared to abnormal and dead larvae at test termination; Table 7, p. 33). Sub-lethal (abnormal) effects included pale coloration, immobility, deformed/underdeveloped body, and scoliosis. However, actual effects were not specified for each treatment level. The NOEC for sub-lethal effects was 1.36 ppm a.i. based on the study authors' statistical analysis (reviewer was unable to statistically verify these results because the replicate data assessed by the study authors were not reported).

Terminal length and wet weights were significantly reduced in the mean-measured 2.44, 3.89, 6.71, and 11.4 ppm a.i. treatment groups compared to pooled control groups (Table 7, p. 33). Terminal lengths were 14.03 and 13.85 mm in the negative and solvent control groups, respectively, and 13.17, 13.89, 12.85, and 9.47 mm in the 0.706, 1.36, 2.44, and 3.89 ppm a.i. treatment groups, respectively. Terminal wet weights (blotted dry) were 41.42 and 38.71 mg in the negative and solvent control groups, respectively, and 36.60, 39.91, 28.80, and 8.73 mg in the 0.706, 1.36, 2.44, and 3.89 ppm a.i. treatment groups, respectively. Growth measurements were not determined for the 6.71 and 11.4 ppm a.i. treatment groups (0% survival). The NOEC for growth was 1.36 ppm a.i.

This chronic-toxicity study is scientifically sound, but it does not fulfill the guideline requirements for a fish early life-stage study (§72-4a) with the Fathead minnow because replicate data for days-to-mean hatch and sub-lethal effects were not submitted and could not be verified by the reviewer. Consequently, this study is classified as SUPPLEMENTAL because it provides information that is useful for risk assessment purposes. Submission of these data may allow the study to be upgraded.

EAD Conclusion:

The EAD is in agreement with the conclusion reported by the study author and the EPA reviewer. Based on parental dry weight, total length and sublethal effects the NOEC for XDE-750 (Aminopyralid) were 1.36 ppm a.i.,

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1.36 ppm a.i. and 1.36 ppm a.i., respectively. These were the most sensitive endpoints. This study is classified as acceptable for use in a risk assessment.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Newly-fertilized embryos, approx. 17-24 hours old
Test Type (Flow through, Static, Static Renewal): Flow-through

Hatch success (Day 5)
NOEC: 11.4 ppm a.i.
LOEC: >11.4 ppm a.i.

Time to hatch (days-to-mean-hatch)
NOEC: 11.4 ppm a.i.
LOEC: >11.4 ppm a.i.

Post-hatch (larval) survival (%; Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Wet weight (Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Length (Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Sub-lethal effects (% normal larvae)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Most Sensitive Endpoint(s): Larvae survival, wet weight, length, and sub-lethal effects.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on the following guidelines: U.S. EPA-FIFRA Standard Evaluation Procedure EPA-540/86-138, Fish Early Life-Stage Test (1986) and OECD Guidelines for Testing of Chemicals, Method 210, "Fish, Early-Life Stage Toxicity Test, (1992). Deviations from U.S. EPA Guideline §72-4a included:

1. The flow-splitting accuracy and the frequency of inspection of the diluter system were not reported.
2. The day that larvae were released from the incubation cups into the test vessels was not reported.
3. The reviewer was unable to statistically verify the endpoints days-to-mean hatch (time-to-hatch), time to swim-up and percent normal larvae (Day 36) because the raw data analyzed by the study authors were not reported.
4. The hardness of the water (53-73 $CaCO_3$/L) was higher than the recommended 40-48 $CaCO_3$/L. The pH range of 6.9-8.2 was greater than the recommended 7.2-7.6.

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Because replicate data for days-to-mean hatch and sub-lethal effects were not reported and conclusions based on this endpoint could not be verified by the reviewer, this study is classified as SUPPLEMENTAL because it provides information that is useful for risk assessment purposes. All other deviations were considered minor.

COMPLIANCE: Signed and dated Quality Assurance, and No Data Confidentiality claims statements were provided. The study was conducted following the Good Laboratory Practice Standards of the US EPA Title 40 CFR Part 160 (Final Rule), OECD ENV/MC/CHEM (98) 17, and EC Directive 99/11/EC (1999; OJ No. L 77/8-21, 23/31/1999).

A. MATERIALS:

1. Test Material XDE-750 (2-pyridinecarboxylic acid, 4-amino-3,6-dichloro)
Description: Solid
Lot No./Batch No. : Lot No. F0031-143, TSN 102319
Purity: 94.5%
Stability of Compound: Relatively consistent concentrations of XDE-750 (Aminopyralid) were recovered from the test solutions sampled from all treatment levels on Days 0, 7, 14, 21, 28, and 36, with reviewer-calculated high-low ratios of 1.02-1.22 (Table 3, pp. 29). Mean-measured recoveries were 90.5-114% of nominal treatment concentrations.
Storage conditions of test chemicals: Room temperature
OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

Physico-chemical properties of XDE-750:

Parameter	Values	Comments
Water solubility at 20 °C		
pH 5	18.85 g cmpd/100 g H2O	Preliminary data
pH 7	17.7 g cmpd/100 g H2O	Preliminary data
pH 9	18.5 g cmpd/100 g H2O	Preliminary data
Vapor Pressure at 25 °C	1.94 x 10-10 mm Hg	
UV absorption	270 nm	
pKa	2.56	
Log Kow	N/A	

2. Test organism:

Species: Fathead minnow, *Pimephales promelas*

Age/embryonic stage at test initiation: Newly-fertilized embryos, 17-24 hours post-fertilization

Method of collection of the fertilized eggs:	N/A (purchased); embryos were shipped to the laboratory, typically embryos are rolled off of spawning substrates
Source:	Aquatic BioSystems, Inc., Fort Collins, CO

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B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range finding study was not performed since this material appeared practically non-toxic to aquatic organisms on an acute basis (LC_{50}/EC_{50} >100 mg/L). The acute LC_{50} value for rainbow trout and Daphnia were reported to be >100 mg/L (1,2). The OECD Guidelines for Testing of Chemicals, Method 210, "Fish, Early Life Stage Toxicity Test", states that concentrations of the substance higher than the 96 hour LC_{50} or 10 mg/L, whichever is lower, need not be tested. Based on this information, the definitive study was set with the highest nominal test concentration at 10.0 mg XDE-750/L (ppm a.i.).

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions: (same as test or not) Feeding (type, source, amount given, frequency): Health: (any mortality observed)	Not reported, parental generation maintained at Aquatic BioSystems, Fort Collins, Colorado, USA	Embryos were spawned on February 19, 2002; from number of paired adults not reported (p. 11).
Number of fertilized eggs/embryos in each treatment at test initiation	100 embryos/treatment, divided into 25 embryos/cup, one cup/replicate aquaria, with four replicates/treatment	*EPA requires minimum of 20 embryos per replicate cup. Minimum of 30 fish per treatment for post-hatch exposure*

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Parameter	Details	Remarks *Criteria*
Concentration of test material: nominal: measured:	0 (negative and solvent controls), 0.780, 1.30, 2.16, 3.60, 6.00, 10.0 ppm a.i. <0.09 (<LOQ, controls), 0.706, 1.36, 2.44, 3.89, 6.71, and 11.4 ppm a.i. Reviewer-determined high:low ratios ranged from 1.02 to 1.22.	Mean-measured concentrations were determined at test initiation, termination, and weekly, and are provided in Table 3, pp. 29. Mean-measured recoveries were 90.5-114% of nominal. *EPA requires a minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.* *- Toxicant conc. must be measured in one tank at each toxicant level every week.* *- One concentration must adversely affect a life stage and one concentration must not affect any life stage.* *OECD requires 5 concentrations spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution must be within ± 20% of the mean measured values.*
Solvent (type, percentage, if used)	Dimethyl formamide (DMF), 0.085 mL/L	*EPA requires that solvent should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.* *OECD requires that solvent must have no effect on survival nor produce any other adverse effects;concentration should not be greater than 0.1 ml/L.*
Number of replicates control: solvent control: treatments:	 4 4 4	*EPA requires 4 replicates per concentration* *EPA/OECD require solvent control when a solubilizing agent has been used.*

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Parameter	Details	Remarks *Criteria*
Test condition: static renewal/flow through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	 Flow-through Intermittent-flow proportional diluter Approximately 9.4 volume replacements/day N/A	A primary feed-stock solution was prepared weekly and mixing chambers were used to dilute the stock solutions with laboratory dilution water. The general operation of the diluter was checked visually at least two times/day during the test. The flow-splitting accuracy and the frequency of inspection of the diluter system were not reported. *Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.* *Toxicant Mixing:* *1) Mixing chamber is recommended but not required;* *2) Aeration should not be used for mixing;* *3) It must be demonstrated that the test solution is completely mixed before intro. into the test system;* *4) Flow splitting accuracy must be within 10%..*
Aeration, if any	None reported.	*Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	36days: 4-day hatching period and 32-day post-hatch period	*EPA requires 32 days post-hatch*

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Parameter	Details	Remarks *Criteria*
Embryo cups, if used type/material: (glass/stainless steel) size: fill volume:	 Glass cylinders with nylon mesh (~360 µm) bottoms 7.5 x 8.5 cm (H x D) Not reported	The embryo cups were suspended in a cylindrical glass test chamber (8.5 x 8.5 cm, H x D), which also had a mesh (~360 µm) bottom. Flow was directed from the delivery tubes in and around the embryos during exposure. The cups were removed on exposure day 12. *EPA requires 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
Test vessel type/material: (glass/stainless steel) size: fill volume:	 Glass aquaria 15 x 10 x 9 cm 850 ml (15-cm depth)	 *EPA/OECD requires all glass or glass with stainless steel frame.*
Source of dilution water	Lake Huron water supplied to the laboratory by the City of Midland Water Treatment Plant prior to municipal treatment. The water is sand-filtered, pH-adjusted with gaseous CO_2, carbon filtered, and UV-treated at the laboratory.	Results of periodic analyses of selected organic and inorganic compounds are provided in Table 1-2, pp. 27-28. *EPA requires natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.* *OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*

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Parameter	Details	Remarks *Criteria*
Water parameters: hardness: pH: TOC: dissolved oxygen: temperature: salinity (for marine or estuarine species): other measurements:	 53-73 mg $CaCO_3$/L 6.9-8.2 <1000 ppb 6.3-9.9 mg/L (78-122% saturation) 24.5-25.5°C N/A See Table 5, p. 31.	Water hardness range was higher than recommended. The pH range was greater than recommended. Alkalinity range throughout the test was 30-43 mg $CaCO_3$/L. Conductivity range throughout the test was 58.7-68.7 µmhos/cm. Residual chlorine concentration was <1 to 7 ppb throughout testing.
interval of water quality measurements:	DO, pH, and temperature were recorded on test days 0, 7, 14, 21, 28, and 36 in each test and control vessel with surviving organisms. Temperature was also measured continuously in one test vessel. Alkalinity, hardness, and conductivity were measured test days 0, 7, 14, 21, 28, and 36 from a control group and one exposure group.	*EPA requires hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 is recommended. DO must be measured at each conc. at least once a week; freshwater parameters in a control and one concentration must be analyzed once a week. Temperature depends upon test species; should not deviate by more than 2°C from appropriate temperature.* *OECD requires DO concentration between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.*

Parameter	Details	Remarks *Criteria*
Post-hatch details: when the post-hatch period began: Number of hatched eggs (alevins)/ treatment released to the test chamber: Day that alevins were released from the incubation cups to the test chamber:	 Day 4, when ≥ 90% of control eggs had hatched All hatched eggs were released to the test chamber. Not reported. Any unhatched embryos were kept in the incubation cups until they hatched, at which time they were released.	 *EPA requires % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.*
Post-hatch Feeding: start date: type/source of feed: amount given: frequency of feeding:	 Within 2 days following 90% hatching of the controls Live brine shrimp nauplii (*Artemia* sp.). 200-475 µL, adjusted to account for losses and supplemented with green algae (*Selenastrum capricornutum*) 2 times/day (Brine shrimp), ≥ 1 time/day (green algae). No feeding for at least 24 hours prior to test termination.	
Lighting	Transitional 16-hour light/8-hour dark photo-period.	Light intensity was 660-714 lux at the middle of the diluter (p. 20). *EPA/OECD requires: 16 hours light, 8 hours dark. Light intensity of 400-800 Lux at surface. Dim or no lighting during embryo incubation.*

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Parameter	Details	Remarks *Criteria*
Stability of chemical in the test system	Verified by analytical determination on Days 0, 7, 14, 21, 28, and 36. Relatively consistent concentrations of XDE-750 (aminopyralid) were recovered, with reviewer-calculated high-low ratios of 1.02-1.22 (Table 3, pp. 29).	Mean-measured recoveries were 90.5-114% of nominal treatment concentrations.
Recovery of chemical: Frequency of measurement: LOD: LOQ:	81.5-118% of nominal Days 0, 7, 14, 21, 28, and 36 Not reported 0.09 ppm a.i.	Based on measured recoveries throughout the exposure period. Results from standards prepared for generation of the calibration curve were not reported in terms of ppm a.i. or % of nominal, only the area response factors were reported (Table 4, p. 30).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	None conducted (fertilized eggs were purchased).	
Other parameters, if any	N/A	

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2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	- Time to hatch - Hatching success - Larvae survival -Overall survival (hatch and larvae combined) - Measurement of growth (length and wet weights) - Behavioral and morphological observations	*EPA minimally requires:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and juveniles;* *- Time to swim-up (if approp.);* *- Measurement of growth;* *- Incidence of pathological or histological effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g.alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects	 Daily Daily Weekly after hatching was complete (Day 5). N/A Day 36 Microscopically verified upon receipt of fertilized eggs. Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes, but insufficient	
Other observations, if any	N/A	

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II. RESULTS AND DISCUSSION

A. MORTALITY:

Hatching success was not statistically-reduced at any treatment level compared to the pooled control (Table 7, p. 33). Hatching success by Day 5 averaged 91 and 99% for the negative and solvent control groups, respectively, and 96, 99, 93, 97, 92, and 89% for the mean-measured 0.706, 1.36, 2.44, 3.89, 6.71, and 11.4 ppm a.i. treatment groups, respectively. The NOEC for hatching success was therefore 11.4 ppm a.i.

Terminal survival of minnow larvae (Day 36) was statistically-reduced at the 2.44 through 11.4 ppm a.i. treatment levels compared to the pooled control (Table 7, p. 33). At Day 36 (study termination), survival was 85.1 and 87.8% in negative and solvent control groups, respectively, and 90.6, 81.7, 58.2, 16.1, 0.0, and 0.0% in the 0.706, 1.36, 2.44, 3.89, 6.71, and 11.4 ppm a.i. treatment groups, respectively. All hatched larvae died at the 6.71 and 11.4 ppm a.i. treatment levels by Days 28 and 14 (24 and 10 days post-hatch), respectively. The NOEC for larval survival was 1.36 ppm a.i.

Table 3: Effect of XDE-750 (Aminopyralid) on Survival of the Fathead Minnow (*Pimphales promelas*).

Treatment, ppm a.i. Mean-Measured (and Nominal) Concentrations	Hatching Success			Juvenile Survival, Day 36[1]	
	No. of Eggs at Study Initiation	Day 5		No.	%
		No.	%		
Negative control	100	91	91	78	85.1
Solvent control	100	99	99	87	87.8
0.706 (0.780)	100	96	96	87	90.6
1.36 (1.30)	100	99	99	81	81.7
2.44 (2.16)	100	93	93	53	58.2*
3.89 (3.60)	100	97	97	15	16.1*
6.71(6.00)	100	92	92	0	0.0*
11.4 (10.0)	100	89	89	0	0.0*
NOEC	11.4 ppm a.i.			1.36 ppm a.i.	
LOEC	>11.4 ppm a.i.			2.44 ppm a.i.	
LC/EC_{50} mg/L	Not reported			Not reported	
Positive control, if used mortality: EC_{50}:	N/A N/A				

[1] Calculated as the number of larvae surviving (termed % Larvae Survival by the study authors) to test termination divided by the total number of embryos hatching successfully (Table 7, p. 33).
*Statistically-different ($p \leq 0.05$) from the pooled control.

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B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Embryos began hatching between Days 2 and 5, and ≥88% hatch occurred in the control (93% pooled control) and all treatment groups by Day 4 (day 0 post-hatch; Appendix F, p. 46). Hatching was verified to be complete on Day 5 in all control and treatment groups. Day-to-mean hatch was 3.3 and 3.0 days in the negative and solvent controls, respectively, and 3.3, 3.0, 3.3, 3.0, 2.5, and 2.8 days for the mean-measured 0.706, 1.36, 2.44, 3.89, 6.71, and 11.4 ppm a.i. treatment groups, respectively. Therefore, based on statistical analysis (Dunnett's test) of the days-to-mean-hatch treatment data compared to the pooled control, the NOEC for time-to-hatch was 11.4 ppm a.i.

Terminal length and wet weights were significantly reduced in the mean-measured 2.44, 3.89, 6.71, and 11.4 ppm a.i. treatment groups compared to pooled control groups (Table 7, p. 33). Terminal lengths were 14.03 and 13.85 mm in the negative and solvent control groups, respectively, and 13.17, 13.89, 12.85, and 9.47 mm in the 0.706, 1.36, 2.44, and 3.89 ppm a.i. treatment groups, respectively. Terminal wet weights (blotted dry) were 41.42 and 38.71 mg in the negative and solvent control groups, respectively, and 36.60, 39.91, 28.80, and 8.73 mg in the 0.706, 1.36, 2.44, and 3.89 ppm a.i. treatment groups, respectively. Growth measurements were not determined for the 6.71 and 11.4 ppm a.i. treatment groups due to a statistically-significant effect on survival. The NOEC for growth parameters was 1.36 ppm a.i.

Statistically-significant treatment-related sub-lethal signs of toxicity were observed at the mean-measured 2.44 through 11.4 ppm a.i. treatment levels (based on % normal compared to abnormal and dead larvae at test termination; Table 7, p. 33). Sub-lethal (abnormal) effects included pale coloration, immobility, deformed/underdeveloped body, and scoliosis, however, actual effects were not specified for each treatment level. The NOEC for sub-lethal effects was 1.36 ppm a.i.

Table 4: Effect of XDE-750 (Aminopyralid) on Time-To-Hatch , Growth (mean±SD) , and Other Sub-Lethal Effects on the Fathead Minnow (*Pimphales promelas*).

Treatment, ppm a.i. measured (and nominal) concentrations	Day-to-Mean-Hatch (days)	Length (mm)	Dry Weight (mg)	% Normal Larvae at Test Termination
Negative control	3.3 ± 0.5	14.03 ± 0.76	41.42 ± 5.54	84.1 ± 10.0
Solvent control	3.0 ± 0	13.85 ± 1.20	38.71 ± 2.46	87.8 ± 6.0
0.706 (0.780)	3.3 ± 0.5	13.17 ± 0.52	36.60 ± 2.13	88.6 ± 1.8
1.36 (1.30)	3.0 ± 0	13.89 ±0.41	39.91 ± 4.01	79.7 ± 7.8
2.44 (2.16)	3.3 ± 0.5	12.85 ± 0.61*	28.80 ± 5.86*	55.9 ± 13.8*
3.89 (3.60)	3.0 ± 0	9.47 ± 0.51*	8.73 ± 2.09*	16.1 ± 9.5*
6.71(6.00)	2.5 ± 0.6	----	----	0.0 ± 0.0*
11.4 (10.0)	2.8 ± 0.5	----	----	0.0 ± 0.0*
NOEC	11.4 ppm a.i.	1.36 ppm a.i.	1.36 ppm a.i.	1.36 ppm a.i.
LOEC	>11.4 ppm a.i.	2.44 ppm a.i.	2.44 ppm a.i.	2.44 ppm a.i.
MATC	>11.4 ppm a.i.	1.82 ppm a.i.	1.82 ppm a.i.	1.82 ppm a.i.
Positive control, if used mortality: EC_{50}:		N/A	N/A	

*Statistically-different ($p \leq 0.05$) from the solvent control using Dunnett's test. While the 0.070 and 0.12 ppm a.i. groups showed significant effects on growth when compared to the solvent control, mean length and weight in these treatment groups were not significantly different from the negative control, and any differences were not considered to be treatment-related.

---- Not determined due to statistically-significant treatment-related effects on survival by test termination.

C. REPORTED STATISTICS:

Endpoints that were analyzed statistically included percent embryos hatched (Day 5), percent larvae survival (post-hatch; Day 36), percent overall survival (pre- and post-hatch combined; Day 36), percent normal larvae at test termination (Day 36), days-to-mean hatch, growth (lengths and wet weights; Day 36).

The percent embryos that hatched, percent normal larvae at test termination, percent larvae that survived and percent overall survival data were arcsine square root transformed and days-to-mean- hatch data were square root transformed to meet the assumptions of ANOVA and the statistically significant treatment-related effects were identified using a one-tailed (lower end) Dunnett's test at a type I error rate of 0.05. Growth data were apparently not transformed and were analyzed statistically using ANOVA and Dunnett's test. All growth related statistical analyses were performed by comparing the treatment groups to the negative control and all survival and hatch related analyses were performed by comparing the treatment groups to a pooled control (p. 21). All NOEC and LOEC values were determined based on the results of the above statistical analyses and the maximum allowable toxicant concentration (MATC) for each endpoint was determined as the geometric mean of the NOEC and LOEC for each endpoint. All statistical analyses were conducted using mean-measured treatment concentrations.

Hatch success (%; Day 5)
NOEC: 11.4 ppm a.i.
LOEC: >11.4 ppm a.i.
MATC: >11.4 ppm a.i.

Time to hatch (days-to-mean-hatch)
NOEC: 11.4 ppm a.i.
LOEC: >11.4 ppm a.i.
MATC: >11.4 ppm a.i.

Post-hatch (larval) survival (%; Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.
MATC: 1.82 ppm a.i.

Wet weight (Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.
MATC: 1.82 ppm a.i.

Length (Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.
MATC: 1.82 ppm a.i.

Sub-lethal effects (% normal larvae)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.
MATC: 1.82 ppm a.i.

Overall survival (% embryos and larvae survival combined; Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.
MATC: 1.82 ppm a.i.

Most Sensitive Endpoint(s): Larvae survival, overall survival, growth and sub-lethal effects.

D. VERIFICATION OF STATISTICAL RESULTS:

Endpoints that were verified statistically included number of embryos hatched (Day 5), percent larvae survival (post-hatch; Day 36), and growth (lengths and wet weights; Day 36). For all endpoints (above) treatment levels were statistically compared to a pooled control because a t-test indicated no significant differences between the negative and solvent control. After confirming normality and homogeneity of variances, NOEC and LOEC values were identified using ANOVA and William's multiple comparison test via TOXSTAT statistical software. The reviewer was unable to statistically verify days-to-mean hatch (time-to-hatch) and the percent normal larvae (Day 36) because the actual values analyzed statistically by the study authors were not reported for either endpoint. The study authors' reported percent overall survival also could not be statistically verified by the reviewer because replicate data in the form of percent normal larvae (Day 36) per replicate were not reported and it was unclear how the study authors derived the reported values for each treatment level from the provided data; apparently dead and abnormal larvae were included in this endpoint (Table 7, p. 33) not just sub-lethal effects.

Hatch success (Day 5)
NOEC: 11.4 ppm a.i.
LOEC: >11.4 ppm a.i.
MATC: >11.4 ppm a.i.

Time to hatch (days-to-mean-hatch)
NOEC: Not verifiable
LOEC: Not verifiable
MATC: Not verifiable

Post-hatch (larval) survival (%; Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Wet weight (Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Length (Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Sub-lethal effects (% normal larvae)
NOEC: Not verifiable
LOEC: Not verifiable

Most Sensitive Endpoint(s): Larvae survival, wet weight, length and sub-lethal effects.

E. STUDY DEFICIENCIES:

The reviewer was unable to statistically verify the endpoints days-to-mean hatch (time-to-hatch), time-to-swim-up, and percent normal larvae (sub-lethal effects) because the raw data were not reported for these endpoints. Consequently, the study authors' reported NOEC and LOEC values for these endpoints are reported in the EXECUTIVE SUMMARY and CONCLUSION sections of this DER.

This study is scientifically valid, however, due to the lack of raw data for days-to-mean hatch (time-to-hatch), time-to-swim-up and sub-lethal effects, this study is classified as SUPPLEMENTAL because it provides information that is useful for risk assessment purposes. Provision of these data may allow the study to be upgraded.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical those of the study authors with the exception of those endpoints noted above and below, which could not be statistically verified by the reviewer.

The study authors' reported percent overall survival could not be statistically verified by the reviewer because data in terms of percent normal larvae (Day 36) per replicate were not reported and it was unclear how the study authors derived the reported values for each treatment level from the provided data; apparently dead and abnormal larvae were included in this endpoint (Table 7, p. 33) not just sub-lethal effects. Consequently, the NOEC, LOEC and MATC values based on the % normal larvae (Day 36) are not reported in the EXECUTIVE SUMMARY and CONCLUSION sections of this DER because it is unclear how they were determined and because they could not be clearly verified by the reviewer using the reported data.

The study authors reported that the maximum loading within the test vessels was 0.113 mg fish/L/day; instantaneous loading was 1.067 g fish/L (p. 21).

EAD comments:

After review of the study data and the US EPA DER, the EAD reviewer is in agreement with the conclusion reached by the US EPA. Deficiences mentioned above are not considered to have impact on the results of this study.

No amendments to the DER are recommended.

G. CONCLUSIONS:

This toxicity study is scientifically sound, but it does not fulfill the guideline requirements for a fish early life-stage study (§72-4a) with the Fathead minnow because replicate data for lack of raw data for days-to-mean hatch (time-to-hatch), time-to-swim-up and sub-lethal effects were not reported and conclusions based on this endpoint could not be verified by the reviewer. Consequently, this study is classified as SUPPLEMENTAL because it provides information that is useful for risk assessment purposes.

Hatch success (Day 5)
NOEC: 11.4 ppm a.i.
LOEC: >11.4 ppm a.i.

Time to hatch (days-to-mean-hatch)
NOEC: 11.4 ppm a.i.
LOEC: >11.4 ppm a.i.

Post-hatch (larval) survival (%; Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Wet weight (Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Length (Day 36)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Sub-lethal effects (% normal larvae)
NOEC: 1.36 ppm a.i.
LOEC: 2.44 ppm a.i.

Most Sensitive Endpoint(s): Larvae survival, overall survival, and growth.

III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.1400: *Fish Early-Life Stage Toxicity Test.*

ASTM Standard E1241-88. 1988. *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish.* American Society for Testing and Materials.

U.S. Environmental Protection Agency. 1986. Standard Evaluation Procedure, *Fish Early Life-Stage Test.* Office of Pesticide Programs. Hazard Evaluation Division. EPA 540/9-86-138.

West, Inc. and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

The SAS System for Windows. 2001. Version 8.2. SAS Institute, Inc., Cary, North Carolina.

APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Embryos hatched (Day 5)
File: 5821hsd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	12.375	2.063	0.776
Within (Error)	25	66.500	2.660	
Total	31	78.875		

Critical F value = 2.49 (0.05,6,25)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Embryos hatched (Day 5)
File: 5821hsd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	23.750	23.750		
2	0.706	24.000	24.000	-0.250	
3	1.36	24.750	24.750	-1.001	
4	2.44	22.750	22.750	1.001	
5	3.89	23.250	23.250	0.501	
6	6.71	24.250	24.250	-0.501	
7	11.4	23.000	23.000	0.751	

Bonferroni T table value = 2.57 (1 Tailed Value, P=0.05, df=25,6)

Embryos hatched (Day 5)
File: 5821hsd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	0.706	4	2.563	10.8	-0.250
3	1.36	4	2.563	10.8	-1.000
4	2.44	4	2.563	10.8	1.000
5	3.89	4	2.563	10.8	0.500
6	6.71	4	2.563	10.8	-0.500
7	11.4	4	2.563	10.8	0.750

196

Embryos hatched (Day 5)
File: 5821hsd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	23.750	23.750	24.063
2	0.706	4	24.000	24.000	24.063
3	1.36	4	24.750	24.750	24.063
4	2.44	4	22.750	22.750	23.417
5	3.89	4	23.250	23.250	23.417
6	6.71	4	24.250	24.250	23.417
7	**11.4**	**4**	**23.000**	**23.000**	**23.000**

Embryos hatched (Day 5)
File: 5821hsd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	24.063				
0.706	24.063	0.313		1.71	k= 1, v=25
1.36	24.063	0.313		1.79	k= 2, v=25
2.44	23.417	0.334		1.82	k= 3, v=25
3.89	23.417	0.334		1.83	k= 4, v=25
6.71	23.417	0.334		1.84	k= 5, v=25
11.4	**23.000**	**0.751**		**1.84**	**k= 6, v=25**

s = 1.631
Note: df used for table values are approximate when v > 20.

Percent larvae survival (day 36)
File: 5821lsd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	16605.666	4151.416	53.010
Within (Error)	19	1487.974	78.314	
Total	23	18093.640		

Critical F value = 2.90 (0.05,4,19)
Since F > Critical F **REJECT Ho:All groups equal**

Percent larvae survival (day 36)
File: 5821lsd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	86.463	86.463		

197

2	0.706	90.625	90.625	-0.768	
3	1.36	81.700	81.700	0.879	
4	2.44	58.200	58.200	5.215	*
5	3.89	16.075	16.075	12.989	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=19,4)

Percent larvae survival (day 36)
File: 5821lsd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	0.706	4	13.190	15.3	-4.162
3	1.36	4	13.190	15.3	4.762
4	2.44	4	13.190	15.3	28.263
5	3.89	4	13.190	15.3	70.388

Percent larvae survival (day 36)
File: 5821lsd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	86.463	86.463	87.850
2	0.706	4	90.625	90.625	87.850
3	**1.36**	**4**	**81.700**	**81.700**	**81.700**
4	2.44	4	58.200	58.200	58.200
5	3.89	4	16.075	16.075	16.075

Percent larvae survival (day 36)
File: 5821lsd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	87.850				
0.706	87.850	0.256		1.73	k= 1, v=19
1.36	**81.700**	**0.879**		**1.81**	**k= 2, v=19**
2.44	58.200	5.215	*	1.84	k= 3, v=19
3.89	16.075	12.989	*	1.85	k= 4, v=19

s = 8.850
Note: df used for table values are approximate when v > 20.

Wet weight (Blotted Dry; mg; Day 36)
File: 5821wd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	3059.569	764.892	47.989

198

Within (Error)	19	302.844	15.939
Total	23	3362.413	

Critical F value = 2.90 (0.05,4,19)
Since F > Critical F **REJECT Ho:All groups equal**

Wet weight (Blotted Dry; Day 36)
File: 5821wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	40.061	40.061		
2	0.706	36.605	36.605	1.414	
3	1.36	39.910	39.910	0.062	
4	2.44	28.805	28.805	4.604	*
5	3.89	8.725	8.725	12.817	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=19,4)

Wet weight (Blotted Dry; Day 36)
File: 5821wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	0.706	4	5.951	14.9	3.456
3	1.36	4	5.951	14.9	0.151
4	2.44	4	5.951	14.9	11.256
5	3.89	4	5.951	14.9	31.336

Wet weight (Blotted Dry; Day 36)
File: 5821wd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	40.061	40.061	40.061
2	0.706	4	36.605	36.605	38.258
3	**1.36**	**4**	**39.910**	**39.910**	**38.258**
4	2.44	4	28.805	28.805	28.805
5	3.89	4	8.725	8.725	8.725

Wet weight (Blotted Dry; Day 36)
File: 5821wd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

199

GRPS 1&2 POOLED	40.061				
0.706	38.258	0.738		1.73	k= 1, v=19
1.36	**38.258**	**0.738**		**1.81**	**k= 2, v=19**
2.44	28.805	4.604	*	1.84	k= 3, v=19
3.89	8.725	12.817	*	1.85	k= 4, v=19

s = 3.992
Note: df used for table values are approximate when v > 20.

Length (Day 36; mm)
File: 5821ld Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	60.091	15.023	30.597
Within (Error)	19	9.336	0.491	
Total	23	69.427		

Critical F value = 2.90 (0.05,4,19)
Since F > Critical F **REJECT Ho:All groups equal**

Length (Day 36)
File: 5821ld Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	13.941	13.941		
2	0.706	13.175	13.175	1.786	
3	1.36	13.890	13.890	0.119	
4	2.44	12.848	12.848	2.549	*
5	3.89	9.465	9.465	10.432	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=19,4)

Length (Day 36)
File: 5821ld Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	0.706	4	1.044	7.5	0.766
3	1.36	4	1.044	7.5	0.051
4	2.44	4	1.044	7.5	1.094
5	3.89	4	1.044	7.5	4.476

Length (Day 36)
File: 5821ld Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	13.941	13.941	13.941
2	0.706	4	13.175	13.175	13.533
3	**1.36**	**4**	**13.890**	**13.890**	**13.533**
4	2.44	4	12.848	12.848	12.848
5	3.89	4	9.465	9.465	9.465

Length (Day 36)
File: 5821ld Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	13.941				
0.706	13.533	0.952		1.73	k= 1, v=19
1.36	**13.533**	**0.952**		**1.81**	**k= 2, v=19**
2.44	12.848	2.548	*	1.84	k= 3, v=19
3.89	9.465	10.428	*	1.85	k= 4, v=19

s = 0.701
Note: df used for table values are approximate when v > 20.

Data Evaluation Report on the Chronic Toxicity of Aminopyralid (XDE-750) to Freshwater Invertebrates - *Daphnia magna.*

PMRA Submission Number{.....} EPA MRID Number 462358-22

Data Requirement:	PMRA DATA CODE	9.3.3
	EPA DP Barcode	D301682
	OECD Data Point	
	EPA MRID	462358-22
	EPA Guideline	§72-4b
	OPPTS Guideline	850.1300

Test material:	XDE-750	**Purity:** 94.5%
Common name	Aminopyralid	
Chemical name:	IUPAC:	2-pyridinecarboxylic acid, 4-amino-3,6-dichloro
	CAS name:	Not reported
	CAS No.:	Not reported
	Synonyms:	XR-750, X660750

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 8/31/04

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation

Signature:
Date: 10/4/04

Primary Reviewer: Brian D. Kiernan, Biologist
EPA/OPP/EFED/ERBIV

Signature:
Date: 11/30/2004 6/7/05

Secondary Reviewer(s): Andrew Wan, EAD
PMRA

Date: 02/08/2005

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

Date Evaluation Completed:

CITATION: Henry, K.A., T.A. Marino, J.L. Staley and E.L. McClymont. 2003. XDE-750: 21-Day Chronic Toxicity Test with the Daphnid, *Daphnia magna* Straus. Unpublished study performed by The Dow Chemical Company, Toxicology & Environmental Research and Consulting, Midland, Michigan. Laboratory Project ID No. 021085. Study submitted by Dow AgroSciences LLC, Indianapolis, Indiana. Study initiated August 14, 2002 and completed January 27, 2003.

EXECUTIVE SUMMARY:

The chronic toxicity of Aminopyralid (XDE-750) to *Daphnia magna* was studied under static renewal conditions for 21 days. Daphnids were exposed to Aminopyralid at nominal concentrations of 0 (negative control), 3.13, 6.25, 12.5, 25.0, 50, and 100 mg a.i./L. The mean-measured treatment concentrations were <0.251 (<LOQ, control), 2.99, 6.16, 12.5, 25.5, 49.8, and 102 mg a.i./L Recoveries were 95.5-102% of nominal for the mean-measured test concentrations, with no evidence of instability.

After 21 days of exposure, cumulative mortality was 0% in the control and treatment groups. The 21-day LC/EC_{50} was estimated as >102 mg a.i./L The mean progeny per surviving adult (reproduction) were 150.6 for the negative control group, compared to 155.1, 151.2, 166.3, 168.8, 185.0, and 184.7 for the 2.99, 6.16, 12.5, 25.5, 49.8, and 102 mg a.i./L test groups, respectively. The EC_{50} for reproduction was estimated as mg a.i./L The mean lengths were 4.24 mm for the negative control group, compared to 4.22, 4.21, 4.20, 4.17, 4.24, and 4.20 mm for the 2.99, 6.16, 12.5, 25.5, 49.8, and 102 mg a.i./L test groups, respectively. The NOEC for mortality, reproduction, and growth (length) were 102 mg a.i./L, the highest concentration tested.

This study is scientifically sound and deviates from the guideline requirements for a chronic toxicity study with freshwater invertebrates [§72-4(b)] but follows OECD guidelines. Due to excessive water hardness, low dissolved oxygen (31%) and reduced replicate size this study is classified as SUPPLEMENTAL.

PMRA: This study is classified as acceptable and satisfies the guideline requirements for a chronic toxicity study with freshwater invertebrates.

Results Synopsis:

Test Organism Age (eg. 1st instar): <24 hours old
Test Type (Flow through, Static, Static Renewal): Static Renewal

Mortality
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: >102 mg a.i./L 95% C.I.:N.A

Mean # Young per Reproductive Day
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
EC_{50}: >102 mg a.i./L 95% C.I.:N.A

Length
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: >102 mg a.i./L 95% C.I.:N.A

Endpoints Affected: None

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The test procedures were based on procedures outlined in the U.S. Environmental Protection Agency, FIFRA Guideline 72-4 and OECD Guideline for Testing Chemicals, Method 211. Deviations from U.S. EPA FIFRA guideline §72-4(b) included:

1. The age and pretest health (including mortality) of the parental stock was not specified.
2. The pH range (6.3-8.7) exceeded the recommended range (7.6-8.0). The water hardness range (154-273 mg/L) exceeded the recommended range (160-180 mg/L).
3. The low dissolved oxygen concentrations (2.9-5.8 mg/L) were measured in the spent control test solutions on day 14.
4. The dilution water measurement of chlorine was not reported.
5. The study design followed OECD guidelines and differed appreciably from EPA guidance. In this study, one daphnid per test chamber was maintained, with 10 replicate chambers per concentration and control. EPA guidance recommends 22 daphnids/level for static renewal studies, where seven test chambers should contain one daphnid each (to collect data on survival, growth, and reproduction), and three test chambers should contain five daphnids each (to collect data on survival only).

This study is classified as SUPPLEMENTAL.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. This study was conducted in compliance with GLP regulations set forth by the U.S. Environmental Protection Agency (40 CFR Part 160), OECD (ENV/MC/CHEM (98) 17), and European Community, Directive 99/11/EC (p.3).

A. MATERIALS:

1. Test Material	Aminopyralid (XDE-750)
Description:	Solid
Lot No./Batch No. :	F0031-143
Purity:	94.5%
Stability of Compound Under Test Conditions:	Verified. The mean measured recoveries (from days 0, 2, 5, 12, 14, 19, and 21 samples) were 95.5-102% of nominal concentrations, with no evidence of instability (Table 3A, pp. 26-27).

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Storage conditions of test chemicals: Not reported.

OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound). The OECD requirements were not reported.

2. Test organism:

Species: *Daphnia magna*

Age of the parental stock: Not reported (test daphnids were <24 hours old).

Source: In-house (Dow Chemical Company) laboratory culture.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A 21-day static renewal range-finding study was conducted at nominal concentrations of 0 (negative control), 0.185, 0.410, 0.911, 2.02, 4.50, and 10.0 mg a.i./L a.i. After 21 days, mortality was ≤20% in all treatment groups, except for the 4.50 mg a.i./L a.i. treatment group which had 75% mortality. No significant effects on the average number of young per adult were observed in the treatment groups. The nominal test concentrations for the definitive test were chosen to incorporate the 4.50 mg a.i./L treatment because of the unexplained mortality.

b. Definitive Study:

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Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period:	Continuous (in-house culture)	
Conditions: (same as test or not)	Same as test	
Feeding:	Mixed diet of *Selenastrum capricornutum* (green algae) and yeast-Ceraphyll-trout chow suspension (YCT) was provided 5 times per week.	
Health: (any mortality observed)	Not reported	
Test condition: static renewal/flow through:	Static renewal	
Type of dilution system- for flow through method.	N/A	*For flow-through study: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.*
Renewal rate for static renewal	3 times per week (Monday, Wednesday, and Friday)	
Aeration, if any	No aeration during testing.	*Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.*
Duration of the test	21 days	*EPA requires 21 days for static renewal*

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Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel)	Borosilicate vessels (covered with sheet of Plexiglas®)	
Size: growth/reproduction test:	120 mL	
survival test:	same	*1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.* *OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 ml of medium in each vessel.*
Fill volume: growth/reproduction test:	90 mL	
survival test:	same	
Source of dilution water	The dilution water was city water from Lake Huron. The water was limed and flocculated with ferric chloride, filtered (sand and carbon), pH-adjusted, and UV-irradiated. The water was autoclaved prior to use.	*Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*

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Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine Interval of water quality measurements	 154-273 mg/L as $CaCO_3$ 6.3-8.7 2.9-10.8 mg/L (31-121% saturation) 19.7-21.1°C <1000 µg/L Not detected (total suspended solids) See Table 1, p. 24 Not detected (Table 2, p. 25) Not reported The DO, temperature, and pH were measured weekly in the freshly prepared bulk test solutions and all the respective spent test solution replicates. The water hardness was measured weekly in the fresh and spent test solutions of the negative control and the 100 mg a.i./L a.i. treatment group.	The pH and water hardness ranges exceeded recommendations. The low dissolved oxygen concentrations were measured in the spent control test solutions on day 14 (Appendix C, pp. 42). *EPA requires:* ***hardness*** *160 to 180 mg/L as $CaCO_3$; OECD requires > 140 mg/L as $CaCO_3$* ***pH*** *7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. OECD requires pH rang 6 - 9 and should not vary more than 1.5 units in any one test.* ***Dissolved Oxygen*** *Renewal: must not drop below 50% for more than 48 hours.* *Flow-through: ≥ 60% through out test.* ***Temperature*** *20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours. OECD requires range 18 - 22°C; temperature should not vary more than ± 2°C.* *OECD requires total organic carbon < 2 mg/L*

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Parameter	Details	Remarks *Criteria*
Number of organisms/replicates: For growth and reproduction: For survival test:	10 daphnids/test level 10 replicate vessels with 1 daphnid per vessel (Not differentiated; same test chambers as above)	Study followed OECD recommended test design, not US EPA. *EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.* *OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.*
Application rates nominal: measured:	0 (negative control), 3.13, 6.25, 12.5, 25.0, 50, and 100 mg a.i./L a.i. <0.251 (<LOQ, control), 2.99, 6.16, 12.5, 25.5, 49.8, and 102 mg a.i./L a.i.	Mean-measured concentrations are provided in Table 3A, pp. 26-27. *EPA requires control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.*
Solvent (type, percentage, if used)	N/A	*EPA requires:* *solvent to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylforma-mide, triethylene glycol, methanol, acetone and ethanol.* *OECD requires ≤ 0.1 ml/L*

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Parameter	Details	Remarks *Criteria*
Lighting	16 hours of light, 8 hours of dark	The light intensity range was 622-925 Lux (p. 19). *EPA/OECD requires: 16 hours light, 8 hours dark.*
Feeding	At test solution renewals, 10 mL of *Selenastrum capricornutum* (217 mg organic carbon/L) and 5 mL of YCT (2010 mg total solids/L) were provided. On non-renewal days, 0.5 mL of the *Selenastrum capricornutum* suspension was provided to each test vessel.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	99.7 ± 2.44% of nominal Days 0, 2, 5, 12, 14, 19, and 21 Not reported 0.251 mg a.i./L a.i.	Based on mean measured test concentrations.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

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2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Data end points measured (list)	- Survival of first-generation daphnids -Length of first-generation daphnids - Progeny per surviving adult (reproduction)	*EPA requires:* *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (recommended) and length (required)* of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs.* **current requirement until the Agency provides specific guidance indicating otherwise (Pesticide Rejection Rate Analysis, p. 132).*
Observation intervals	Mortality of first-generation daphnids was recorded daily and juvenile production was recorded three times per week (Monday, Wednesday, and Friday). The daphnid length was determined at test termination (day 21).	
Water quality was acceptable?	Yes	
Were raw data included?	Yes, sufficient.	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 21 days of exposure, cumulative mortality was 0% in the control and treatment groups (Table 6, p. 30). The 21-day LC/EC_{50} was estimated as >102 mg a.i./L a.i. and the NOEC for mortality was 102 mg a.i./L a.i.

Table 1: Effect of Aminopyralid (XDE-750) on Survival, Growth, and Reproduction of Daphnia sp.

Mean-Measured Treatment Concentrations (mg a.i./L) (Nominal Conc.)	Mortality (Dead or Immobile)		Mean Length (mm)	Reproduction (Mean Progeny per Surviving Adult)
	No. Dead	%		
Negative control	0	0	4.24 ± 00.7	150.6 ± 21.1
2.99 (3.13)	0	0	4.22 ± 0.05	155.1 ± 43.1
6.16 (6.25)	0	0	4.21 ± 0.08	151.2 ± 34.3
12.5 (12.5)	0	0	4.20 ± 0.10	166.3 ± 32.0
25.5 (25.0)	0	0	4.17 ± 0.03	168.8 ± 18.8
49.8 (50.0)	0	0	4.24 ± 0.12	185.0 ± 24.3
102 (100)	0	0	4.20 ± 0.05	184.7 ± 19.7
NOEC, mg a.i./L (nominal)	102 (100)		102 (100)	102 (100)
LOEC, mg a.i./L(nominal)	>102 (>100)		>102 (>100)	>102 (>100)
LC_{50}/EC_{50} (95% C.I.), mg a.i./L (nominal)	>102 (>100)		>102 (>100)	>102 (>100)

B. EFFECT ON REPRODUCTION AND GROWTH:

The mean progeny per surviving adult (reproduction) were 150.6 for the negative control group, compared to 155.1, 151.2, 166.3, 168.8, 185.0, and 184.7 for the 2.99, 6.16, 12.5, 25.5, 49.8, and 102 mg a.i./L a.i. test groups, respectively. The EC_{50} for reproduction was estimated as >102 mg a.i./L a.i. (Table 6, p. 30). The NOEC for reproduction was 102 mg a.i./L.

The mean lengths were 4.24 mm for the negative control group, compared to 4.22, 4.21, 4.20, 4.17, 4.24, and 4.20 mm for the 2.99, 6.16, 12.5, 25.5, 49.8, and 102 mg a.i./L a.i. test groups, respectively. The NOEC for length was 102 mg a.i./L.

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C. REPORTED STATISTICS:

The statistical endpoints included parental mortality, the progeny per surviving adult, and terminal length (of parental daphnids). Survival data (LC_{50}) were not analyzed because no mortality occurred during the test. Analyses included Bartlett's Test (evaluation of homogeneity) and Shapiro-Wilk's test (assessment of normality). The one-tailed Dunnett's test determined differences in treatment groups compared to the control. The EC_{50} (reproductive and growth data) was estimated based on significance data. The NOEC and LOEC were estimated based on results from the Steel's test and the Wilcoxon test. Mean-measured values were used in all estimations.

Mortality
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: >102 mg a.i./L 95% C.I.:N.A

Mean # Young per Reproductive Day
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: >102 mg a.i./L 95% C.I.:N.A

Length
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: >102 mg a.i./L 95% C.I.:N.A

Endpoints Affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

The NOEC for mortality and reproduction could be visually determined, as there were no reductions from control. The NOEC for length was verified using the non-parametric Kruskal-Wallis test via TOXSTAT statistical software.

Mortality
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: > 102 mg a.i./L 95% C.I.:N.A

Mean # Young per Reproductive Day
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: > 102 mg a.i./L 95% C.I.:N.A

Length
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: > 102 mg a.i./L 95% C.I.:N.A

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Endpoints Affected: None

E. STUDY DEFICIENCIES:

The reduced replicate size (10 reps per treatment vs. 22 recommended reps per treatment) reduced the statistical power and, thus, the ability to detect potential significant differences if they existed. The water hardness was too high and the dissolved oxygen fell to 31% for an unspecified period.

Although the PMRA-EAD reviewer agrees with the USEPA's assessment of this study, a new study would not be expected to reveal any new information as there were no mortality in the controls.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'. The reviewer based toxicity values on the mean-measured treatment concentrations, rather than the corresponding nominal treatment concentrations as reported by the study author.

G. CONCLUSIONS:

This study is scientifically sound and deviates from the guideline requirements for a chronic toxicity study with freshwater invertebrates [§72-4(b)] but follows OECD guidelines. The water hardness was too high and the dissolved oxygen fell to 31% for an unspecified period. Due to these deviations, this study is classified as SUPPLEMENTAL.

PMRA: This study is classified as acceptable and satisfies the guideline requirements for a chronic toxicity study with freshwater invertebrates.

Mortality
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: > 102 mg a.i./L 95% C.I.:N.A

Mean # Young per Reproductive Day
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: > 102 mg a.i./L 95% C.I.:N.A

Length
NOEC: 102 mg a.i./L
LOEC: >102 mg a.i./L
LC/EC_{50}: > 102 mg a.i./L 95% C.I.:N.A

Endpoints Affected: None

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III. REFERENCES:

Organisation for Economic Cooperation and Development. OECD Guideline for Testing Chemicals. Method 211 "*Daphnia magna* Reproduction Test." Adopted September 21, 1998.

Environmental Protection Agency. Hazard Evaluation Division: Standard Evaluation Procedure *Daphnia magna* Life Cycle (21-Day Renewal) Chronic Toxicity Test. EPA 540/9-86-141, June 1987.

OECD Series on Principles on Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM (98)17.

EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

Dow AgroSciences LLC, Test Substance Distribution Certificate. TSN102319, Dow AgroSciences LLC, Indianapolis, Indiana.

Certificate of Analysis for Test Substance, TSN102319. Lab Report Number DECO GL-AL MD-2000-005682, Analytical Sciences Laboratory, The Dow Chemical Company, 25 October 2000.

Product Technology Information Platform (PTIP) Database. Dow AgroSciences LLC, Indianapolis, Indiana.

Marino, T.A., Hales, C.A., McClymont, E.L. and Yaroch A.M. (2001). XDE-750 Herbicide: An Acute Toxicity Study with the Daphnia, *Daphnia magna* Straus. Toxicology & Environmental Research and Consulting (TERC) Study Number: 0110479. The Dow Chemical Company, Midland, Michigan.

Shapiro, S.S. and M.B. Wilk. (1965). "An Analysis of Variance Test for Normality (complete samples)", *Biometrika*, 52, 591-611.

Winer, B.J. (1971). *Statistical Methods in Experimental Design* (2nd Ed.) McGraw-Hill, New York, New York, 1971.

Steel, R.G.D. (1959). A Multiple Comparison Rank Sum Test: Treatments versus Control, *Biometrics*, 15:560-572. 1959.

Hollander, M. and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*; John Wiley: New York, New York.

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APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

length
File: 58221 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	4.239	4.239	429.000
2	2.99	4.218	4.218	376.500
3	6.16	4.213	4.213	355.500
4	12.5	4.201	4.201	374.500
5	25.5	4.174	4.174	226.500
6	49.8	4.243	4.243	389.500
7	102	4.202	4.202	333.500

Calculated H Value = -53.597 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

length
File: 58221 Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 5	0 4	0 7	0 3	0 2	0 1	0 6
5	25.5	4.174	4.174	\						
4	12.5	4.201	4.201	.	\					
7	102	4.202	4.202	.	.	\				
3	6.16	4.213	4.213	.	.	.	\			
2	2.99	4.218	4.218	.	.	.	.	\		
1	control	4.239	4.239	.	.	.	.	.	\	
6	49.8	4.243	4.243	.	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 8.874

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DATA EVALUATION RECORD
MIDGE CHRONIC TOXICITY STUDY
USEPA:Non Guideline PMRA DACO: 9.3.4

1. **CHEMICAL:** Aminopyralid PC Code No.: 005100

2. **TEST MATERIAL:** XR-750 Technical (Syn.: XDE-750) Purity: 94.5%

3. **CITATION:**

Author: Putt, A.E.

Title: XDE-750 - The Full Life-Cycle Toxicity To Midge (*Chironomus riparius*) Under Static Conditions

Study Completion Date: May 2, 2002

Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, MA 02571-1075

Sponsor: The Dow Chemical Company
for Dow AgroSciences, LLC
1803 Building
Midland, MI 48674

Laboratory Report ID: 12550.6195

MRID No.: 462358-23 PMRA Submission #: 2004-0789

DP Barcode: D301682

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: **Date:** 10/8/04

APPROVED BY: Gregory Hess, Staff Scientist, Dynamac Corporation

Signature: **Date:** 10/12/04

5. **APPROVED BY:** Brian D. Kiernan, Biologist, OPP/EFED/ERBIV

Signature: 6/16/05 **Date:** 12/14/2004

APPROVED BY: 213, EAD, PMRA

Signature: **Date:** January 28, 2005

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6. STUDY PARAMETERS:

Age of Test Organism:	1st Instar, 2 days old
Definitive Test Duration:	28 days
Study Method:	Static
Type of Concentrations:	Nominal

7. CONCLUSIONS:

The 28-day chronic toxicity of XR-750 Technical (Synonym: XDE-750 Tech., aminopyralid) to a midge, *Chironomus riparius*, was studied under static conditions in water-spiked exposures (sediment was not spiked). Endpoints assessed were the percent emergence (combined sexes) and development rates (male, female, and combined sexes). Ash-free dry weights were not assessed in this study.

The nominal test concentrations were 0 (negative control), 63, 130, 250, 500, and 1000 ppm a.i. Mean-measured treatment concentrations were 58, 123, 247, 520, and 973 ppm a.i. for the overlying water, and recoveries ranged from 87-112% of nominal concentrations during the study. Pore water and sediment concentrations were determined at the 63, 250, and 1000 ppm a.i. test levels only. Recoveries in pore water were 17-18% of nominal overlying water concentrations on Day 0, and increased to 81-93% of nominal at 7 and 28 Days. Recoveries in sediment were 7-15% of nominal at Day 0, 35-40% at Day 7, and 16-68% at Day 28. Treatment endpoints are expressed in terms of measured pore water concentrations averaged over the entire exposure period, i.e., 40, 82, 158, 315 and 630 mg a.i./L.

A statistically-significant treatment-related reduction in mean percent emergence (the most sensitive endpoint) was observed at the 158 ppm a.i. treatment levels compared to the negative control. Mean percent emergence was 94% for the control group, compared to 88, 86, 80, 75, and 0% at the sediment-exposures 40, 82, 158, 315 and 630 ppm a.i. treatment levels, respectively. The NOEC for percent emergence was 82 ppm a.i.. The 28-day EC_{50}, based on sediment concentrations and midge emergence, was 4,032 ppm a.i..

The mean development rate of male midge in the 315 ppm a.i. level was statistically different from the mean development rate of the male control organisms (0.0582 versus 0.0625). No other statistical differences on development rates were observed. The NOEC for development rate was 158 ppm a.i.

This study was designed to fulfill proposed OECD Draft Guideline 219 (February 2001), and does not fulfill any current U.S. EPA guideline requirements. This study is classified as SUPPLEMENTAL, and provides information on the chronic toxicity of XR-750 Technical (aminopyralid) to sediment-dwelling invertebrates (*Chironomus riparius*). PMRA classifies this study as acceptable.

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Results Synopsis:
Based on Mean Pore Water Concentrations (63% of nominal)
Percent Emergence (Combined sexes)
NOEC: 82 ppm a.i.
LOEC: 158 ppm a.i.
EC_{50}:4,032 ppm a.i. 95% C.I.: 200-210,000
Slope: 0.77±0.46

Development Rate (Males)
NOEC: 158 ppm a.i.
LOEC: 315 ppm a.i.

Development Rate (Females)
NOEC: 315 ppm a.i.
LOEC: >315 ppm a.i.

Development Rate (Combined sexes)
NOEC: 315 ppm a.i.
LOEC: >315 ppm a.i.

Endpoints affected: Percent emergence and male development rate
Most sensitive endpoint: Percent emergence

8. ADEQUACY OF THE STUDY:

A. Classification: USEPA: Supplemental PMRA: Acceptable

B. Rationale: This study was not designed to fulfill any current U.S. EPA guideline.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following sources were used as guidance in evaluating this study, and deviations from these guidance documents are listed below:

U.S. EPA. 1996. Ecological Effects Test Guidelines, OPPTS 850.1735 (Public Draft), EPA-712-C-96-354. April 1996.

U.S. EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates. Office of Research and Development and Office of Water, Washington, DC EPA/600/R-99/064. March 2000.

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1. The study was initiated with first instar, whereas second to third instar are recommended.

2. The water temperature of 19-21°C was slightly lower than the recommended 22°C.

3. The pH ranges exceeded 0.4 units for all groups (including control). Initial pH measurements on Days 0 and 1 in the 500 and 1000 ppm a.i. levels ranged from 2.8 to 5.6.

4. Initial measurements of length and weight should have been provided for a sub-set, and terminal ash-free dry weights should have been determined at study termination.

5. Sediments were not analyzed for cation exchange capacity. total volatile sulfides, BOD, COD, Eh, total inorganic carbon, total volatile solids, acid volatile sulfides, metals, oil and grease, and petroleum hydrocarbons; these analyses are suggested in the guidance documents.

6. A physical description and water solubility of the test material were not reported.

7. The test chemical was mixed into stock solutions and added to the overlying water instead of the soil as recommended.

8. The ratio of sediment:overlying water in the test systems (75 mL:300 mL) differed from recommendations (100 mL:175 mL).

9. The test vessels were covered by clear plastic plates instead of glass covers as recommended for static tests.

10. The overlying water was not renewed during testing.

11. Only four replicate vessels were used to collect biological data, instead of the eight recommended.

12. Sediment and pore water test concentrations were not analyzed at every nominal treatment level.

10. SUBMISSION PURPOSE: This study was submitted to provide information on the toxicity of XR-750 Technical (aminopyralid) to sediment-dwelling chironomids for the purpose of pesticide registration (new chemical).

11. MATERIALS AND METHODS:

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A. Test Organisms

Guideline Criteria	Reported Information
Species Chironomus tentans *Other species which can be used are Hyalella azteca, Chironomus riparius, Daphnia sp., Ceriodaphnia sp.* (Specific criteria for these species are not listed in this report)	*Chironomus riparius*
Life Stage Second to third instar larvae (about 10 d old larvae with at least 50% at third instar.	1st instar, 2 days old.
Supplier Brood stock can be obtained from laboratory, commercial, or government sources.(Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Obtained from laboratory cultures.
All organisms from the same source?	Yes.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Brood stock must be acclimated to culture water gradually from transport water to 100% culture water; water temperature exchange rate not to exceed 2°C within 24 hr; Avoid unnecessary stress, crowding and rapid temperature and water quality changes.	Continuous breeding cultures were maintained in laboratory well water from the same source as the dilution water (in the definitive study). Egg masses were removed from the culture aquaria 5 days prior to test initiation, and hatched midge larvae were reared at 19°C in culture bowls for 2 days to provide first-instar larvae for use during exposure.
Feeding Feeding should begin on day 0 and continue through day 9 unless food is not being eaten.	Daily during rearing, midge larvae were fed a finely-ground suspension of flaked fish food at 10 mg/mL.

Guideline Criteria	Reported Information
Pretest Mortality A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality of midge larvae was observed 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (Overlying water) and sediment Soft reconstituted water or water from a natural source, **not** de-chlorinated tap water. [Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details)].	Overlying water was from the same source as the culture water (laboratory well water). Artificial sediment was prepared in the laboratory by combining 71.7% industrial sand, 20% kaolin clay, and 8.3% sphagnum peat.
Does water support test animals without observable signs of stress?	Midges have successfully survived and reproduced over several generations in the dilution water.
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	pH levels declined greatly during the definitive study at the two highest test concentrations.
Water Temperature 23°C ± 1°C. Daily mean test temperature Must not deviate more than ±1°C and instantaneous temperature must be within ±. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	Test water temperature was maintained at 19-21°C. Temperature was measured daily in overlying water in each replicate vessel of each treatment level and control. Raw data were not provided.

Guideline Criteria	Reported Information
pH Not specified, but should be appropriate to the test species and should not deviate more than 0.4 pH units.	pH ranged from 7.1-8.1 for the control and 63 through 250 ppm a.i. levels, 3.3-8.0 for the 500 ppm a.i. level, and 2.8-7.9 for the 1000 ppm a.i. level. pH was measured in each replicate vessel of each treatment level and control on Days -1, 0, 1, and 28. Raw data were not provided.
Dissolved Oxygen Should be measured at the beginning and end of short term tests. DO should be >40 percent and <100 percent saturation.	DO ranged from 7.0-9.5 mg/L. DO was measured daily in overlying water in each replicate vessel of each treatment level and control. It was reported that 5.4 mg/L is equivalent to 60% saturation at 20°C. Raw data were not provided.
Total Hardness Prefer 40 - 200 mg/L as $CaCO_3$.	160-220 mg/L $CaCO_3$, as measured at study initiation and termination in a composite sample from the control and 1000 ppm a.i. levels.
Conductivity Not specified, but should be amenable to the test species.	440-650 µmhos/cm, as measured at study initiation and termination in a composite sample from the control and 1000 ppm a.i. levels.
Sediment Characterization All sediment must be characterized for: pH, organic carbon content (TOC), total volatile sulfides, particle size distribution (% sand, silt, clay), and percent water content.	pH: 7.5 TOC: 1.8% Total volatile sulfides: Not reported Particle size distribution: 77% sand, 6% silt, and 17% clay Water holding capacity: 11.3% at 1/3 bar

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Guideline Criteria	Reported Information
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	Not reported
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	XR-750 Technical Synonyms: XDE-750 Technical; aminopyralid Lot no.: F0031-143 (TSN 102319) Purity: 94.5% A physical description and water solubility were not reported.
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	Three primary stock solutions (1000 ppm a.i.) were prepared directly in laboratory well water (concentrations adjusted for purity). The stock solutions were ultrasonicated for 2 hours and stirred overnight. Flasks were protected from light (using aluminum foil), and were observed to be pale yellow with no undissolved material. A negative control was included in the test.

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Guideline Criteria	Reported Information
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; Sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	Not applicable, as the sediment was not spiked. Applications were made to the overlying water, and test concentrations were based on toxicity information obtained from preliminary experiments. Twenty-four hours following addition of organisms to the test systems and suspension of aeration, the appropriate volume of overlying water (range of 19 to 300 mL) was removed from each test vessel, and replaced with an equivalent volume of stock solution. The overlying water was then gently stirred to aid in distribution, and aeration (1 to 3 bubbles/second) was resumed.
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100ml of sediment and 175 ml of overlying water.	1. Glass beakers 2. 600 mL; containing a 75-mL (1.5-cm) layer of sediment and 300 mL of overlying water.
Covers Static: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	Test vessels covered by clear plastic plates.
Type of Dilution System Must provide reproducible supply of toxicant.	N/A - Static system.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A - Static system.

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Guideline Criteria	Reported Information
Aeration Dilution water should be vigorously aerated so that dissolved oxygen in the overlying water remains above 40% saturation. In static systems, overlying water may be gently aerated through a 1-mL pipet located not closer than 2 cm from the sediment surface; Test organisms should not added 12 to 24h; Water quality characteristics should be measured before test organisms are added.	Test solutions were gently aerated (1 to 3 bubbles/second) 5 days prior to addition of the test organisms, suspended for a 24-hour period after the addition of midges, and continued throughout the duration of the exposure period. No further details were provided.
Photoperiod 16 hours light, 8 hours dark with a 15-30 min transition period and illuminance of about 100 to 1000 lux.	16 hours light, 8 hours dark. Light intensity ranged from 50 to 80 footcandles (538 to 861 lux).
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	No co-solvents were used.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be throughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment	Test containers were prepared with sediment and overlying water 5 days prior to treatment (p. 17). The sediment was covered with a turbulence reducer (modified plastic disk) during the introduction of the overlying water.

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Guideline Criteria	Reported Information
Renewal of Overlying Water: Renewal is required and flow rates should not differ by more than 10% in any two test chambers and should begin on day -1.	None performed.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	On Day -1, twenty midge larvae were impartially added to each of four replicate test vessels/level. No other details were reported.
Range Finding Test	A 24-day preliminary range-finding experiment was initiated with 2-day old midge larvae and nominal overlying water XDE-750 concentrations of 0 (negative control), 0.10, 1.0, 10, 100, and 1000 ppm a.i. (p. 23). After 24 days, the mean percent emergence was 82% for the control group, compared to 82, 70, 83, 85, and 0% for the toxicant levels, respectively. The mean development rate at the highest level could not be determined as no emergence was observed. At the remaining levels, the mean development rate was 0.0588, 0.0636, 0.0632, and 0.0596, respectively, compared to 0.0604 for the control group.
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	All replicate test vessels were observed daily. Observations of midge emergence and abnormal behavior were made and the physical characteristics of the test solutions were recorded. Starting on Day 10 and thereafter, a daily check of emerged midges was made.

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Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative control), 63, 130, 250, 500, and 1000 ppm a.i. Aqueous solubility was not reported.
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	20 midge larvae/chamber, with 8 replicate chambers per level. Four replicates were prepared for biological response and water quality measurements, and four replicates were prepared for chemical analysis of the overlying water.
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding Midges in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin® suspension daily. A drop in d.o. level below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until d.o. levels increase.	From Days -1 through 10, midges were fed 0.50 mL of finely-ground flaked fish food suspension (10 mg/mL) daily. From Days 11 through 28, 1.0 mL was offered.

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Guideline Criteria	Reported Information
Water Parameter Measurements Overlying Water Quality should measure conductivity, hardness, pH, alkalinity, and ammonia in all treatments at beginning and end of a test and should not vary by more than 50% within a treatment during the test.	DO and temperature were measured daily in each replicate vessel of each treatment and control level. The temperature was also continuously monitored in one replicate vessel of the 250 ppm a.i. group. The pH was measured on Days -1, 0, 1, and 28 in each replicate vessel of each treatment and control level. Total hardness, total alkalinity, specific conductance, and ammonia concentrations were determined at test initiation and termination in a composite sample from the highest treatment level and control solution.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	XDE-750 Technical (Syn.: XR-750) concentrations were measured in the overlying water from all treatment and control levels at Days 0 (1 hour), 7, and 28. Concentrations were also determined from the sediment and pore water of the 63, 250, and 1000 ppm a.i. treatment levels on Days 0 (1 hour), 7, and 28.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.

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Guideline Criteria	Reported Information
Control Mortality Must be ≤ 30% in the sediment at end of the test.	Negative control: 6% mortality (580) This value was reviewer-interpreted from emergence data. Mortality data were not reported.
Percent Recovery of Chemical: 1) % of nominal;	1) In overlying water: 87-112% of nominal at 0, 7, and 28 days for all concentration levels. In pore water: 17-18% of nominal on Day 0, and 81-93% at 7 and 28 Days (63, 250, and 1000 ppm a.i. test levels). In sediment: 7-15% of nominal at Day 0, 35-40% at Day 7, and 16-68% at Day 28 (63, 250, and 1000 mg a.i./L test levels; reviewer-calculated).
2) Procedural recovery;	2) In aqueous QC samples: 94.1-103% of nominal concentrations. In sediment QC samples: 77.4-111% of nominal concentrations.
3) Limit of quantitation (LOQ)	3) LOQ = 0.40, 1.0, and 0.84 ppm a.i. (1-hour, Day 7, and Day 28, respectively)
Data Endpoints - Survival of Larvae - Ash-free dry weight (AFDW) should be determined by pooling all living organisms from a replicate and drying to a constant weight (e.g. 60°C for 24 h)	- Percent emerged (combined sexes) - Development rate (male, female, and combined sexes)
Raw data included?	Yes

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Effects Data

Toxicant Concentration				Cumulative Number Dead (and %)	Mean Dry Weight per midge (mg)
Nominal (ppm a.i.)	Measured (Day 28)				
	Sediment (ppm a.i.)	Pore Water (ppm a.i.)	Overlying Water (ppm a.i.)		
Control	ND	ND	<0.84	5/80 (6)	ND
63	10	53	55	10/80 (12)	ND
130	ND	ND	120	11/80 (14)	ND
250	91	230	240	16/80 (20)	ND
500	ND	ND	470	20/80 (25)	ND
1000	680	930	940	80/80 (100)	ND

ND - Not determined.

Nominal Concentrations (ppm a.i.)	Percent Emerged (%)	Mean Development Rate (1/days)		
		Male	Female	Combined
Control	94	0.0625	0.0546	0.0581
63	88	0.0590	0.0557	0.0572
130	86	0.0611	0.0537	0.0570
250	80*	0.0598	0.0555	0.0579
500	75*	0.0582*	0.0522	0.0557
1000	0*	N/A	N/A	N/A

* Statistically different from control group.

Other Significant Results:

The mean percent emergence at the nominal 250, 500, and 1000 ppm a.i. levels was statistically different from the mean percent emergence of the control organisms. The NOEC for percent emergence was 130 ppm a.i. The mean development rate of male midge in the 500 ppm a.i. level was statistically different from the mean development rate of the male control organisms. No other statistical differences were observed. The NOEC for development rate was 250 ppm a.i., based on overlying water concentrations

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The 28-day EC_{50} (with 95% C.I.), based on nominal concentrations and midge emergence (the most sensitive endpoint), was 680 ppm a.i. (243 ppm sediment).

B. Statistical Results

Method: Endpoints assessed included percent midge emergence and development rate (male, female, and combined sexes). Percent emergence data were arcsine transformed prior to analysis. Analyses were performed using the mean replicate organism response and nominal concentrations using a computer program (Gulley, et al., 1989).

Data were assessed for normality using the Shapiro-Wilks Test for normality and for homogeneity of variance using Bartlett's Test. Percent emergence and development rate data passed both tests, and were therefore analyzed using the William's Test.

The EC_{50} (with 95% C.I.) was calculated for percent emergence using linear interpolation.

Based on Nominal Concentrations in the Overlying Water
Percent Emergence (Combined sexes)
NOEC: 130 ppm a.i.
LOEC: 250 ppm a.i.
EC_{50}: 680 ppm a.i. 95% C.I.: 640 to 720 ppm a.i.
Slope: Not reported

Development Rate (Males)
NOEC: 250 ppm a.i.
LOEC: 500 ppm a.i.

Development Rate (Females)
NOEC: 500 ppm a.i.
LOEC: >500 ppm a.i.

Development Rate (Combined sexes)
NOEC: 500 ppm a.i.
LOEC: >500 ppm a.i.

Endpoints affected: Percent emergence and male development rate
Most sensitive endpoint: Percent emergence

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13. VERIFICATION OF STATISTICAL RESULTS:

Method: After confirming normality and homogeneity of variances, percent emergence and development rate (male, female, and combined sexes) data were assessed for treatment-related effects compared to the negative control using ANOVA and William's multiple comparison test via TOXSTAT statistical software. An EC50 (with 95% C.I.) was determined using the probit method via NUTHATCH statistical software for percent emergence. The reviewer excluded the nominal 1000 ppm a.i. treatment group from all statistical analyses given the 0% emergence by 28 days. All toxicity values are reported as pore water concentrations as a percentage of overlying water, based on mean recovery across all samples at the nominal 63, 250 and 1000 treatment levels (63%).

Based on Mean Pore Water Concentrations (63% of nominal)

Percent Emergence (Combined sexes)
NOEC: 82 ppm a.i.
LOEC: 158 ppm a.i.
EC_{50}:4032 ppm a.i. 95% C.I.: 200-210,000
Slope: 0.77±0.46

Development Rate (Males)
NOEC: 158 ppm a.i.
LOEC: 315 ppm a.i.

Development Rate (Females)
NOEC: 315 ppm a.i.
LOEC: >315 ppm a.i.

Development Rate (Combined sexes)
NOEC: 315 ppm a.i.
LOEC: >315 ppm a.i.

Endpoints affected: Percent emergence and male development rate
Most sensitive endpoint: Percent emergence

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study author's with the exception of the EC_{50} value based on percent emergence data and the fact that the reported toxicity values were all determined in terms of the nominal overlying water treatment concentrations rather than the mean sediment concentrations. All toxicity values reported in the CONCLUSION section of this DER are reviewer-determined because they are based on the pore water concentrations.

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This study was not designed to fulfill any current U.S. EPA FIFRA guideline, however, the study does provide information that may be useful for risk assessment purposes..

Initial pH measurements on Day 0 and 1 at the 500 and 1000 ppm a.i. levels ranged from 2.8 to 5.6 and were appreciably lower than the control pH at the same intervals. The low pH is due to the concentration of XDE-750 in the solution, and a pH of 3.5 has been reported to cause 100% mortality of first instar larvae of *Chironomus tentans* (Townsend et al., 1981) and indicates that the reduction in pH of the exposure solutions caused by the test substance may have contributed to the observed reduction in midge survival at these treatment levels.

This study was conducted in compliance with all pertinent OECD GLP regulations with the following exceptions: routine water, food, and sediment contaminant screening analyses for pesticides, PCBs, and toxic metals were conducted using standard U.S. EPA procedures, and were not collected in accordance with GLP procedures (i.e., no distinct protocol, Study Director, etc.).

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15. REFERENCES:

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Weber, C.I., *et al.* (eds.). 1989. Short-term methods for estimating the full life-cycle toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

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16. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Percent Emergence (Combined Sexes; Day 28)
File: 5823ed Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	832.500	208.125	2.291
Within (Error)	15	1362.500	90.833	
Total	19	2195.000		

Critical F value = 3.06 (0.05,4,15)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Percent Emergence (Combined Sexes; Day 28)
File: 5823ed Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	93.750	93.750		
2	58	87.500	87.500	0.927	
3	123	86.250	86.250	1.113	
4	247	80.000	80.000	2.040	
5	520	75.000	75.000	2.782	*

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

Percent Emergence (Combined Sexes; Day 28)
File: 5823ed Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	58	4	15.904	17.0	6.250
3	123	4	15.904	17.0	7.500
4	247	4	15.904	17.0	13.750
5	520	4	15.904	17.0	18.750

Percent Emergence (Combined Sexes; Day 28)
File: 5823ed Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

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GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	93.750	93.750	93.750
2	58	4	87.500	87.500	87.500
3	**123**	**4**	**86.250**	**86.250**	**86.250**
4	247	4	80.000	80.000	80.000
5	520	4	75.000	75.000	75.000

Percent Emergence (Combined Sexes; Day 28)
File: 5823ed Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	93.750				
58	87.500	0.927		1.75	k= 1, v=15
123	**86.250**	**1.113**		**1.84**	**k= 2, v=15**
247	80.000	2.040	*	1.87	k= 3, v=15
520	75.000	2.782	*	1.88	k= 4, v=15

s = 9.531
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	47.	1.8	1.2E+03	0.67	0.039
EC10	1.4E+02	17.	1.1E+03	0.43	0.13
EC25	8.5E+02	2.0E+02	3.7E+03	0.30	0.23
EC50	**6.4E+03**	**2.0E+02**	**2.1E+05**	**0.72**	**0.031**

Slope = 0.770 Std.Err. = 0.462

Goodness of fit: p = 0.94 based on DF= 2.0 15.

5823ED : Percent Emergence (Combined Sexes; Day 28)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	93.8	93.7	0.0727	100.	0.00
58.0	4.00	87.5	88.3	-0.760	94.2	5.78
123.	4.00	86.3	85.0	1.30	90.7	9.31
247.	4.00	80.0	80.7	-0.730	86.2	13.8
520.	4.00	75.0	74.9	0.119	79.9	20.1

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

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!!!Warning: EC50 not bracketed by doses evaluated.

Development Rate Male (Day 28)
File: 5823mdd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.0047	0.0012	2.000
Within (Error)	15	0.0089	0.0006	
Total	19	0.0136		

Critical F value = 3.06 (0.05,4,15)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Development Rate Male (Day 28)
File: 5823mdd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.625	0.625		
2	58	0.590	0.590	2.035	
3	123	0.611	0.611	0.808	
4	247	0.599	0.599	1.530	
5	520	0.582	0.582	2.497	*

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

Development Rate Male (Day 28)
File: 5823mdd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	58	4	0.041	6.5	0.035
3	123	4	0.041	6.5	0.014
4	247	4	0.041	6.5	0.027
5	520	4	0.041	6.5	0.043

Development Rate Male (Day 28)
File: 5823mdd Transform: NO TRANSFORMATION

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	0.625	0.625	0.625
2	58	4	0.590	0.590	0.600
3	123	4	0.611	0.611	0.600
4	247	4	0.599	0.599	0.599
5	520	4	0.582	0.582	0.582

Development Rate Male (Day 28)
File: 5823mdd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.625				
58	0.600	1.434		1.75	k= 1, v=15
123	0.600	1.434		1.84	k= 2, v=15
247	0.599	1.543		1.87	k= 3, v=15
520	0.582	2.518	*	1.88	k= 4, v=15

s = 0.024
Note: df used for table values are approximate when v > 20.

Development Rate Female (Day 28)
File: 5823fdd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.0033	0.0008	1.333
Within (Error)	15	0.0090	0.0006	
Total	19	0.0123		

Critical F value = 3.06 (0.05,4,15)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Development Rate Female (Day 28)
File: 5823fdd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.546	0.546		
2	58	0.557	0.557	-0.635	

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3	123	0.538	0.538	0.491
4	247	0.556	0.556	-0.548
5	520	0.522	0.522	1.371

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

Development Rate Female (Day 28)
File: 5823fdd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	58	4	0.041	7.5	-0.011
3	123	4	0.041	7.5	0.008
4	247	4	0.041	7.5	-0.010
5	520	4	0.041	7.5	0.024

Development Rate Female (Day 28)
File: 5823fdd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	0.546	0.546	0.552
2	58	4	0.557	0.557	0.552
3	123	4	0.538	0.538	0.547
4	247	4	0.556	0.556	0.547
5	**520**	**4**	**0.522**	**0.522**	**0.522**

Development Rate Female (Day 28)
File: 5823fdd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.552				
58	0.552	0.318		1.75	k= 1, v=15
123	0.547	0.029		1.84	k= 2, v=15
247	0.547	0.029		1.87	k= 3, v=15
520	**0.522**	**1.371**		**1.88**	**k= 4, v=15**

s = 0.024
Note: df used for table values are approximate when v > 20.

Development Rate Male and Female Comb. (Day 28)

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File: 5823mfd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.0015	0.0004	0.800
Within (Error)	15	0.0079	0.0005	
Total	19	0.0094		

Critical F value = 3.06 (0.05,4,15)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Development Rate Male and Female Comb. (Day 28)
File: 5823mfd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.581	0.581		
2	58	0.572	0.572	0.617	
3	123	0.570	0.570	0.712	
4	247	0.579	0.579	0.126	
5	520	0.557	0.557	1.565	

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

Development Rate Male and Female Comb. (Day 28)
File: 5823mfd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	58	4	0.037	6.4	0.010
3	123	4	0.037	6.4	0.011
4	247	4	0.037	6.4	0.002
5	520	4	0.037	6.4	0.025

Development Rate Male and Female Comb. (Day 28)
File: 5823mfd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

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1	neg control	4	0.581	0.581	0.581
2	58	4	0.572	0.572	0.574
3	123	4	0.570	0.570	0.574
4	247	4	0.579	0.579	0.574
5	**520**	**4**	**0.557**	**0.557**	**0.557**

Development Rate Male and Female Comb. (Day 28)
File: 5823mfd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.581				
58	0.574	0.471		1.75	k= 1, v=15
123	0.574	0.471		1.84	k= 2, v=15
247	0.574	0.471		1.87	k= 3, v=15
520	**0.557**	**1.520**		**1.88**	**k= 4, v=15**

s = 0.023
Note: df used for table values are approximate when v > 20.

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DATA EVALUATION RECORD
SEEDLING EMERGENCE EC_{25} TEST
§123-1(a) (TIER II) PMRA DACO: 9.8.4.2

1. **CHEMICAL:** Aminopyralid — PC Code No.: 005100

2. **TEST MATERIAL:** XDE-750 as the GF-871 (formulation) — Purity: 40.6%

3. **CITATION:**

Author: Aufderheide, J.

Title: Effect of GF-871 on Seedling Emergence and Growth of Selected Non-Target Terrestrial Plants (Tier II)

Study Completion Date: January 21, 2004

Laboratory: ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Sponsor: Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268

Laboratory Report ID: 48322

MRID No.: 462358-24 PMRA Submission#: 2004-0790

DP Barcode: D301682

4. **REVIEWED BY:** John Marton, Staff Scientist, Dynamac Corporation **Date:** 8/17/04

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation **Date:** 10/6/04

5. **APPROVED BY:** Brian D. Kiernan, Biologist, OPP/EFED/ERBIV **Date:** 12/08/2004

Signature: 6/16/05

Monika Engel PMRA-EAD **Date:** February 7, 2005

Signature:

2030245

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: Dicots: *Cucumis sativus, Lactuca sativa, Brassica napus, Raphanus sativus, Glycine max, and Beta vulgaris altissima*
Monocots: *Echinochloa spec, Zea mays, Allium cepa, and Triticum aestivum*

Definitive Study Duration: 22 days

Type of Concentrations: Nominal

7. CONCLUSIONS:

Seedling emergence was studied ten non-target crop species after pre-emergent application of XDE-750 as the GF-871 formulation (Aminopyralid). The ten species tested were cucumber, lettuce, oilseed rape, radish, soybean, sugar beet, barnyard grass, corn, onion, and wheat. Cucumber, soybean, and sugar beet were tested at nominal rates of 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.8, 3.61, 7.21, 14.4, 28.9, and 57.7 g a.i./ha. Rape and radish were tested at rates of 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.8, 3.61, 7.21, 14.4, 28.9, 57.7, and 230.8 g a.i./ha. Lettuce was tested at rates of 0.11, 0.23, 0.45, 0.90, 1.8, 3.61, 7.21, 14.4, 28.9, 57.7, and 230.8 g a.i./ha. Onion was tested at rates of 0.23, 0.45, 0.90, 1.8, 3.61, 7.21, 14.4, 28.9, 57.7, and 230.8 g a.i./ha. Barnyard grass, corn, and wheat were tested at rates of 3.61, 7.21, 14.4, 28.9, 57.7, and 230.8 g a.i./ha.

The most sensitive species was soybean, a dicot, with an EC_{25} of 2.7 g a.i./ha (0.002 lb a.i./A) based on fresh shoot weight; the NOEC for soybean fresh weight was 0.9 g a.i./ha (0.008 lb a.i./A). The most sensitive monocot was onion, based on fresh shoot weight, with an EC_{25} of 29 g a.i./ha (0.026 lb a.i./A); the EC_{05} for onion fresh weight was 13 g a.i./ha (0.01 lb a.i./A). Due to statistical variation, the derived NOEC for onion was below the calculated EC_{25} and per Agency guidance the EC_{05} is used for risk assessments. Note that units are active ingredient, not acid equivalents.

This study is classified as Supplemental. This study is scientifically sound, but it does not fulfill the guideline requirements for a seedling emergence study (Subdivision J, §123-1a (TIER II)) because soil surface watering occurred without report of test substance mobility characteristics and Thiram was applied to sugar beet without further explanation.

EAD Conclusion:

The EAD is in agreement with the conclusions reported by the study author and the EPA reviewer. The most sensitive dicot was soybean with an EC_{25} of 2.7 g a.i./ha and a NOEC of 0.9 g a.i./ha. based on fresh shoot weight. The most sensitive monocot was onion with an EC_{25} of 29 g a.i./ha. Due to statistical variation, the derived NOEC for onion was below

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the calculated EC_{25}, thus the EC_{05} is 13 g a.i./ha based on fresh shoot weight.

Most sensitive dicot: Soybean
Most sensitive parameter: Fresh weight
NOEC: 0.9 g a.i./ha (0.0008 lb a.i./A)
EC_{05}: 0.91 g a.i./ha (0.0008 lb a.i./A) 95% C.I.: 0.59-1.4 g a.i./ha (0.0005-0.001 lb a.i./A)
EC_{25}: 2.7 g a.i./ha (0.002 lb a.i./A) 95% C.I.: 2.0-3.6 g a.i./ha (0.002-0.003 lb a.i./A)
Slope: 2.08±0.159

Most sensitive monocot: Onion
Most sensitive parameter: Fresh weight
NOEC: $>EC_{25}$
EC_{05}: 13 g a.i./ha (0.011 lb a.i./A) 95% C.I.: 4.3-37 g a.i./ha (0.004-0.03 lb a.i./A)
EC_{25}: 29 g a.i./ha (0.026 lb a.i./A) 95% C.I.: 16-54 g a.i./ha (0.014-0.05 lb a.i./A)
Slope: 2.62±0.768

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This study is scientifically sound but does not fulfill the guideline requirements for a seedling emergence study (Subdivision J, §123-1 (TIER II)) because of failure to provide information on the solubility, volatility, and Kd value of the test material to determine what effect (if any) soil-surface watering had on the mobility of the test material during the study, as well as failure to provide an explanation as to why sugar beet was treated with Thiram.

C. Repairability: The information should be provided regarding the test mobility characteristics, as well as an explanation regarding the use of Thiram on sugarbeet.

9. GUIDELINE DEVIATIONS:

Sugar beet was treated with the fungicide Thiram and no explanation was provided as to why this was deemed necessary. The seeds and seedlings were top-watered daily during the first four days and some species received a minimal amount of top-watering once or twice more during the study. No details were provided regarding the mobility of the test substance to determine the effect (if any) of top-watering.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the phytotoxicity to non-target crop species after pre-emergent application of Aminopyralid for the purpose of chemical registration.

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11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	Dicots: cucumber, oilseed rape, radish, soybean sugar beet, and lettuce Monocots: corn, barnyard grass, onion and wheat
Number of plants per repetition:	Cucumber, Oilseed rape, Radish, Soybean, Sugar Beet, and Corn: 40 seeds/rep, 5 seeds/pot, 2 pots/rep, 4 reps/treatment level Barnyard Grass, Onion, and Wheat: 30 seeds/rep, 5 seeds/pot, 1 pot/rep, 6 reps/treatment level Corn: 36 seeds/rep, 3 seeds/pot, 2 pots/rep, 6 reps/treatment level
Source of seed and historical % germination of seed:	See Table 1 p. 21 for seed source information and historical % germination.

B. Test System

Guideline Criteria	Reported Information
Solvent:	80% non-ionic surfactant
Site of test:	Barnyard grass, corn, cucumber, soybean, radish: On-site Greenhouse 7. Lettuce, onion, oilseed rape, sugar beet, and wheat: On-site Greenhouse 8.

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Guideline Criteria	Reported Information
Planting method/type of pot:	The planting containers were round plastic pots (16.5 cm x 11.5 cm x 10 cm). Cucumber, corn, wheat and soybean were planted at approximately 20 mm. Radish, barnyard grass, and sugar beet were planted at approximately 13 mm. Oilseed rape, lettuce, and onion were planted at approximately 6 mm. The growth medium was silt loam soil with organic content of approximately 2.7% and an approximate pH of 7.0.
Method of application:	An overhead track sprayer was used for application.
Method of watering:	All pots were top-watered daily during the first four days of testing and with some species, a minimal amount of top watering was needed once or twice more during the testing. Pots were also sub-irrigated throughout the study.
Growth stage at application:	Soil surface

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x	2x
Doses: At least 5	0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.43, 28.85, 57.70, 115.8, and 230.8 g a.i./ha The application rate range was adjusted according to the expected sensitivity to the test material.
Controls: Negative and solvent	Negative control (deionized water)

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Guideline Criteria	Reported Information
Replicates per dose: At least 3	4 replicates
Test duration: 14 days	22 days
Were observations made at least weekly?	Yes
Maximum dosage rate:	The maximum dosage rate for the study was 230.8 g a.i./ha (nominal).

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was a NOEC observed for each species?	Yes
Phytotoxic observations:	Phytotoxic observations were reported as "visual injury," on a scale from 0-100%. Onion, soybean, sugar beet, lettuce and oilseed rape were the only species that experience substantial visual injury (>30%).
Were initial chemical concentrations measured? (Optional)	Yes. Initial concentrations were measured for the nominal application rates of 58.8, 118, and 235 g/ha; mean measured concentrations ranged from 102-103% of nominal.
Were adequate raw data included?	Replicate emergence, shoot height, and fresh shoot weight data were reported.

Results for the most sensitive parameter of each species

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Results Synopsis

Seedling Emergence

Crop	Day 21 Emergence		Shoot Length		Shoot Weight		Percent Survival		Most Sensitive Parameter
	NOEC	EC_{25}	NOEC	EC_{25}	NOEC	EC_{25}	NOEC	EC_{25}	
Barnyard Grass	230.8	>230.8	230.8	>230.8	230.8	>230.8	230.8	>230.8	None
Corn	>230.8	>230.8	230.8	>230.8	230.8	>230.8	230.8	>230.8	None
Onion	57.7	24.4	28.9	46.5	57.7	50.7	230.8	>230.8	Shoot Length
Wheat	230.8	>230.8	230.8	>230.8	230.8	>230.8	230.8	>230.8	None
Cucumber	57.7	>57.7	57.7	>57.7	57.7	>57.7	57.7	>57.7	None
Soybean	7.21	16.3	3.61	5.63	0.90	2.62	28.9	46.0	Shoot Weight
Sugar beet	57.7	>57.7	7.21	23.7	57.7	16.2	57.7	>57.7	Shoot Weight
Lettuce	57.7	76.4	28.9	36.8	28.9	23.8	28.9	37.2	Shoot Weight
Oilseed rape	230.8	>230.8	230.8	>230.8	57.7	>57.7	230.8	>230.8	Shoot Weight
Radish	230.8	>230.8	230.8	>230.8	230.8	>230.8	230.8	>230.8	None

ND = Not determined
* Units are g a.i./ha

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Morphological Observations (negative percent reductions indicate promoted growth)

Barnyard Grass:
The application rate range for barnyard grass included a negative control, 3.61, 7.21, 14.4, 28.9, 57.7, 115.8, and 230.8 g a.i./ha. The percent emergence for the control and treatment levels was 70, 58, 60, 45, 70, 73, 65, and 73% respectively. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 545, 593, 578, 582, 559, 548, 559, and 525 mm respectively, which indicated a -9, -6, -7, -3, 0, -3, and 4% inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 24.1, 23.3, 24.4, 19.3, 20.5, 22.4, 18.8, and 18.7 g, respectively, which indicated a -9, -14, 10, 4, -5, 12, and 13% inhibition for the respective treatment levels, when compared to the control. Only the highest treatment level (230.8 g a.i./ha) had a visual injury rating (8%).

Corn:
The application rate range for corn included a negative control, 3.61, 7.21, 14.4, 28.9, 57.7, 115.8, and 230.8 g a.i./ha. The percent emergence for the control and treatment levels was 100, 100, 100, 100, 100, 98, 98, and 100% respectively. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 973, 998, 999, 938, 956, 1040, 974, and 936 mm respectively, which indicated a -3, -3, 4, 2, -7, 0, and 4% inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 226, 240, 227, 226, 219, 239, 229, and 215 g, respectively, which indicated a -6, -1, 0, 3, -6, -1, and 5% inhibition for the respective treatment levels, when compared to the control. No visual injury was observed for the control or any of the treatment levels.

Onion:
The application rate range for onion included a negative control, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, 57.7, 115.8, and 230.8 g a.i./ha. The percent emergence for the control and treatment levels was 80, 93, 85, 73, 78, 75, 88, 55, 68, 60, 13, and 5%, respectively. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 973, 998, 999, 938, 956, 1040, 974, and 936 mm respectively, which indicated a 0, 8, 5, 8, -2, 8, -5, 16, 25, 27, and 53% inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 226, 240, 227, 226, 219, 239, 229, and 215 g, respectively, which indicated a -76, -48, -19, -28, -41, -45, 3, -3, 26, 86, and 98% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 8, 0, 0, 3, 0, 3, 3, 18, 13, 33, 90, and 98% respectively.

Wheat:

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The application rate range for wheat included a negative control, 3.61, 7.21, 14.4, 28.9, 57.7, 115.8, and 230.8 g a.i./ha. The percent emergence for the control and treatment levels was 100, 100, 98, 98, 93, 93, 98, and 93% respectively. The percent survival was 100% for the control and all treatment levels except for the 14.4 g a.i./ha treatment level which had a survival percent of 97%. The mean shoot length for the control and treatment levels was 343, 332, 345, 351, 356, 356, 362, and 364 mm respectively, which indicated a 3, 0, -2, -4, -4, -6, and -6%inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 10.4, 9.76, 10.1, 10.5, 11.2, 10.4, 11.2, and 11.0 g, respectively, which indicated a 6, 3, -1, -8, 0, -8, and -6%inhibition for the respective treatment levels, when compared to the control. Only the highest treatment level (230.8 g a.i./ha) had a visual injury rating (3%).

Cucumber:
The application rate range for cucumber included a negative control, 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, and 57.7 g a.i./ha. The percent emergence for the control and treatment levels was 98, 95, 95, 90, 93, 85, 95, 80, 85, 98, 88, 83, and 88% respectively. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 233, 240, 209, 208, 233, 228, 221, 194, 211, 195, 233, 257, and 287 mm respectively, which indicated a -3, 10, 10, 0, 2, 17, 9, 16, 0, -10, and -23% inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 179, 183, 174, 171, 177, 179, 171, 160, 174, 175, 174, 172, and 151 g, respectively, which indicated a -2, 3, 4, 1, 0, 4, 11, 3, 2, 3, 4, and 15% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 0, 0, 0, 0, 0, 5, 5, 0, 0, 8, 10, and 20% respectively.

Soybean:
The application rate range for soybean included a negative control, 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, and 57.7 g a.i./ha. The percent emergence for the control and treatment levels was 85, 93, 90, 98, 95, 98, 95, 88, 95, 98, 70, 65, and 30% respectively. The percent survival for the control and treatment levels was 100, 100, 100, 100, 100, 100, 100, 100, 97, 100, 92, 83, and 74% respectively. The mean shoot length for the control and treatment levels was 237, 246, 236, 233, 238, 236, 235, 231, 223, 149, 88, 45, and 44 mm respectively, which indicated a -4, 1, 2, 0, 0, 1, 3, 6, 37, 63, 81, and 82% inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 68.2, 67.7, 67.6, 68.3, 67.9, 69.6, 66.3, 49.5, 48.2, 33.2, 9.11, 5.13, and 1.65 g, respectively, which indicated a 1, 1, 0, 0, -2, 3, 27, 29, 51, 87, 92, and 98 % inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 3, 0, 0, 3, 0, 10, 20, 25, 40, 55, 65, and 83% respectively.

Sugar beet:
The application rate range for sugar beet included a negative control, 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, and 57.7 g a.i./ha. The percent emergence for the control and treatment levels was 65, 83, 70, 70, 85, 83, 70, 75, 80, 80, 78, 75, and 75% respectively. The percent survival for the control and treatment levels was 92, 100, 100, 100, 92, 100, 97, 100, 100, 100, 97, 100, and 94% respectively. The mean shoot length for the control and treatment levels was 146, 152, 152, 145, 150, 146, 149, 139, 149, 144, 119, 100, and 70 mm respectively, which indicated a -4, -4, 0, -3, 0, -2, 5, -2, 2, 18, 31, and 52%inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 23.6, 34.0, 29.5, 28.1, 30.3, 29.2, 27.4, 20.0, 30.5, 27.8, 19.5, 12.3, and 5.25 g, respectively, which indicated a -44, -25, -19, -28, -24, -16, 15, -29, -17, 17, 48, and 78 % inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 0, 0, 0, 0, 0, 3, 0, 10, 18, 35, 50, and 70% respectively.

Lettuce:
The application rate range for lettuce included a negative control, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, 57.7, and 230.8 g a.i./ha. The percent emergence for the control and treatment levels was 80, 83, 80, 95, 95, 83, 75, 90, 95, 80, 73, and 25% respectively. The percent survival for the control and treatment levels was 97, 100, 100, 100, 100, 100, 100, 100, 100, 90, 35, and 0% respectively. The mean shoot length for the control and treatment levels was 51, 52, 50, 52, 52, 50, 51, 52, 55, 44, 40, and 0 mm respectively, which indicated a -2, 2, -2, -2, 2, 0, -2, -7, 14, 22, and 100% inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 3.00, 3.33, 3.23, 4.32, 3.88, 3.01, 2.86, 3.32, 3.21, 1.71, 0.517, and 0 g, respectively, which indicated a -11, -8, -44, -29, 0, 5, -11, -7, 43, 83, and 100 % inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 0, 0, 0, 0, 0, 0, 0, 5, 35, 88, 100% respectively.

Oilseed rape:
The application rate range for oilseed rape included a negative control, 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, 57.7, and 230.8 g a.i./ha. The percent emergence for the control and treatment levels was 78, 90, 83, 90, 80, 85, 85, 78, 85, 78, 93, 83, 85, and 75% respectively. The percent survival for the control and treatment levels was 100, 98, 98, 97, 96, 98, 97, 100, 97, 100, 97, 100, 95, and 100% respectively. The mean shoot length for the control and treatment levels was 223, 197, 225, 209, 198, 206, 203, 211, 216, 209, 194, 222, 225, and 179 mm respectively, which indicated a 12, -1, 7, 12, 8, 9, 6, 3, 7, 13, 0, -1, and 20% inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 3.00, 3.33, 3.23, 4.32, 3.88, 3.01, 2.86, 3.32, 3.21, 1.71, 0.517, and 0 g,

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respectively, which indicated a -11, -8, -44, -29, 0, 5, -11, -7, 43, 83, and 100 % inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 5, 0, 0, 8, 0, 0, 3, 0, 0, 13, 25, 28, and 43% respectively.

Radish:
The application rate range for radish included a negative control, 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, 57.7, and 230.8 g a.i./ha. The percent emergence for the control and treatment levels was 100, 100, 98, 98, 100, 100, 98, 100, 100, 98, 100, 100, 95, and 100% respectively. The percent survival was 100% for both the control and treatment levels. The mean shoot length for the control and treatment levels was 158, 147, 152, 155, 153, 134, 151, 150, 152, 145, 147, 154, 152, and 153 mm respectively, which indicated a 7, 4, 2, 3, 15, 5, 5, 4, 8, 7, 3, 4, and 3% inhibition for the respective treatment levels, when compared to the control. The mean shoot weight for the control and the treatment levels was 48.6, 40.8, 46.0, 44.8, 44.5, 35.8, 43.8, 42.6, 45.0, 41.0, 41.1, 43.6, 40.4, and 42.0 g, respectively, which indicated a 16, 5, 8, 8, 26, 10, 12, 7, 16, 15, 10, 17, and 13 % inhibition for the respective treatment levels, when compared to the control. No visual injury was observed except for the two highest treatment levels of 57.7 and 230.8 g a.i./ha which had visual injury ratings of 8 and 15% respectively.

Statistical Results

Statistical Method: The means and standard deviations were calculated for the percent emergence, phytotoxicity ratings, shoot length, and dry weight data. Statistical analysis of the concentration versus effect data was performed using SAS for Windows or Minitab software.

Most sensitive monocot: Onion
Most sensitive parameter: Emergence
NOEC: 57.7 g a.i./ha
EC_{25}: 24.4 g a.i./ha 95% C.I.: 14.4-39.7 g a.i./ha
EC_{50}: 57.0 g a.i./ha 95% C.I.: 34.9-107 g a.i./ha
Slope: Not reported

Most sensitive dicot: Soybean
Most sensitive parameter: Shoot Weight
NOEC: 0.90 g a.i./ha
EC_{25}: 2.62 g a.i./ha 95% C.I.: 1.98-3.31 g a.i./ha

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EC_{50}: 5.74 g a.i./ha 95% C.I.: 4.74-6.70 g a.i./ha

13. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Seedling emergence, shoot length, and fresh weight data were analyzed to determine if they satisfied the assumptions of ANOVA (i.e., normal distribution and homogeneity of variances) for all species exhibiting at least a 5% reduction in response. If they did, the NOEC was determined using ANOVA, followed by Bonferroni's t-test (unequal replicates, non-monotonic response), Dunnett's test (equal replicates, non-monotonic response), or William's test (monotonic response). If the data did not meet these assumptions, transformations (e.g., square-root, inverse square-root, or natural log) were attempted. If these transformations were successful, the NOEC was determined using a method described above. If the transformations were not successful, the NOEC was determined using the non-parametric Kruskal-Wallis test. These analyses were conducted using TOXSTAT statistical software. The EC_{05} and EC_{25} values and their 95% confidence intervals and slopes were determined using the Probit method via Nuthatch statistical software.

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Results synopsis

Crop	Emergence*			Shoot Length			Fresh Weight			Most Sensitive Parameter
	NOEC	EC_{05}	EC_{25}	NOEC	EC_{05}	EC_{25}	NOEC	EC_{05}	EC_{25}	
Barnyard Grass	230.8	ND	>230.8	230.8	>230.8	>230.8	230.8	30	>230.8	None
Corn	230.8	>230.8	>230.8	230.8	>230.8	>230.8	230.8	>230.8	>230.8	None
Onion	57.7	24	46[b]	28.9	16	93[b]	57.7	**13**	**29**[a]	Fresh Weight
Wheat	230.8	44	>230.8	230.8	>230.8	>230.8	230.8	ND	>230.8	None
Cucumber	>57.7	0.014	>57.7	57.7	ND	>57.7	57.7	41	>57.7	None
Soybean	7.21	5.8	17[b]	3.61	1.1	4.4[a]	**0.9**	0.91	**2.7**[b]	Fresh Weight
Sugar Beet	57.7	>57.7	>57.7	7.21	6.0	21[a]	14.43[a]	5.7	14[a]	Fresh Weight
Lettuce	57.7	31	76	>57.7[b]	22	60[b]	14.43[a]	11	20[a]	Fresh Weight
Oilseed Rape	230.8	>230.8	>230.8	230.8	0.0008	>230.8	57.7	4.9	49[a]	Fresh Weight
Radish	230.8	>230.8	>230.8	230.8	ND	>230.8	230.8	$8.0e^{-8}$	>230.8	None

[a] The revicwer's estimate was lower than the study authors'.
[b] The reviewer's estimate was higher than the study authors'.
*units are g a.i./ha
ND=The EC_x value could not be determined using the Probit method.
Values in bold are the most sensitive endpoints for risk assessment.

EC_x values, confidence intervals, and slopes

Species	Emergence*					Shoot Length*				
	EC_{05}	Confidence Interval	EC_{25}	Confidence Interval	Slope	EC_{05}	Confidence Interval	EC_{25}	Confidence Interval	Slope
Barnyard Grass	ND	N/A	>230.8	N/A	N/A	>230.8	N/A	>230.8	N/A	N/A
Corn	>230.8	N/A	>230.8	N/A	N/A	>230.8	N/A	>230.8	N/A	N/A
Onion	24	12-46	46[b]	30-70	3.40±0.676	16	1.6-160	93[b]	40-220	1.25±0.787
Wheat	44	0.28-7100	>230.8	N/A	0.288±0.253	>230.8	N/A	>230.8	N/A	N/A
Cucumber	0.014	$4.0e^{-9}$-$5.1e^{4}$	>57.7	N/A	0.140±0.102	ND	N/A	>57.7	N/A	N/A
Soybean	5.8	2.7-13	17[b]	11-25	2.12±0.416	1.1	0.64-2.0	4.4[a]	3.1-6.1	1.64±0.145
Sugar Beet	>57.7	N/A	>57.7	N/A	N/A	6.0	3.8-9.6	21[a]	18-26	1.75±0.202
Lettuce	31	14-68	76	49-120	2.52±0.516	22	7.6-64	60[b]	35-100	2.21±1.34
Oilseed Rape	>230.8	N/A	>230.8	N/A	N/A	0.0008	$1.1e^{26}$-$5.8e^{19}$	>230.8	N/A	0.0525±0.10
Radish	>230.8	N/A	>230.8	N/A	N/A	ND	N/A	>230.8	N/A	N/A

[a]The reviewer's estimate was lower than the study authors'.
[b] The reviewer's estimate was higher than the study authors'.
*units are g a.i./ha
ND=The EC_x value could not be determined using the Probit method.

Species	Fresh Weight*				
	EC_{05}	Confidence Interval	EC_{25}	Confidence Interval	Slope
Barnyard Grass	30	0.14-6.6e[3]	>230.8	N/A	0.822±0.984
Corn	>230.8	N/A	>230.8	N/A	N/A
Onion	13	4.3-37	29[a]	16-54	2.62±0.768
Wheat	ND	N/A	>230.8	N/A	N/A
Cucumber	41	18-91	>57.7	N/A	3.53±3.96
Soybean	0.91	0.59-1.4	2.7[b]	2.0-3.6	2.08±0.159
Sugar Beet	5.7	2.7-12	14[a]	8.9-21	2.55±0.493
Lettuce	11	6.0-22	20[a]	14-30	3.87±1.01
Oilseed Rape	4.9	0.35-68	49[a]	16-150	0.972±0.372
Radish	8.0e[-8]	1.4e[-30]-4.7e[15]	>230.8	N/A	0.0821±0.1

[a]The reviewer's estimate was lower than the study authors'.
[b] The reviewer's estimate was higher than the study authors'.
*units are g a.i./ha
ND=The EC_x value could not be determined using the Probit method.

Most sensitive dicot: Soybean
Most sensitive parameter: Fresh weight
NOEC: 0.9 g a.i./ha (0.008 lb a.i./A)
EC_{05}: 0.91 g a.i./ha (0.008 lb a.i./A) 95% C.I.: 0.59-1.4 g a.i./ha (0.0005-0.001 lb a.i./A)
EC_{25}: 2.7 g a.i./ha (0.002 lb a.i./A) 95% C.I.: 2.0-3.6 g a.i./ha (0.002-0.003 lb a.i./A)
Slope: 2.08±0.159

Most sensitive monocot: Onion
Most sensitive parameter: Fresh weight
EC_{05}: 13 g a.i./ha (0.01 lb a.i./A) 95% C.I.: 4.3-37 g a.i./ha (0.004-0.03 lb a.i./A)
EC_{25}: 29 g a.i./ha (0.026 lb a.i./A) 95% C.I.: 16-54 g a.i./ha (0.014-0.05 lb a.i./A)
Slope: 2.62±0.768

14. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the most sensitive species (soybean, a dicot and onion, a monocot) were similar to the study author's; however, the reviewer's analysis determined that onion fresh weight was more sensitive than onion emergence. Differences between the reviewer's and the study authors' estimates can be attributed to the different statistical methods which were used to derive these estimates. Because the reviewer's analysis provided EC_{05} values and slopes for all estimates, the reviewer's values are reported in the Conclusions section. The reviewer has also provided the toxicity values for the most sensitive monocot and dicot species in units of lb a.i./A.

The definitive study was conducted from July 2 to July 24, 2003. The average temperatures for Greenhouse 7 ranged from 15.2 to 35.3°C and the relative humidity % ranged from 42 to 94%. The average temperatures for Greenhouse 8 ranged from 16.0 to 36.6°C and the relative humidity ranged from 34 to 93%. Natural sunlight was the only source of light during the treatment exposures, and ranged from 347-879 $\mu Em^{-2}s^{-1}$.

This study was conducted in accordance with USEPA Good Laboratory Practice Regulations (Title 40, Part 160) and included a Quality Assurance statement.

EAD Comments:
After review of the study data and the US EPA DER, the reviewer is in agreement with the conclusion reached by the US EPA, with the recommendation that the results for the sugar beet be omitted due to possible interference from Thiram use.

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15. REFERENCES:

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APPENDIX I. OUTPUT FROM REVIEWER'S STATISTICAL VERIFICATION:

barnyard grass emergence
File: 5824ge Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	25.469	3.638	1.888
Within (Error)	24	46.250	1.927	
Total	31	71.719		

Critical F value = 2.42 (0.05,7,24)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

barnyard grass emergence
File: 5824ge Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	7.000	7.000		
2	3.61	5.750	5.750	1.273	
3	7.21	6.000	6.000	1.019	
4	14.43	4.500	4.500	2.547	*
5	28.9	7.000	7.000	0.000	
6	57.7	7.250	7.250	-0.255	
7	115.4	6.500	6.500	0.509	
8	230.8	7.250	7.250	-0.255	

Dunnett table value = 2.48 (1 Tailed Value, P=0.05, df=24,7)

barnyard grass emergence
File: 5824ge Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	3.61	4	2.434	34.8	1.250
3	7.21	4	2.434	34.8	1.000
4	14.43	4	2.434	34.8	2.500
5	28.9	4	2.434	34.8	0.000
6	57.7	4	2.434	34.8	-0.250
7	115.4	4	2.434	34.8	0.500
8	230.8	4	2.434	34.8	-0.250

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barnyard grass emergence
File: 5824ge Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	7.000	7.000	5.813
2	3.61	4	5.750	5.750	5.813
3	7.21	4	6.000	6.000	5.813
4	14.43	4	4.500	4.500	5.813
5	28.9	4	7.000	7.000	6.917
6	57.7	4	7.250	7.250	6.917
7	115.4	4	6.500	6.500	6.917
8	230.8	4	7.250	7.250	7.250

barnyard grass emergence
File: 5824ge Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	5.813				
3.61	5.813	1.210		1.71	k= 1, v=24
7.21	5.813	1.210		1.79	k= 2, v=24
14.43	5.813	1.210		1.82	k= 3, v=24
28.9	6.917	0.085		1.83	k= 4, v=24
57.7	6.917	0.085		1.84	k= 5, v=24
115.4	6.917	0.085		1.84	k= 6, v=24
230.8	7.250	0.255		1.85	k= 7, v=24

s = 1.388
Note: df used for table values are approximate when v > 20.

ECx

!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.
onion emergence
File: 5824ie Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	49.000	5.444	2.016
Within (Error)	30	81.000	2.700	
Total	39	130.000		

Critical F value = 2.21 (0.05,9,30)

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Since F < Critical F FAIL TO REJECT Ho:All groups equal

onion emergence
File: 5824ie Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	8.000	8.000		
2	0.23	9.250	9.250	-1.076	
3	0.45	8.500	8.500	-0.430	
4	0.9	7.250	7.250	0.645	
5	1.8	7.750	7.750	0.215	
6	3.61	7.500	7.500	0.430	
7	7.21	8.500	8.500	-0.430	
8	14.43	5.500	5.500	2.152	
9	28.9	6.750	6.750	1.076	
10	57.7	6.000	6.000	1.721	

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

onion emergence
File: 5824ie Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	2.951	36.9	-1.250
3	0.45	4	2.951	36.9	-0.500
4	0.9	4	2.951	36.9	0.750
5	1.8	4	2.951	36.9	0.250
6	3.61	4	2.951	36.9	0.500
7	7.21	4	2.951	36.9	-0.500
8	14.43	4	2.951	36.9	2.500
9	28.9	4	2.951	36.9	1.250
10	57.7	4	2.951	36.9	2.000

onion emergence
File: 5824ie Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	8.000	8.000	8.625
2	0.23	4	9.250	9.250	8.625
3	0.45	4	8.500	8.500	8.500
4	0.9	4	7.250	7.250	7.750
5	1.8	4	7.750	7.750	7.750

6	3.61	4	7.500	7.500	7.750
7	7.21	4	8.500	8.500	7.750
8	14.43	4	5.500	5.500	6.125
9	28.9	4	6.750	6.750	6.125
10	57.7	4	6.000	6.000	6.000

onion emergence
File: 5824ie Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	8.625				
0.23	8.625	0.538		1.70	k= 1, v=30
0.45	8.500	0.430		1.78	k= 2, v=30
0.9	7.750	0.215		1.80	k= 3, v=30
1.8	7.750	0.215		1.81	k= 4, v=30
3.61	7.750	0.215		1.82	k= 5, v=30
7.21	7.750	0.215		1.83	k= 6, v=30
14.43	6.125	1.614		1.83	k= 7, v=30
28.9	6.125	1.614		1.83	k= 8, v=30
57.7	6.000	1.721		1.83	k= 9, v=30

s = 1.643
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	24.	12.	46.	0.14	0.51
EC10	30.	17.	54.	0.12	0.56
EC25	46.	30.	70.	0.092	0.65
EC50	72.	54.	96.	0.062	0.75

Slope = 3.40 Std.Err. = 0.676

Goodness of fit: p = 0.32 based on DF= 9.0 36.

5824IE : onion emergence

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	8.00	7.81	0.188	100.	0.00
0.230	4.00	9.25	7.81	1.44	100.	2.27e-14
0.450	4.00	8.50	7.81	0.688	100.	3.08e-12
0.900	4.00	7.25	7.81	-0.562	100.	4.57e-09
1.80	4.00	7.75	7.81	-0.0623	100.	2.42e-06
3.61	4.00	7.50	7.81	-0.312	100.	0.000474
7.21	4.00	8.50	7.81	0.690	100.	0.0328
14.4	4.00	5.50	7.75	-2.25	99.1	0.860
28.9	4.00	6.75	7.13	-0.379	91.3	8.74
57.7	4.00	6.00	4.93	1.07	63.1	36.9

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115.	4.00	1.25	1.92	-0.670	24.6	75.4
231.	4.00	0.500	0.340	0.160	4.35	95.7

wheat emergence
File: 5824we Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	10.000	10.000	86.000
2	3.61	10.000	10.000	86.000
3	7.21	9.750	9.750	71.000
4	14.43	9.750	9.750	71.000
5	28.9	9.250	9.250	41.000
6	57.7	9.250	9.250	51.000
7	115.4	9.750	9.750	71.000
8	230.8	9.250	9.250	51.000

Calculated H Value = 8.396 Critical H Value Table = 14.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

wheat emergence
File: 5824we Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 5	0 6	0 8	0 4	0 7	0 3	0 1	0 2
5	28.9	9.250	9.250	\							
6	57.7	9.250	9.250	.	\						
8	230.8	9.250	9.250	.	.	\					
4	14.43	9.750	9.750	.	.	.	\				
7	115.4	9.750	9.750	.	.	.	.	\			
3	7.21	9.750	9.750	.	.	.	.	.	\		
1	control	10.000	10.000	.	.	.	.	.	.	\	
2	3.61	10.000	10.000	.	.	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,8) = 3.124 SE = 5.388
Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	44.	0.28	7.1E+03	1.1	0.0062
EC10	8.1E+02	9.7	6.7E+04	0.94	0.012
EC25	1.0E+05	1.2	9.1E+09	2.4	1.1E-05
EC50	2.2E+07	0.022	2.2E+16	4.4	1.0E-09

Slope = 0.288 Std.Err. = 0.253

Goodness of fit: p = 0.56 based on DF= 5.0 24.

5824WE : wheat emergence

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Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	10.0	10.0	-0.0387	100.	0.00
3.61	4.00	10.0	9.79	0.213	97.5	2.50
7.21	4.00	9.75	9.73	0.0181	96.9	3.06
14.4	4.00	9.75	9.67	0.0835	96.3	3.71
28.9	4.00	9.25	9.59	-0.340	95.5	4.47
57.7	4.00	9.25	9.50	-0.252	94.7	5.35
115.	4.00	9.75	9.40	0.350	93.6	6.36
231.	4.00	9.25	9.28	-0.0341	92.5	7.52

!!!Warning: EC10 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

cucumber emergence
File: 5824ce Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	14.100	1.567	2.185
Within (Error)	30	21.500	0.717	
Total	39	35.600		

Critical F value = 2.21 (0.05,9,30)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

cucumber emergence
File: 5824ce Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	9.750	9.750		
2	0.23	9.250	9.250	0.835	
3	0.45	8.500	8.500	2.088	
4	0.9	9.500	9.500	0.418	
5	1.8	8.000	8.000	2.923	*
6	3.61	8.500	8.500	2.088	
7	7.21	9.750	9.750	0.000	
8	14.43	8.750	8.750	1.670	
9	28.9	8.250	8.250	2.505	
10	57.7	8.750	8.750	1.670	

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

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cucumber emergence
File: 5824ce Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	1.521	15.6	0.500
3	0.45	4	1.521	15.6	1.250
4	0.9	4	1.521	15.6	0.250
5	1.8	4	1.521	15.6	1.750
6	3.61	4	1.521	15.6	1.250
7	7.21	4	1.521	15.6	0.000
8	14.43	4	1.521	15.6	1.000
9	28.9	4	1.521	15.6	1.500
10	57.7	4	1.521	15.6	1.000

cucumber emergence
File: 5824ce Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	9.750	9.750	9.750
2	0.23	4	9.250	9.250	9.250
3	0.45	4	8.500	8.500	9.000
4	0.9	4	9.500	9.500	9.000
5	1.8	4	8.000	8.000	8.750
6	3.61	4	8.500	8.500	8.750
7	7.21	4	9.750	9.750	8.750
8	14.43	4	8.750	8.750	8.750
9	28.9	4	8.250	8.250	8.500
10	57.7	4	8.750	8.750	8.500

cucumber emergence
File: 5824ce Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	9.750				
0.23	9.250	0.835		1.70	k= 1, v=30
0.45	9.000	1.253		1.78	k= 2, v=30
0.9	9.000	1.253		1.80	k= 3, v=30
1.8	8.750	1.671		1.81	k= 4, v=30
3.61	8.750	1.671		1.82	k= 5, v=30
7.21	8.750	1.671		1.83	k= 6, v=30
14.43	8.750	1.671		1.83	k= 7, v=30

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28.9	8.500	2.088	*	1.83	k= 8, v=30
57.7	8.500	2.088	*	1.83	k= 9, v=30

s = 0.847
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.014	4.0E-09	5.1E+04	3.3	2.8E-07
EC10	5.7	0.00094	3.5E+04	1.9	0.00017
EC25	1.3E+05	0.31	5.3E+10	2.8	2.4E-06
EC50	8.7E+09	0.0070	1.1E+22	6.0	8.0E-13

Slope = 0.140 Std.Err. = 0.102

Goodness of fit: p = 0.13 based on DF= 10. 39.

5824CE : cucumber emergence

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	9.75	9.79	-0.0378	100.	0.00
0.0280	4.00	9.50	9.26	0.244	94.6	5.44
0.0560	4.00	9.50	9.21	0.291	94.1	5.92
0.110	4.00	9.00	9.16	-0.160	93.6	6.41
0.230	4.00	9.25	9.10	0.147	93.0	6.99
0.450	4.00	8.50	9.05	-0.548	92.4	7.56
0.900	4.00	9.50	8.99	0.512	91.8	8.17
1.80	4.00	8.00	8.92	-0.924	91.2	8.83
3.61	4.00	8.50	8.86	-0.356	90.5	9.52
7.21	4.00	9.75	8.78	0.965	89.7	10.3
14.4	4.00	8.75	8.71	0.0410	89.0	11.0
28.9	4.00	8.25	8.63	-0.379	88.2	11.8
57.7	4.00	8.75	8.55	0.204	87.3	12.7

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

soybean emergence
File: 5824se Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	191.100	21.233	10.272
Within (Error)	30	62.000	2.067	
Total	39	253.100		

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Critical F value = 2.21 (0.05,9,30)
Since F > Critical F REJECT Ho:All groups equal

soybean emergence
File: 5824se Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	9.500	9.500		
2	0.23	9.500	9.500	0.000	
3	0.45	9.750	9.750	-0.246	
4	0.9	9.500	9.500	0.000	
5	1.8	8.750	8.750	0.738	
6	3.61	9.500	9.500	0.000	
7	7.21	9.750	9.750	-0.246	
8	14.43	6.500	6.500	2.951	*
9	28.9	5.750	5.750	3.689	*
10	57.7	3.000	3.000	6.394	*

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

soybean emergence
File: 5824se Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	2.582	27.2	0.000
3	0.45	4	2.582	27.2	-0.250
4	0.9	4	2.582	27.2	0.000
5	1.8	4	2.582	27.2	0.750
6	3.61	4	2.582	27.2	0.000
7	7.21	4	2.582	27.2	-0.250
8	14.43	4	2.582	27.2	3.000
9	28.9	4	2.582	27.2	3.750
10	57.7	4	2.582	27.2	6.500

soybean emergence
File: 5824se Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	9.500	9.500	9.583
2	0.23	4	9.500	9.500	9.583
3	0.45	4	9.750	9.750	9.583
4	0.9	4	9.500	9.500	9.500

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5	1.8	4	8.750	8.750	9.333
6	3.61	4	9.500	9.500	9.333
7	7.21	4	9.750	9.750	9.333
8	14.43	4	6.500	6.500	6.500
9	28.9	4	5.750	5.750	5.750
10	57.7	4	3.000	3.000	3.000

soybean emergence
File: 5824se Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	9.583				
0.23	9.583	0.082		1.70	k= 1, v=30
0.45	9.583	0.082		1.78	k= 2, v=30
0.9	9.500	0.000		1.80	k= 3, v=30
1.8	9.333	0.164		1.81	k= 4, v=30
3.61	9.333	0.164		1.82	k= 5, v=30
7.21	9.333	0.164		1.83	k= 6, v=30
14.43	6.500	2.951	*	1.83	k= 7, v=30
28.9	5.750	3.689	*	1.83	k= 8, v=30
57.7	3.000	6.394	*	1.83	k= 9, v=30

s = 1.438
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	5.8	2.7	13.	0.17	0.46
EC10	8.7	4.6	16.	0.14	0.53
EC25	17.	11.	25.	0.087	0.67
EC50	35.	28.	44.	0.050	0.79

Slope = 2.12 Std.Err. = 0.416

Goodness of fit: p = 0.90 based on DF= 10. 39.

5824SE : soybean emergence

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	9.50	9.45	0.0512	100.	0.00
0.0280	4.00	9.25	9.45	-0.199	100.	2.74e-09
0.0560	4.00	9.00	9.45	-0.449	100.	1.61e-07
0.110	4.00	9.75	9.45	0.301	100.	5.82e-06
0.230	4.00	9.50	9.45	0.0512	100.	0.000191
0.450	4.00	9.75	9.45	0.301	100.	0.00312
0.900	4.00	9.50	9.45	0.0548	100.	0.0382
1.80	4.00	8.75	9.42	-0.669	99.7	0.318
3.61	4.00	9.50	9.28	0.225	98.2	1.84

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7.21	4.00	9.75	8.76	0.993	92.7	7.33
14.4	4.00	6.50	7.48	-0.985	79.2	20.8
28.9	4.00	5.75	5.38	0.369	56.9	43.1
57.7	4.00	3.00	3.05	-0.0466	32.2	67.8

lettuce emergence
File: 5824le Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	155.125	17.236	11.953
Within (Error)	30	43.250	1.442	
Total	39	198.375		

Critical F value = 2.21 (0.05,9,30)
Since F > Critical F REJECT Ho:All groups equal

lettuce emergence
File: 5824le Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	8.000	8.000		
2	0.45	9.500	9.500	-1.767	
3	0.9	9.500	9.500	-1.767	
4	1.8	8.250	8.250	-0.294	
5	3.61	7.500	7.500	0.589	
6	7.21	9.000	9.000	-1.178	
7	14.43	9.500	9.500	-1.767	
8	28.9	7.750	7.750	0.294	
9	57.7	7.250	7.250	0.883	
10	230.8	2.500	2.500	6.477	*

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

lettuce emergence
File: 5824le Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.45	4	2.157	27.0	-1.500
3	0.9	4	2.157	27.0	-1.500
4	1.8	4	2.157	27.0	-0.250
5	3.61	4	2.157	27.0	0.500
6	7.21	4	2.157	27.0	-1.000

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7	14.43	4	2.157	27.0	-1.500
8	28.9	4	2.157	27.0	0.250
9	57.7	4	2.157	27.0	0.750
10	230.8	4	2.157	27.0	5.500

lettuce emergence
File: 5824le Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	8.000	8.000	9.000
2	0.45	4	9.500	9.500	9.000
3	0.9	4	9.500	9.500	9.000
4	1.8	4	8.250	8.250	8.563
5	3.61	4	7.500	7.500	8.563
6	7.21	4	9.000	9.000	8.563
7	14.43	4	9.500	9.500	8.563
8	28.9	4	7.750	7.750	7.750
9	57.7	4	7.250	7.250	7.250
10	230.8	4	2.500	2.500	2.500

lettuce emergence
File: 5824le Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	9.000				
0.45	9.000	1.178		1.70	k= 1, v=30
0.9	9.000	1.178		1.78	k= 2, v=30
1.8	8.563	0.663		1.80	k= 3, v=30
3.61	8.563	0.663		1.81	k= 4, v=30
7.21	8.563	0.663		1.82	k= 5, v=30
14.43	8.563	0.663		1.83	k= 6, v=30
28.9	7.750	0.294		1.83	k= 7, v=30
57.7	7.250	0.883		1.83	k= 8, v=30
230.8	2.500	6.478	*	1.83	k= 9, v=30

s = 1.201
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	31.	14.	68.	0.17	0.46
EC10	43.	23.	83.	0.14	0.52
EC25	76.	49.	1.2E+02	0.096	0.64
EC50	1.4E+02	1.1E+02	1.8E+02	0.056	0.77

Slope = 2.52 Std.Err. = 0.516

Goodness of fit: p = 0.33 based on DF= 9.0 36.

5824LE : lettuce emergence

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	8.00	8.58	-0.576	100.	0.00
0.110	4.00	8.25	8.58	-0.326	100.	2.28e-13
0.230	4.00	8.00	8.58	-0.576	100.	1.07e-10
0.450	4.00	9.50	8.58	0.924	100.	1.59e-08
0.900	4.00	9.50	8.58	0.924	100.	1.60e-06
1.80	4.00	8.25	8.58	-0.326	100.	9.18e-05
3.61	4.00	7.50	8.58	-1.08	100.	0.00306
7.21	4.00	9.00	8.57	0.429	99.9	0.0577
14.4	4.00	9.50	8.52	0.979	99.4	0.639
28.9	4.00	7.75	8.22	-0.467	95.8	4.19
57.7	4.00	7.25	7.15	0.0955	83.4	16.6
231.	4.00	2.50	2.50	-0.00275	29.2	70.8

onion se height
File: 5824il Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	4629.179	514.353	2.624
Within (Error)	30	5880.240	196.008	
Total	39	10509.419		

Critical F value = 2.21 (0.05,9,30)
Since F > Critical F REJECT Ho:All groups equal

onion se height
File: 5824il Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	126.100	126.100		
2	0.23	126.600	126.600	-0.051	
3	0.45	115.625	115.625	1.058	
4	0.90	119.400	119.400	0.677	
5	1.8	116.400	116.400	0.980	
6	3.61	128.650	128.650	-0.258	
7	7.21	115.475	115.475	1.073	
8	14.43	132.275	132.275	-0.624	
9	28.9	105.500	105.500	2.081	
10	57.7	95.025	95.025	3.139	*

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Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

onion se height
File: 5824il Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	25.145	19.9	-0.500
3	0.45	4	25.145	19.9	10.475
4	0.90	4	25.145	19.9	6.700
5	1.8	4	25.145	19.9	9.700
6	3.61	4	25.145	19.9	-2.550
7	7.21	4	25.145	19.9	10.625
8	14.43	4	25.145	19.9	-6.175
9	28.9	4	25.145	19.9	20.600
10	57.7	4	25.145	19.9	31.075

onion se height
File: 5824il Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	126.100	126.100	126.350
2	0.23	4	126.600	126.600	126.350
3	0.45	4	115.625	115.625	121.304
4	0.90	4	119.400	119.400	121.304
5	1.8	4	116.400	116.400	121.304
6	3.61	4	128.650	128.650	121.304
7	7.21	4	115.475	115.475	121.304
8	14.43	4	132.275	132.275	121.304
9	28.9	4	105.500	105.500	105.500
10	57.7	4	95.025	95.025	95.025

onion se height
File: 5824il Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	126.350				
0.23	126.350	0.025		1.70	k= 1, v=30
0.45	121.304	0.484		1.78	k= 2, v=30
0.90	121.304	0.484		1.80	k= 3, v=30
1.8	121.304	0.484		1.81	k= 4, v=30
3.61	121.304	0.484		1.82	k= 5, v=30

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7.21	121.304	0.484		1.83	k= 6, v=30
14.43	121.304	0.484		1.83	k= 7, v=30
28.9	105.500	2.081	*	1.83	k= 8, v=30
57.7	95.025	3.139	*	1.83	k= 9, v=30

s = 14.000
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	16.	1.6	1.6E+02	0.49	0.10
EC10	31.	6.6	1.4E+02	0.33	0.22
EC25	93.	40.	2.2E+02	0.18	0.43
EC50	3.2E+02	49.	2.1E+03	0.41	0.15

Slope = 1.25 Std.Err. = 0.787

Goodness of fit: p = 0.88 based on DF= 8.0 32.

5824IL : onion se height

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	126.	123.	3.44	100.	0.00
0.230	4.00	127.	123.	3.94	100.	0.00402
0.450	4.00	116.	123.	-7.02	100.	0.0173
0.900	4.00	119.	123.	-3.18	99.9	0.0686
1.80	4.00	116.	122.	-5.97	99.8	0.238
3.61	4.00	129.	122.	6.88	99.3	0.725
7.21	4.00	115.	120.	-4.82	98.1	1.93
14.4	4.00	132.	117.	15.2	95.5	4.55
28.9	4.00	106.	111.	-5.55	90.5	9.47
57.7	4.00	95.0	101.	-6.22	82.5	17.5
116.	3.00	91.7	87.2	4.43	71.1	28.9

!!!Warning: EC50 not bracketed by doses evaluated.

cucumber se length
File: 5824cl Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	9232.845	1025.872	1.045
Within (Error)	30	29459.302	981.977	
Total	39	38692.148		

Critical F value = 2.21 (0.05,9,30)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

275

cucumber se length
File: 5824cl Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	232.750	232.750		
2	0.028	240.050	240.050	-0.329	
3	0.56	209.000	209.000	1.072	
4	0.11	208.525	208.525	1.093	
5	0.23	232.975	232.975	-0.010	
6	0.45	227.850	227.850	0.221	
7	0.9	221.400	221.400	0.512	
8	1.8	194.200	194.200	1.740	
9	3.61	213.675	213.675	0.861	
10	7.21	195.250	195.250	1.692	

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

cucumber se length
File: 5824cl Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.028	4	56.282	24.2	-7.300
3	0.56	4	56.282	24.2	23.750
4	0.11	4	56.282	24.2	24.225
5	0.23	4	56.282	24.2	-0.225
6	0.45	4	56.282	24.2	4.900
7	0.9	4	56.282	24.2	11.350
8	1.8	4	56.282	24.2	38.550
9	3.61	4	56.282	24.2	19.075
10	7.21	4	56.282	24.2	37.500

cucumber se length
File: 5824cl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	232.750	232.750	236.400
2	0.028	4	240.050	240.050	236.400
3	0.56	4	209.000	209.000	219.950
4	0.11	4	208.525	208.525	219.950
5	0.23	4	232.975	232.975	219.950
6	0.45	4	227.850	227.850	219.950
7	0.9	4	221.400	221.400	219.950
8	1.8	4	194.200	194.200	203.938

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9	3.61	4	213.675	213.675	203.938
10	7.21	4	195.250	195.250	195.250

cucumber se length
File: 5824cl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	236.400				
0.028	236.400	0.165		1.70	k= 1, v=30
0.56	219.950	0.578		1.78	k= 2, v=30
0.11	219.950	0.578		1.80	k= 3, v=30
0.23	219.950	0.578		1.81	k= 4, v=30
0.45	219.950	0.578		1.82	k= 5, v=30
0.9	219.950	0.578		1.83	k= 6, v=30
1.8	203.938	1.300		1.83	k= 7, v=30
3.61	203.938	1.300		1.83	k= 8, v=30
7.21	195.250	1.692		1.83	k= 9, v=30

s = 31.337
Note: df used for table values are approximate when v > 20.

ECx
!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.

soybean se length
File: 5824sl Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	251680.619	27964.513	57.723
Within (Error)	30	14533.740	484.458	
Total	39	266214.359		

Critical F value = 2.21 (0.05,9,30)
Since F > Critical F REJECT Ho:All groups equal

soybean se length
File: 5824sl Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	237.375	237.375		

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2	0.23	238.200	238.200	-0.053	
3	0.45	236.325	236.325	0.067	
4	0.9	235.375	235.375	0.129	
5	1.8	231.175	231.175	0.398	
6	3.61	222.500	222.500	0.956	
7	7.21	148.875	148.875	5.686	*
8	14.43	88.175	88.175	9.586	*
9	28.9	45.425	45.425	12.333	*
10	57.7	43.625	43.625	12.449	*

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

soybean se length
File: 5824sl Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	39.532	16.7	-0.825
3	0.45	4	39.532	16.7	1.050
4	0.9	4	39.532	16.7	2.000
5	1.8	4	39.532	16.7	6.200
6	3.61	4	39.532	16.7	14.875
7	7.21	4	39.532	16.7	88.500
8	14.43	4	39.532	16.7	149.200
9	28.9	4	39.532	16.7	191.950
10	57.7	4	39.532	16.7	193.750

soybean se length
File: 5824sl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	237.375	237.375	237.788
2	0.23	4	238.200	238.200	237.788
3	0.45	4	236.325	236.325	236.325
4	0.9	4	235.375	235.375	235.375
5	1.8	4	231.175	231.175	231.175
6	3.61	4	222.500	222.500	222.500
7	7.21	4	148.875	148.875	148.875
8	14.43	4	88.175	88.175	88.175
9	28.9	4	45.425	45.425	45.425
10	57.7	4	43.625	43.625	43.625

soybean se length
File: 5824sl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

278

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	237.788				
0.23	237.788	0.027		1.70	k= 1, v=30
0.45	236.325	0.067		1.78	k= 2, v=30
0.9	235.375	0.129		1.80	k= 3, v=30
1.8	231.175	0.398		1.81	k= 4, v=30
3.61	222.500	0.956		1.82	k= 5, v=30
7.21	148.875	5.686	*	1.83	k= 6, v=30
14.43	88.175	9.586	*	1.83	k= 7, v=30
28.9	45.425	12.333	*	1.83	k= 8, v=30
57.7	43.625	12.449	*	1.83	k= 9, v=30

s = 22.010
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	1.1	0.64	2.0	0.12	0.57
EC10	1.9	1.2	3.0	0.10	0.62
EC25	4.4	3.1	6.1	0.074	0.71
EC50	11.	9.1	14.	0.046	0.81

Slope = 1.64 Std.Err. = 0.145

!!!Poor fit: p = 0.018 based on DF= 10. 39.

5824SL : soybean se length

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	237.	242.	-4.98	100.	0.00
0.0280	4.00	246.	242.	3.87	100.	0.000989
0.0560	4.00	236.	242.	-6.27	100.	0.00802
0.110	4.00	233.	242.	-9.54	100.	0.0494
0.230	4.00	238.	242.	-3.48	99.7	0.281
0.450	4.00	236.	240.	-3.38	98.9	1.10
0.900	4.00	235.	234.	1.74	96.4	3.60
1.80	4.00	231.	219.	12.0	90.4	9.58
3.61	4.00	223.	192.	30.7	79.1	20.9
7.21	4.00	149.	151.	-2.62	62.5	37.5
14.4	4.00	88.2	104.	-16.2	43.1	56.9
28.9	4.00	45.4	61.0	-15.6	25.2	74.8
57.7	4.00	43.6	29.8	13.8	12.3	87.7

sugarbeet se length
File: 5824ul Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	25993.999	2888.222	23.277

279

Within (Error)	30	3722.405	124.080
Total	39	29716.404	

Critical F value = 2.21 (0.05,9,30)
Since F > Critical F REJECT Ho:All groups equal

sugarbeet se length
File: 5824ul Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	145.750	145.750		
2	0.23	150.425	150.425	-0.594	
3	0.45	145.550	145.550	0.025	
4	0.9	149.025	149.025	-0.416	
5	1.8	139.150	139.150	0.838	
6	3.61	148.525	148.525	-0.352	
7	7.21	143.550	143.550	0.279	
8	14.43	119.275	119.275	3.361	*
9	28.9	100.375	100.375	5.761	*
10	57.7	69.675	69.675	9.658	*

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

sugarbeet se length
File: 5824ul Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	20.006	13.7	-4.675
3	0.45	4	20.006	13.7	0.200
4	0.9	4	20.006	13.7	-3.275
5	1.8	4	20.006	13.7	6.600
6	3.61	4	20.006	13.7	-2.775
7	7.21	4	20.006	13.7	2.200
8	14.43	4	20.006	13.7	26.475
9	28.9	4	20.006	13.7	45.375
10	57.7	4	20.006	13.7	76.075

sugarbeet se length
File: 5824ul Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED

280

	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	control	4	145.750	145.750	148.088
2	0.23	4	150.425	150.425	148.088
3	0.45	4	145.550	145.550	147.288
4	0.9	4	149.025	149.025	147.288
5	1.8	4	139.150	139.150	143.838
6	3.61	4	148.525	148.525	143.838
7	7.21	4	143.550	143.550	143.550
8	14.43	4	119.275	119.275	119.275
9	28.9	4	100.375	100.375	100.375
10	57.7	4	69.675	69.675	69.675

sugarbeet se length
File: 5824ul Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	148.088				
0.23	148.088	0.297		1.70	k= 1, v=30
0.45	147.288	0.195		1.78	k= 2, v=30
0.9	147.288	0.195		1.80	k= 3, v=30
1.8	143.838	0.243		1.81	k= 4, v=30
3.61	143.838	0.243		1.82	k= 5, v=30
7.21	143.550	0.279		1.83	k= 6, v=30
14.43	119.275	3.361	*	1.83	k= 7, v=30
28.9	100.375	5.761	*	1.83	k= 8, v=30
57.7	69.675	9.658	*	1.83	k= 9, v=30

s = 11.139
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	6.0	3.8	9.6	0.10	0.63
EC10	9.7	6.7	14.	0.078	0.70
EC25	21.	18.	26.	0.044	0.82
EC50	52.	45.	60.	0.032	0.86

Slope = 1.75 Std.Err. = 0.202

Goodness of fit: p = 0.83 based on DF= 10. 39.

5824UL : sugarbeet se length

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	146.	148.	-2.36	100.	0.00
0.0280	4.00	152.	148.	3.57	100.	5.06e-07
0.0560	4.00	152.	148.	3.62	100.	9.89e-06

0.110	4.00	145.	148.	-2.68	100.	0.000138
0.230	4.00	150.	148.	2.32	100.	0.00184
0.450	4.00	146.	148.	-2.54	100.	0.0150
0.900	4.00	149.	148.	1.07	99.9	0.101
1.80	4.00	139.	147.	-8.18	99.5	0.522
3.61	4.00	149.	145.	3.54	97.9	2.11
7.21	4.00	144.	138.	5.24	93.4	6.62
14.4	4.00	119.	124.	-4.50	83.6	16.4
28.9	4.00	100.	99.7	0.689	67.3	32.7
57.7	4.00	69.7	69.5	0.210	46.9	53.1

lettuce se length
File: 582411 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	459.057	51.006	1.130
Within (Error)	28	1264.112	45.147	
Total	37	1723.170		

Critical F value = 2.24 (0.05,9,28)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

lettuce se length
File: 582411 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	50.525	50.525		
2	0.23	49.675	49.675	0.179	
3	0.45	51.825	51.825	-0.274	
4	0.9	51.950	51.950	-0.300	
5	1.8	49.750	49.750	0.163	
6	3.61	50.950	50.950	-0.089	
7	7.21	52.050	52.050	-0.321	
8	14.43	54.800	54.800	-0.900	
9	28.9	44.250	44.250	1.321	
10	57.7	40.400	40.400	1.740	

Bonferroni T table value = 2.72 (1 Tailed Value, P=0.05, df=28,9)

lettuce se length
File: 582411 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL

282

1	control	4			
2	0.23	4	12.923	25.6	0.850
3	0.45	4	12.923	25.6	-1.300
4	0.9	4	12.923	25.6	-1.425
5	1.8	4	12.923	25.6	0.775
6	3.61	4	12.923	25.6	-0.425
7	7.21	4	12.923	25.6	-1.525
8	14.43	4	12.923	25.6	-4.275
9	28.9	4	12.923	25.6	6.275
10	57.7	2	15.828	31.3	10.125

lettuce se length
File: 582411 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	50.525	50.525	51.441
2	0.23	4	49.675	49.675	51.441
3	0.45	4	51.825	51.825	51.441
4	0.9	4	51.950	51.950	51.441
5	1.8	4	49.750	49.750	51.441
6	3.61	4	50.950	50.950	51.441
7	7.21	4	52.050	52.050	51.441
8	14.43	4	54.800	54.800	51.441
9	28.9	4	44.250	44.250	44.250
10	57.7	2	40.400	40.400	40.400

lettuce se length
File: 582411 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	51.441				
0.23	51.441	0.193		1.70	k= 1, v=28
0.45	51.441	0.193		1.78	k= 2, v=28
0.9	51.441	0.193		1.81	k= 3, v=28
1.8	51.441	0.193		1.82	k= 4, v=28
3.61	51.441	0.193		1.83	k= 5, v=28
7.21	51.441	0.193		1.83	k= 6, v=28
14.43	51.441	0.193		1.83	k= 7, v=28
28.9	44.250	1.321		1.84	k= 8, v=28
57.7	40.400	1.740		1.84	k= 9, v=28

s = 6.719
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate

283

EC5	22.	7.6	64.	0.23	0.34
EC10	32.	16.	63.	0.15	0.51
EC25	60.	35.	1.0E+02	0.12	0.58
EC50	1.2E+02	35.	4.2E+02	0.26	0.29

Slope = 2.21 Std.Err. = 1.34

Goodness of fit: p = 0.90 based on DF= 8.0 31.

5824LL : lettuce se length

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	50.5	51.4	-0.845	100.	0.00
0.110	4.00	51.8	51.4	0.430	100.	8.36e-10
0.230	4.00	49.7	51.4	-1.70	100.	8.53e-08
0.450	4.00	51.8	51.4	0.455	100.	3.75e-06
0.900	4.00	52.0	51.4	0.580	100.	0.000122
1.80	4.00	49.8	51.4	-1.62	100.	0.00259
3.61	4.00	51.0	51.4	-0.402	100.	0.0364
7.21	4.00	52.0	51.2	0.850	99.7	0.332
14.4	4.00	54.8	50.3	4.47	98.0	2.03
28.9	4.00	44.3	47.1	-2.82	91.6	8.37
57.7	2.00	40.4	39.2	1.19	76.3	23.7

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

rape se length
File: 5824rl Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	7628.689	847.632	1.482
Within (Error)	30	17155.815	571.861	
Total	39	24784.504		

Critical F value = 2.21 (0.05,9,30)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

rape se length
File: 5824rl Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	223.425	223.425		

284

2	0.45	206.250	206.250	1.016
3	0.9	202.900	202.900	1.214
4	1.8	211.025	211.025	0.733
5	3.61	216.225	216.225	0.426
6	7.21	208.725	208.725	0.869
7	14.43	193.725	193.725	1.756
8	28.9	222.450	222.450	0.058
9	57.7	225.025	225.025	-0.095
10	230.8	178.550	178.550	2.654

Bonferroni T table value = 2.71 (1 Tailed Value, P=0.05, df=30,9)

rape se length
File: 5824rl Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.45	4	45.774	20.5	17.175
3	0.9	4	45.774	20.5	20.525
4	1.8	4	45.774	20.5	12.400
5	3.61	4	45.774	20.5	7.200
6	7.21	4	45.774	20.5	14.700
7	14.43	4	45.774	20.5	29.700
8	28.9	4	45.774	20.5	0.975
9	57.7	4	45.774	20.5	-1.600
10	230.8	4	45.774	20.5	44.875

rape se length
File: 5824rl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	223.425	223.425	223.425
2	0.45	4	206.250	206.250	210.791
3	0.9	4	202.900	202.900	210.791
4	1.8	4	211.025	211.025	210.791
5	3.61	4	216.225	216.225	210.791
6	7.21	4	208.725	208.725	210.791
7	14.43	4	193.725	193.725	210.791
8	28.9	4	222.450	222.450	210.791
9	57.7	4	225.025	225.025	210.791
10	230.8	4	178.550	178.550	178.550

rape se length
File: 5824rl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

285

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	223.425				
0.45	210.791	0.747		1.70	k= 1, v=30
0.9	210.791	0.747		1.78	k= 2, v=30
1.8	210.791	0.747		1.80	k= 3, v=30
3.61	210.791	0.747		1.81	k= 4, v=30
7.21	210.791	0.747		1.82	k= 5, v=30
14.43	210.791	0.747		1.83	k= 6, v=30
28.9	210.791	0.747		1.83	k= 7, v=30
57.7	210.791	0.747		1.83	k= 8, v=30
230.8	178.550	2.654	*	1.83	k= 9, v=30

s = 23.914
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.00079	1.1E-26	5.8E+19	11.	1.4E-23
EC10	6.6E+03	5.4E-13	8.3E+19	8.0	8.1E-17
EC25	2.5E+15	3.3E-38	1.8E+68	26.	1.3E-53
EC50	1.8E+28	2.2E-72	1.4E+128	50.	1.2E-100

Slope = 0.0525 Std.Err. = 0.0976

Goodness of fit: p = 0.15 based on DF= 11. 42.

5824RL : rape se length

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	223.	223.	0.338	100.	0.00
0.0280	4.00	197.	210.	-13.2	94.1	5.90
0.0560	4.00	224.	210.	14.9	93.9	6.08
0.110	4.00	209.	209.	-0.272	93.7	6.27
0.230	4.00	198.	209.	-11.1	93.5	6.48
0.450	4.00	206.	208.	-1.94	93.3	6.68
0.900	4.00	203.	208.	-4.83	93.1	6.88
1.80	4.00	211.	207.	3.77	92.9	7.10
3.61	4.00	216.	207.	9.45	92.7	7.31
7.21	4.00	209.	206.	2.45	92.5	7.53
14.4	4.00	194.	206.	-12.0	92.2	7.76
28.9	4.00	222.	205.	17.2	92.0	7.99
57.7	4.00	225.	205.	20.3	91.8	8.23
231.	4.00	179.	204.	-25.1	91.3	8.72

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

286

radish se length
File: 5824dl Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	1602.965	178.107	1.286
Within (Error)	30	4155.262	138.509	
Total	39	5758.228		

Critical F value = 2.21 (0.05,9,30)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

radish se length
File: 5824dl Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	158.300	158.300		
2	0.028	146.750	146.750	1.388	
3	0.056	152.325	152.325	0.718	
4	0.11	155.075	155.075	0.388	
5	0.23	152.900	152.900	0.649	
6	0.45	134.025	134.025	2.917	*
7	0.9	150.850	150.850	0.895	
8	1.8	150.050	150.050	0.991	
9	3.61	152.250	152.250	0.727	
10	7.21	145.400	145.400	1.550	

Bonferroni T table value = 2.71 (1 Tailed Value, P=0.05, df=30,9)

radish se length
File: 5824dl Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.028	4	22.527	14.2	11.550
3	0.056	4	22.527	14.2	5.975
4	0.11	4	22.527	14.2	3.225
5	0.23	4	22.527	14.2	5.400
6	0.45	4	22.527	14.2	24.275
7	0.9	4	22.527	14.2	7.450
8	1.8	4	22.527	14.2	8.250
9	3.61	4	22.527	14.2	6.050
10	7.21	4	22.527	14.2	12.900

287

radish se length
File: 5824dl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	158.300	158.300	158.300
2	0.028	4	146.750	146.750	151.763
3	0.056	4	152.325	152.325	151.763
4	0.11	4	155.075	155.075	151.763
5	0.23	4	152.900	152.900	151.763
6	0.45	4	134.025	134.025	146.794
7	0.9	4	150.850	150.850	146.794
8	1.8	4	150.050	150.050	146.794
9	3.61	4	152.250	152.250	146.794
10	7.21	4	145.400	145.400	145.400

radish se length
File: 5824dl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	158.300				
0.028	151.763	0.786		1.70	k= 1, v=30
0.056	151.763	0.786		1.78	k= 2, v=30
0.11	151.763	0.786		1.80	k= 3, v=30
0.23	151.763	0.786		1.81	k= 4, v=30
0.45	146.794	1.383		1.82	k= 5, v=30
0.9	146.794	1.383		1.83	k= 6, v=30
1.8	146.794	1.383		1.83	k= 7, v=30
3.61	146.794	1.383		1.83	k= 8, v=30
7.21	145.400	1.550		1.83	k= 9, v=30

s = 11.769
Note: df used for table values are approximate when v > 20.

ECx

!!!Failure#1: near-singular matrix, model possibly unsuitable.

barnyard grass
File: 5824gw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	130.367	18.624	0.992
Within (Error)	24	450.665	18.778	

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Total	31	581.032

Critical F value = 2.42 (0.05,7,24)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

barnyard grass
File: 5824gw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	21.388	21.388		
2	3.61	23.348	23.348	-0.640	
3	7.21	24.436	24.436	-0.995	
4	14.43	19.315	19.315	0.677	
5	28.9	20.466	20.466	0.301	
6	57.7	22.396	22.396	-0.329	
7	115.8	18.836	18.836	0.833	
8	230.8	18.665	18.665	0.889	

Dunnett table value = 2.48 (1 Tailed Value, P=0.05, df=24,7)

barnyard grass
File: 5824gw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	3.61	4	7.599	35.5	-1.960
3	7.21	4	7.599	35.5	-3.048
4	14.43	4	7.599	35.5	2.073
5	28.9	4	7.599	35.5	0.922
6	57.7	4	7.599	35.5	-1.008
7	115.8	4	7.599	35.5	2.552
8	230.8	4	7.599	35.5	2.723

barnyard grass
File: 5824gw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	21.388	21.388	23.057
2	3.61	4	23.348	23.348	23.057
3	7.21	4	24.436	24.436	23.057
4	14.43	4	19.315	19.315	20.725

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5	28.9	4	20.466	20.466	20.725
6	57.7	4	22.396	22.396	20.725
7	115.8	4	18.836	18.836	18.836
8	230.8	4	18.665	18.665	18.665

barnyard grass
File: 5824gw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	23.057				
3.61	23.057	0.545		1.71	k= 1, v=24
7.21	23.057	0.545		1.79	k= 2, v=24
14.43	20.725	0.216		1.82	k= 3, v=24
28.9	20.725	0.216		1.83	k= 4, v=24
57.7	20.725	0.216		1.84	k= 5, v=24
115.8	18.836	0.833		1.84	k= 6, v=24
230.8	18.665	0.889		1.85	k= 7, v=24

s = 4.333
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	30.	0.14	6.6E+03	1.1	0.0045
EC10	83.	3.4	2.1E+03	0.68	0.040
EC25	4.6E+02	34.	6.1E+03	0.55	0.075
EC50	3.0E+03	4.2	2.2E+06	1.4	0.0014

Slope = 0.822 Std.Err. = 0.984

Goodness of fit: p = 0.57 based on DF= 5.0 24.

5824GW : barnyard grass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	21.4	22.5	-1.07	100.	0.00
3.61	4.00	23.3	22.3	1.08	99.2	0.815
7.21	4.00	24.4	22.1	2.33	98.4	1.56
14.4	4.00	19.3	21.8	-2.51	97.2	2.82
28.9	4.00	20.5	21.4	-0.901	95.2	4.85
57.7	4.00	22.4	20.7	1.71	92.1	7.88
116.	4.00	18.8	19.7	-0.877	87.8	12.2
231.	4.00	18.7	18.4	0.234	82.1	17.9

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

290

onion se weight
File: 5824iw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	4.123	0.458	4.164
Within (Error)	29	3.198	0.110	
Total	38	7.321		

Critical F value = 2.22 (0.05,9,29)
Since F > Critical F REJECT Ho:All groups equal

onion se weight
File: 5824iw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.931	0.931		
2	0.45	1.383	1.383	-1.931	
3	0.9	1.110	1.110	-0.764	
4	1.8	1.193	1.193	-1.117	
5	3.61	1.314	1.314	-1.633	
6	7.21	1.345	1.345	-1.766	
7	14.43	0.905	0.905	0.111	
8	28.9	0.954	0.954	-0.100	
9	57.7	0.685	0.685	1.048	
10	115.8	0.178	0.178	2.969	*

Bonferroni T table value = 2.71 (1 Tailed Value, P=0.05, df=29,9)

onion se weight
File: 5824iw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.45	4	0.636	68.4	-0.453
3	0.9	4	0.636	68.4	-0.179
4	1.8	4	0.636	68.4	-0.262
5	3.61	4	0.636	68.4	-0.383
6	7.21	4	0.636	68.4	-0.414
7	14.43	4	0.636	68.4	0.026
8	28.9	4	0.636	68.4	-0.024
9	57.7	4	0.636	68.4	0.246
10	115.8	3	0.687	73.9	0.752

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onion se weight
File: 5824iw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	0.931	0.931	1.212
2	0.45	4	1.383	1.383	1.212
3	0.9	4	1.110	1.110	1.212
4	1.8	4	1.193	1.193	1.212
5	3.61	4	1.314	1.314	1.212
6	7.21	4	1.345	1.345	1.212
7	14.43	4	0.905	0.905	0.929
8	28.9	4	0.954	0.954	0.929
9	57.7	4	0.685	0.685	0.685
10	115.8	3	0.178	0.178	0.178

onion se weight
File: 5824iw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	1.212				
0.45	1.212	1.200		1.70	k= 1, v=29
0.9	1.212	1.200		1.78	k= 2, v=29
1.8	1.212	1.200		1.81	k= 3, v=29
3.61	1.212	1.200		1.82	k= 4, v=29
7.21	1.212	1.200		1.83	k= 5, v=29
14.43	0.929	0.005		1.83	k= 6, v=29
28.9	0.929	0.005		1.83	k= 7, v=29
57.7	0.685	1.047		1.84	k= 8, v=29
115.8	0.178	2.965	*	1.84	k= 9, v=29

s = 0.332
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	13.	4.3	37.	0.23	0.34
EC10	17.	7.1	42.	0.19	0.41
EC25	29.	16.	54.	0.13	0.54
EC50	53.	37.	77.	0.080	0.69

Slope = 2.62 Std.Err. = 0.768

Goodness of fit: p = 0.15 based on DF= 8.0 32.

5824IW : onion se weight

292

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.930	1.25	-0.319	100.	0.00
0.230	4.00	1.64	1.25	0.391	100.	3.03e-08
0.450	4.00	1.38	1.25	0.134	100.	2.89e-06
0.900	4.00	1.11	1.25	-0.140	100.	0.000176
1.80	4.00	1.19	1.25	-0.0568	100.	0.00592
3.61	4.00	1.31	1.25	0.0655	99.9	0.111
7.21	4.00	1.34	1.23	0.110	98.8	1.16
14.4	4.00	0.904	1.16	-0.259	93.1	6.91
28.9	4.00	0.954	0.944	0.00961	75.6	24.4
57.7	4.00	0.685	0.578	0.106	46.3	53.7
116.	3.00	0.178	0.235	-0.0566	18.8	81.2

wheat se weight
File: 5824ww Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	7.411	1.059	0.681
Within (Error)	24	37.293	1.554	
Total	31	44.704		

Critical F value = 2.42 (0.05,7,24)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

wheat se weight
File: 5824ww Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	10.378	10.378		
2	3.61	9.763	9.763	0.698	
3	7.21	10.075	10.075	0.344	
4	14.43	10.520	10.520	-0.161	
5	28.9	11.171	11.171	-0.899	
6	57.7	10.397	10.397	-0.021	
7	115.8	11.176	11.176	-0.905	
8	230.8	10.970	10.970	-0.672	

Dunnett table value = 2.48 (1 Tailed Value, P=0.05, df=24,7)

wheat se weight
File: 5824ww Transform: NO TRANSFORMATION

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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	3.61	4	2.186	21.1	0.615
3	7.21	4	2.186	21.1	0.304
4	14.43	4	2.186	21.1	-0.141
5	28.9	4	2.186	21.1	-0.793
6	57.7	4	2.186	21.1	-0.019
7	115.8	4	2.186	21.1	-0.798
8	230.8	4	2.186	21.1	-0.592

wheat se weight
File: 5824ww Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	10.378	10.378	10.071
2	3.61	4	9.763	9.763	10.071
3	7.21	4	10.075	10.075	10.075
4	14.43	4	10.520	10.520	10.520
5	28.9	4	11.171	11.171	10.784
6	57.7	4	10.397	10.397	10.784
7	115.8	4	11.176	11.176	11.073
8	230.8	4	10.970	10.970	11.073

wheat se weight
File: 5824ww Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	10.071				
3.61	10.071	0.349		1.71	k= 1, v=24
7.21	10.075	0.344		1.79	k= 2, v=24
14.43	10.520	0.161		1.82	k= 3, v=24
28.9	10.784	0.460		1.83	k= 4, v=24
57.7	10.784	0.460		1.84	k= 5, v=24
115.8	11.073	0.788		1.84	k= 6, v=24
230.8	11.073	0.788		1.85	k= 7, v=24

s = 1.247
Note: df used for table values are approximate when v > 20.

ECx
!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.

294

cucumber se weight
File: 5824cw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	2877.444	319.716	1.099
Within (Error)	30	8729.126	290.971	
Total	39	11606.570		

Critical F value = 2.21 (0.05,9,30)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

cucumber se weight
File: 5824cw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	178.803	178.803		
2	0.23	176.981	176.981	0.151	
3	0.45	179.090	179.090	-0.024	
4	0.9	171.070	171.070	0.641	
5	1.8	159.873	159.873	1.569	
6	3.61	173.683	173.683	0.424	
7	7.21	174.912	174.912	0.323	
8	14.43	174.348	174.348	0.369	
9	28.9	171.653	171.653	0.593	
10	57.7	151.013	151.013	2.304	

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

cucumber se weight
File: 5824cw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	30.637	17.1	1.821
3	0.45	4	30.637	17.1	-0.287
4	0.9	4	30.637	17.1	7.733
5	1.8	4	30.637	17.1	18.930
6	3.61	4	30.637	17.1	5.120
7	7.21	4	30.637	17.1	3.890
8	14.43	4	30.637	17.1	4.455
9	28.9	4	30.637	17.1	7.150
10	57.7	4	30.637	17.1	27.790

295

cucumber se weight
File: 5824cw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	178.803	178.803	178.803
2	0.23	4	176.981	176.981	178.036
3	0.45	4	179.090	179.090	178.036
4	0.9	4	171.070	171.070	171.070
5	1.8	4	159.873	159.873	170.894
6	3.61	4	173.683	173.683	170.894
7	7.21	4	174.912	174.912	170.894
8	14.43	4	174.348	174.348	170.894
9	28.9	4	171.653	171.653	170.894
10	57.7	4	151.013	151.013	151.013

cucumber se weight
File: 5824cw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	178.803				
0.23	178.036	0.064		1.70	k= 1, v=30
0.45	178.036	0.064		1.78	k= 2, v=30
0.9	171.070	0.641		1.80	k= 3, v=30
1.8	170.894	0.656		1.81	k= 4, v=30
3.61	170.894	0.656		1.82	k= 5, v=30
7.21	170.894	0.656		1.83	k= 6, v=30
14.43	170.894	0.656		1.83	k= 7, v=30
28.9	170.894	0.656		1.83	k= 8, v=30
57.7	151.013	2.304	*	1.83	k= 9, v=30

s = 17.058
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	41.	18.	91.	0.17	0.45
EC10	52.	37.	73.	0.074	0.71
EC25	77.	38.	1.5E+02	0.15	0.49
EC50	1.2E+02	22.	6.3E+02	0.36	0.19

Slope = 3.53 Std.Err. = 3.96

Goodness of fit: p = 0.82 based on DF= 10. 39.

5824CW : cucumber se weight

296

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	179.	174.	4.52	100.	0.00
0.0280	4.00	183.	174.	8.86	100.	1.63e-14
0.0560	4.00	174.	174.	0.0314	100.	1.63e-14
0.110	4.00	171.	174.	-3.49	100.	1.63e-14
0.230	4.00	177.	174.	2.70	100.	1.63e-14
0.450	4.00	179.	174.	4.81	100.	1.63e-14
0.900	4.00	171.	174.	-3.21	100.	3.29e-12
1.80	4.00	160.	174.	-14.4	100.	6.31e-09
3.61	4.00	174.	174.	-0.599	100.	4.08e-06
7.21	4.00	175.	174.	0.633	100.	0.000847
14.4	4.00	174.	174.	0.171	99.9	0.0604
28.9	4.00	172.	172.	-0.0203	98.5	1.50
57.7	4.00	151.	151.	0.00691	86.6	13.4

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

soybean se weight
File: 5824sw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	27813.420	3090.380	56.675
Within (Error)	30	1635.852	54.528	
Total	39	29449.272		

Critical F value = 2.21 (0.05,9,30)
Since F > Critical F REJECT Ho:All groups equal

soybean se weight
File: 5824sw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	68.241	68.241		
2	0.23	67.927	67.927	0.060	
3	0.45	69.566	69.566	-0.254	
4	0.9	66.330	66.330	0.366	
5	1.8	49.494	49.494	3.590	*
6	3.61	48.163	48.163	3.845	*
7	7.21	33.209	33.209	6.709	*
8	14.43	9.112	9.112	11.324	*
9	28.9	5.125	5.125	12.088	*
10	57.7	1.650	1.650	12.753	*

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Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

soybean se weight
File: 5824sw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	13.263	19.4	0.314
3	0.45	4	13.263	19.4	-1.325
4	0.9	4	13.263	19.4	1.911
5	1.8	4	13.263	19.4	18.747
6	3.61	4	13.263	19.4	20.078
7	7.21	4	13.263	19.4	35.033
8	14.43	4	13.263	19.4	59.129
9	28.9	4	13.263	19.4	63.116
10	57.7	4	13.263	19.4	66.591

soybean se weight
File: 5824sw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	68.241	68.241	68.578
2	0.23	4	67.927	67.927	68.578
3	0.45	4	69.566	69.566	68.578
4	0.9	4	66.330	66.330	66.330
5	1.8	4	49.494	49.494	49.494
6	3.61	4	48.163	48.163	48.163
7	7.21	4	33.209	33.209	33.209
8	14.43	4	9.112	9.112	9.112
9	28.9	4	5.125	5.125	5.125
10	57.7	4	1.650	1.650	1.650

soybean se weight
File: 5824sw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	68.578				
0.23	68.578	0.065		1.70	k= 1, v=30
0.45	68.578	0.065		1.78	k= 2, v=30
0.9	66.330	0.366		1.80	k= 3, v=30
1.8	49.494	3.590	*	1.81	k= 4, v=30

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3.61	48.163	3.845	*	1.82	k= 5, v=30
7.21	33.209	6.709	*	1.83	k= 6, v=30
14.43	9.112	11.324	*	1.83	k= 7, v=30
28.9	5.125	12.088	*	1.83	k= 8, v=30
57.7	1.650	12.753	*	1.83	k= 9, v=30

s = 7.384
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.91	0.59	1.4	0.094	0.65
EC10	1.4	0.94	2.0	0.081	0.69
EC25	2.7	2.0	3.6	0.061	0.75
EC50	5.7	4.7	6.9	0.042	0.82

Slope = 2.08 Std.Err. = 0.159

Goodness of fit: p = 0.24 based on DF= 10. 39.

5824SW : soybean se weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	68.2	68.0	0.195	100.	0.00
0.0280	4.00	67.7	68.0	-0.309	100.	8.42e-05
0.0560	4.00	67.6	68.0	-0.428	100.	0.00157
0.110	4.00	68.3	68.0	0.245	100.	0.0190
0.230	4.00	67.9	67.9	0.0131	99.8	0.193
0.450	4.00	69.6	67.3	2.28	98.9	1.12
0.900	4.00	66.3	64.7	1.59	95.1	4.86
1.80	4.00	49.5	57.8	-8.30	84.9	15.1
3.61	4.00	48.2	44.8	3.41	65.8	34.2
7.21	4.00	33.2	28.2	5.05	41.4	58.6
14.4	4.00	9.11	13.6	-4.46	19.9	80.1
28.9	4.00	5.13	4.82	0.307	7.08	92.9
57.7	4.00	1.65	1.24	0.415	1.82	98.2

sugarbeet se weight
File: 5824uw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	2553.111	283.679	8.252
Within (Error)	30	1031.262	34.375	
Total	39	3584.373		

Critical F value = 2.21 (0.05,9,30)
Since F > Critical F REJECT Ho:All groups equal

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sugarbeet se weight
File: 5824uw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	23.641	23.641		
2	0.23	30.269	30.269	-1.599	
3	0.45	29.243	29.243	-1.351	
4	0.9	27.384	27.384	-0.903	
5	1.8	20.004	20.004	0.877	
6	3.61	30.507	30.507	-1.656	
7	7.21	27.761	27.761	-0.994	
8	14.43	19.522	19.522	0.994	
9	28.9	12.348	12.348	2.724	*
10	57.7	5.254	5.254	4.435	*

Dunnett table value = 2.54 (1 Tailed Value, P=0.05, df=30,9)

sugarbeet se weight
File: 5824uw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	10.530	44.5	-6.629
3	0.45	4	10.530	44.5	-5.603
4	0.9	4	10.530	44.5	-3.744
5	1.8	4	10.530	44.5	3.636
6	3.61	4	10.530	44.5	-6.867
7	7.21	4	10.530	44.5	-4.120
8	14.43	4	10.530	44.5	4.119
9	28.9	4	10.530	44.5	11.292
10	57.7	4	10.530	44.5	18.387

sugarbeet se weight
File: 5824uw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	23.641	23.641	27.718
2	0.23	4	30.269	30.269	27.718
3	0.45	4	29.243	29.243	27.718
4	0.9	4	27.384	27.384	27.384
5	1.8	4	20.004	20.004	26.091
6	3.61	4	30.507	30.507	26.091
7	7.21	4	27.761	27.761	26.091

300

8	14.43	4	19.522	19.522	19.522
9	28.9	4	12.348	12.348	12.348
10	57.7	4	5.254	5.254	5.254

sugarbeet se weight
File: 5824uw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	27.718				
0.23	27.718	0.983		1.70	k= 1, v=30
0.45	27.718	0.983		1.78	k= 2, v=30
0.9	27.384	0.903		1.80	k= 3, v=30
1.8	26.091	0.591		1.81	k= 4, v=30
3.61	26.091	0.591		1.82	k= 5, v=30
7.21	26.091	0.591		1.83	k= 6, v=30
14.43	19.522	0.994		1.83	k= 7, v=30
28.9	12.348	2.724	*	1.83	k= 8, v=30
57.7	5.254	4.435	*	1.83	k= 9, v=30

s = 5.863
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	5.7	2.7	12.	0.16	0.48
EC10	8.0	4.3	15.	0.13	0.54
EC25	14.	8.9	21.	0.093	0.65
EC50	25.	19.	33.	0.056	0.77

Slope = 2.55 Std.Err. = 0.493

Goodness of fit: p = 0.13 based on DF= 10. 39.

5824UW : sugarbeet se weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	23.6	28.2	-4.57	100.	0.00
0.0280	4.00	34.0	28.2	5.76	100.	2.39e-12
0.0560	4.00	29.5	28.2	1.31	100.	6.46e-10
0.110	4.00	28.1	28.2	-0.134	100.	8.62e-08
0.230	4.00	30.3	28.2	2.06	100.	9.70e-06
0.450	4.00	29.2	28.2	1.03	100.	0.000406
0.900	4.00	27.4	28.2	-0.822	100.	0.0110
1.80	4.00	20.0	28.2	-8.16	99.8	0.171
3.61	4.00	30.5	27.8	2.74	98.4	1.55
7.21	4.00	27.8	25.9	1.87	91.8	8.23
14.4	4.00	19.5	20.7	-1.15	73.3	26.7
28.9	4.00	12.3	12.4	-0.101	44.1	55.9

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57.7	4.00	5.25	5.09	0.161	18.1	81.9

lettuce se weight
File: 5824lw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	29.827	3.314	5.675
Within (Error)	28	16.343	0.584	
Total	37	46.170		

Critical F value = 2.24 (0.05,9,28)
Since F > Critical F REJECT Ho:All groups equal

lettuce se weight
File: 5824lw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	3.003	3.003		
2	0.23	3.228	3.228	-0.416	
3	0.45	4.315	4.315	-2.428	
4	0.9	3.881	3.881	-1.624	
5	1.8	3.012	3.012	-0.016	
6	3.61	2.859	2.859	0.267	
7	7.21	3.322	3.322	-0.590	
8	14.43	3.206	3.206	-0.376	
9	28.9	1.712	1.712	2.389	
10	57.7	0.516	0.516	3.757	*

Bonferroni T table value = 2.72 (1 Tailed Value, P=0.05, df=28,9)

lettuce se weight
File: 5824lw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.23	4	1.470	48.9	-0.225
3	0.45	4	1.470	48.9	-1.312
4	0.9	4	1.470	48.9	-0.878
5	1.8	4	1.470	48.9	-0.009
6	3.61	4	1.470	48.9	0.144
7	7.21	4	1.470	48.9	-0.319
8	14.43	4	1.470	48.9	-0.203
9	28.9	4	1.470	48.9	1.291

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10	57.7	2	1.800	59.9	2.486

lettuce se weight
File: 5824lw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	3.003	3.003	3.606
2	0.23	4	3.228	3.228	3.606
3	0.45	4	4.315	4.315	3.606
4	0.9	4	3.881	3.881	3.606
5	1.8	4	3.012	3.012	3.099
6	3.61	4	2.859	2.859	3.099
7	7.21	4	3.322	3.322	3.099
8	14.43	4	3.206	3.206	3.099
9	28.9	4	1.712	1.712	1.712
10	57.7	4	0.516	0.516	0.516

lettuce se weight
File: 5824lw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	3.606				
0.23	3.606	1.117		1.70	k= 1, v=28
0.45	3.606	1.117		1.78	k= 2, v=28
0.9	3.606	1.117		1.81	k= 3, v=28
1.8	3.099	0.179		1.82	k= 4, v=28
3.61	3.099	0.179		1.83	k= 5, v=28
7.21	3.099	0.179		1.83	k= 6, v=28
14.43	3.099	0.179		1.83	k= 7, v=28
28.9	1.712	2.390	*	1.84	k= 8, v=28
57.7	0.516	3.758	*	1.84	k= 9, v=28

s = 0.764
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	11.	6.0	22.	0.14	0.52
EC10	14.	8.2	24.	0.12	0.58
EC25	20.	14.	30.	0.081	0.69
EC50	30.	24.	39.	0.051	0.79

Slope = 3.87 Std.Err. = 1.01

Goodness of fit: p = 0.27 based on DF= 8.0 31.

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5824LW : lettuce se weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	3.00	3.39	-0.382	100.	0.00
0.110	4.00	3.33	3.39	-0.0564	100.	2.62e-14
0.230	4.00	3.23	3.39	-0.158	100.	1.31e-14
0.450	4.00	4.31	3.39	0.930	100.	7.26e-11
0.900	4.00	3.88	3.39	0.495	100.	1.67e-07
1.80	4.00	3.01	3.39	-0.374	100.	0.000102
3.61	4.00	2.86	3.38	-0.526	100.	0.0173
7.21	4.00	3.32	3.36	-0.0368	99.2	0.784
14.4	4.00	3.21	3.03	0.179	89.4	10.6
28.9	4.00	1.71	1.80	-0.0919	53.3	46.7
57.7	2.00	0.516	0.474	0.0423	14.0	86.0

rape se weight
File: 5824rw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	2162.856	240.317	2.803
Within (Error)	30	2572.388	85.746	
Total	39	4735.244		

Critical F value = 2.21 (0.05,9,30)
Since F > Critical F REJECT Ho:All groups equal

rape se weight
File: 5824rw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	47.417	47.417		
2	0.45	40.681	40.681	1.029	
3	0.9	39.125	39.125	1.267	
4	1.8	35.994	35.994	1.745	
5	3.61	42.296	42.296	0.782	
6	7.21	34.692	34.692	1.944	
7	14.43	31.415	31.415	2.444	
8	28.9	32.810	32.810	2.231	
9	57.7	36.943	36.943	1.600	
10	230.8	18.529	18.529	4.412	*

Bonferroni T table value = 2.71 (1 Tailed Value, P=0.05, df=30,9)

304

rape se weight
File: 5824rw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.45	4	17.725	37.4	6.736
3	0.9	4	17.725	37.4	8.293
4	1.8	4	17.725	37.4	11.423
5	3.61	4	17.725	37.4	5.121
6	7.21	4	17.725	37.4	12.726
7	14.43	4	17.725	37.4	16.003
8	28.9	4	17.725	37.4	14.607
9	57.7	4	17.725	37.4	10.475
10	230.8	4	17.725	37.4	28.888

rape se weight
File: 5824rw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	47.417	47.417	47.417
2	0.45	4	40.681	40.681	40.681
3	0.9	4	39.125	39.125	39.138
4	1.8	4	35.994	35.994	39.138
5	3.61	4	42.296	42.296	39.138
6	7.21	4	34.692	34.692	34.692
7	14.43	4	31.415	31.415	33.722
8	28.9	4	32.810	32.810	33.722
9	57.7	4	36.943	36.943	33.722
10	230.8	4	18.529	18.529	18.529

rape se weight
File: 5824rw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	47.417				
0.45	40.681	1.029		1.70	k= 1, v=30
0.9	39.138	1.264		1.78	k= 2, v=30
1.8	39.138	1.264		1.80	k= 3, v=30
3.61	39.138	1.264		1.81	k= 4, v=30
7.21	34.692	1.944	*	1.82	k= 5, v=30
14.43	33.722	2.092	*	1.83	k= 6, v=30
28.9	33.722	2.092	*	1.83	k= 7, v=30
57.7	33.722	2.092	*	1.83	k= 8, v=30

305

230.8	18.529	4.412	*	1.83	k= 9, v=30

s = 9.260
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	4.9	0.35	68.	0.57	0.072
EC10	12.	1.5	87.	0.44	0.13
EC25	49.	16.	1.5E+02	0.25	0.32
EC50	2.4E+02	87.	6.7E+02	0.22	0.36

Slope = 0.972 Std.Err. = 0.372

Goodness of fit: p = 0.50 based on DF= 11. 42.

5824RW : rape se weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	47.4	40.9	6.53	100.	0.00
0.0280	4.00	40.0	40.9	-0.839	100.	0.00663
0.110	4.00	40.4	40.9	-0.430	99.9	0.0589
0.230	4.00	34.7	40.8	-6.09	99.8	0.168
0.450	4.00	40.7	40.7	-0.0444	99.6	0.403
0.560	4.00	47.5	40.7	6.85	99.5	0.527
0.900	4.00	39.1	40.5	-1.39	99.1	0.920
1.80	4.00	36.0	40.1	-4.10	98.1	1.95
3.61	4.00	42.3	39.3	2.97	96.2	3.83
7.21	4.00	34.7	38.0	-3.36	93.0	6.95
14.4	4.00	31.4	36.1	-4.66	88.2	11.8
28.9	4.00	32.8	33.3	-0.484	81.4	18.6
57.7	4.00	36.9	29.7	7.24	72.6	27.4
231.	4.00	18.5	20.7	-2.19	50.7	49.3

!!!Warning: EC50 not bracketed by doses evaluated.

radish se weight
File: 5824dw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	874.798	97.200	1.199
Within (Error)	30	2431.517	81.051	
Total	39	3306.315		

Critical F value = 2.21 (0.05,9,30)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

306

radish se weight
File: 5824dw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	48.622	48.622		
2	0.45	35.811	35.811	2.012	
3	0.9	43.847	43.847	0.750	
4	1.8	42.643	42.643	0.939	
5	3.61	45.039	45.039	0.563	
6	7.21	36.422	36.422	1.916	
7	14.43	32.201	32.201	2.579	
8	28.9	43.615	43.615	0.786	
9	57.7	40.445	40.445	1.284	
10	230.8	42.040	42.040	1.034	

Bonferroni T table value = 2.71 (1 Tailed Value, P=0.05, df=30,9)

radish se weight
File: 5824dw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.45	4	17.233	35.4	12.811
3	0.9	4	17.233	35.4	4.775
4	1.8	4	17.233	35.4	5.978
5	3.61	4	17.233	35.4	3.582
6	7.21	4	17.233	35.4	12.200
7	14.43	4	17.233	35.4	16.420
8	28.9	4	17.233	35.4	5.006
9	57.7	4	17.233	35.4	8.176
10	230.8	4	17.233	35.4	6.582

radish se weight
File: 5824dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	48.622	48.622	48.622
2	0.45	4	35.811	35.811	41.835
3	0.9	4	43.847	43.847	41.835
4	1.8	4	42.643	42.643	41.835
5	3.61	4	45.039	45.039	41.835
6	7.21	4	36.422	36.422	38.945
7	14.43	4	32.201	32.201	38.945
8	28.9	4	43.615	43.615	38.945

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9	57.7	4	40.445	40.445	38.945
10	230.8	4	42.040	42.040	38.945

radish se weight
File: 5824dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	48.622				
0.45	41.835	1.066		1.70	k= 1, v=30
0.9	41.835	1.066		1.78	k= 2, v=30
1.8	41.835	1.066		1.80	k= 3, v=30
3.61	41.835	1.066		1.81	k= 4, v=30
7.21	38.945	1.520		1.82	k= 5, v=30
14.43	38.945	1.520		1.83	k= 6, v=30
28.9	38.945	1.520		1.83	k= 7, v=30
57.7	38.945	1.520		1.83	k= 8, v=30
230.8	38.945	1.520		1.83	k= 9, v=30

s = 9.003
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	8.0E-08	1.4E-30	4.7E+15	11.	1.7E-23
EC10	0.0021	6.5E-17	7.0E+10	6.7	3.1E-14
EC25	5.3E+04	1.5E-06	1.9E+15	5.3	2.8E-11
EC50	8.8E+12	3.5E-15	2.2E+40	14.	4.0E-28

Slope = 0.0821 Std.Err. = 0.0968

Goodness of fit: p = 0.49 based on DF= 11. 42.

5824DW : radish se weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	48.6	48.7	-0.112	100.	0.00
0.0280	4.00	40.8	43.0	-2.24	88.3	11.7
0.0560	4.00	46.0	42.8	3.22	87.8	12.2
0.110	4.00	45.0	42.5	2.48	87.3	12.7
0.230	4.00	44.5	42.3	2.22	86.8	13.2
0.450	4.00	35.8	42.0	-6.21	86.2	13.8
0.900	4.00	43.8	41.8	2.09	85.7	14.3
1.80	4.00	42.6	41.5	1.16	85.1	14.9
3.61	4.00	45.0	41.2	3.84	84.5	15.5
7.21	4.00	36.4	40.9	-4.49	83.9	16.1
14.4	4.00	32.2	40.6	-8.41	83.3	16.7
28.9	4.00	43.6	40.3	3.31	82.7	17.3
57.7	4.00	40.4	40.0	0.451	82.1	17.9

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```
     231.       4.00       42.0       39.3       2.69       80.7       19.3
!!!Warning: EC5 not bracketed by doses evaluated.
!!!Warning: EC10 not bracketed by doses evaluated.
!!!Warning: EC25 not bracketed by doses evaluated.
!!!Warning: EC50 not bracketed by doses evaluated.
```


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DATA EVALUATION RECORD
VEGETATIVE VIGOR EC_{25} TEST
§123-1(B) (TIER II) PMRA DACO:9.8.4

1. **CHEMICAL:** Aminopyralid — PC Code No.: 005100

2. **TEST MATERIAL:** XDE-750 as GF-871 (formulation) — Purity: 40.6%

3. **CITATION:**

Author: Aufderheide, J

Title: Effect of GF-871 on Vegetative Vigor of Selected Non-Target Terrestrial Plants (Tier II)

Study Completion Date: January 21, 2004

Laboratory: ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Sponsor: Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268

Laboratory Report ID: 48323

MRID No.: 462358-25 PMRA Submission #: 2004-0790

DP Barcode: D301682

4. **REVIEWED BY:** John Marton, Staff Scientist, Dynamac Corporation **Date:** 8/18/04

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation **Date:** 10/10/04

5. **APPROVED BY:** Brian D. Kiernan, Biologist, OPP/EFED/ERBIV **Date:** 12/08/2004

Signature: 6/16/05

Monika Engel, PMRA-EAD **Date:** February 7, 2005

Signature:

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: Dicots: *Cucumis sativus, Lactuca sativa, Brassica napus, Raphanus sativus, Glycine max, and Beta vulgaris altissima*
Monocots: *Echinochloa spec, Zea mays, Allium cepa, and Triticum aestivum*

Definitive Study Duration: 21 days

Type of Concentrations: Nominal

7. CONCLUSIONS:

Vegetative vigor was studied ten non-target crop species after post-emergent application of XDE-750 as the GF-871 formulation (Aminopyralid). The ten species tested were cucumber, lettuce, oilseed rape, radish, soybean, sugar beet, barnyard grass, corn, onion, and wheat. Species were tested based on expected sensitivity ranging from 0.028 to 230.8 g a.i./ha.

The most sensitive species was soybean, a dicot, with an EC_{25} of 0.75 g a.i./ha ($6.6e^{-4}$ lb a.i./A) based on shoot length; the NOEC for soybean shoot length was 0.45 g a.i./ha ($4.0e^{-4}$ lb a.i./A). The most sensitive monocot was onion, based on fresh shoot weight, with an EC_{25} of 53 g a.i./ha (0.05 lb a.i./A); the NOEC for onion fresh weight was 1.8 g a.i./ha ($1.6e^{-3}$ lb a.i./A). Note that units are active ingredient, not acid equivalents.

This study is classified as Supplemental. This study is scientifically sound, but it does not fulfill the guideline requirements for a vegetative vigor study (Subdivision J, §123-1b (TIER II)) because Thiram was applied to sugar beet without further explanation. Both corn and radish were grown under very low light conditions, which may have affected the results.

EAD Conclusion:

The EAD is in agreement with the conclusions reported by the study author and the EPA reviewer. The most sensitive dicot was soybean with an EC_{25} of 0.75 g a.i./ha and a NOEC of 0.45 g a.i./ha. based on fresh shoot length. The most sensitive monocot was onion with an EC_{25} of 53 g a.i./ha and a NOEC of 1.8 g a.i./ha based on fresh weight.

Most sensitive dicot: Soybean
Most sensitive parameter: Shoot length
NOEC: 0.45 g a.i./ha ($4.0e^{-4}$ lb a.i./A)
EC_{05}: 0.027 g a.i./ha ($2.4e^{-5}$ lb a.i./A)
95% C.I.: 0.0053-0.14 g a.i./ha ($4.7e^{-6}$-$1.2e^{-4}$ lb a.i./A)

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EC_{25}: 0.75 g a.i./ha ($6.6e^{-4}$ lb a.i./A)
95% C.I.: 0.29-1.9 g a.i./ha ($2.6e^{-4}$-$1.7e^{-3}$ lb a.i./A)
Slope: 0.676±0.0759

Most sensitive monocot: Onion
Most sensitive parameter: Fresh weight
NOEC: 1.8 g a.i./ha ($1.6e^{-3}$ lb a.i./A)
EC_{05}: 0.012 g a.i./ha ($1.0e^{-5}$ lb a.i./A)
95% C.I.: $2.0e^{-8}$-$7.4e^{3}$ g a.i./ha ($1.8e^{-11}$-6.51 lb a.i./A)
EC_{25}: 53 g a.i./ha (0.05 lb a.i./A)
95% C.I.: 0.40-7200 g a.i./ha ($3.5e^{-4}$-6.3 lb a.i./A)
Slope: 0.266±0.167

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This study is scientifically sound but does not fulfill the guideline requirements for a vegetative vigor study (Subdivision J, §123-1b (TIER II)) because of failure to provide an explanation as to why sugar beet was treated with Thiram. Furthermore, low light levels may have affected the results.

C. Repairability: An explanation regarding the use of Thiram on sugarbeet should be provided. There is no repairability regarding low light levels.

9. GUIDELINE DEVIATIONS:

Sugar beet was treated with the pesticide Thiram and no explanation was provided as to why this was deemed necessary.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the phytotoxicity of post-emergent application of Aminopyralid to non-target crop species for the purpose of chemical registration.

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11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	Dicots: cucumber, oilseed rape, radish, soybean sugar beet, and lettuce Monocots: corn, barnyard grass, onion, and wheat
Number of plants per repetition:	Cucumber, Oilseed rape, Radish, Soybean, Sugar Beet, and Corn: 36 plants/rep total, 2 plants/pot, 3 pots/rep, 6 reps/treatment level Barnyard Grass, Onion, and Wheat: 30 plants/rep, 5 plants/pot, 1 pot/rep, 6 reps/treatment level Lettuce: 36 plants/rep, 3 plants/pot, 2 pots/rep, 6 reps/treatment level
Source of seed and historical % germination of seed:	See Table 1 p. 21 for seed source information and historical % germination.

B. Test System

Guideline Criteria	Reported Information
Solvent:	80% non-ionic surfactant
Site of test:	Corn and Radish: On-site Greenhouse 3 Cucumber and Barnyard grass: On-site Greenhouse 5 Oilseed rape, Soybean, and Wheat: On-site Greenhouse 7 Lettuce, Onion, and Sugar beet: On-site Greenhouse 8.

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Guideline Criteria	Reported Information
Planting method/type of pot:	The planting containers were square plastic pots (10 cm x 10 cm x 12 cm). Cucumber, corn and soybean were planted at approximately 20 mm. Radish, barnyard grass, and wheat were planted at approximately 13 mm. Oilseed rape, sugar beet, lettuce, and onion were planted at approximately 6 mm. The growth medium was silt loam soil with organic content of approximately 2.7% and an approximate pH of 7.0.
Method of application:	An overhead track sprayer was used for application.
Method of watering:	The pots were bottom-watered through sub-irrigation. Minimal top watering was performed on Day 3, and care was taken not to wet the foliage.
Growth stage at application:	1-4 leaf stage (see pp. 12).

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x	2x
Doses: At least 5	0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.43, 28.85, 57.70, 115.4, and 230.8 g a.i./ha The application rate range was adjusted according to the expected sensitivity to the test material.
Controls: Negative and solvent	Negative control (deionized water)
Replicates per dose: At least 3	6 replicates

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Guideline Criteria	Reported Information
Test duration: 14 days	21 days
Were observations made at least weekly?	Yes
Maximum dosage rate:	The maximum dosage rate for the study was 230.8 g a.i./ha (nominal).

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was a NOEC observed for each species?	Yes
Phytotoxic observations:	Phytotoxic observations were reported as "visual injury," on a scale from 0-100%. All dicot species experienced significant visual damage (≥30%).
Were initial chemical concentrations measured? (Optional)	Yes. Initial concentrations were measured for the nominal application rates of 58.8, 118, and 235 g/ha; mean measured concentrations ranged from 103-104% of nominal.
Were adequate raw data included?	Replicate survival, shoot height, and fresh shoot weight data were reported.

Results for the most sensitive parameter of each species

Results Synopsis

Vegetative Vigor

Crop	Plant Height*		Fresh Weight*		Most Sensitive Parameter
	NOEC	EC_{25}	NOEC	EC_{25}	
Barnyard Grass	≥230.8	≥230.8	≥230.8	≥230.8	None
Corn	≥230.8	≥230.8	≥230.8	≥230.8	None

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Crop	Plant Height*		Fresh Weight*		Most Sensitive Parameter
	NOEC	EC_{25}	NOEC	EC_{25}	
Onion	57.7	≥230.8	57.7	78.2	Fresh Weight
Wheat	≥230.8	≥230.8	≥230.8	≥230.8	None
Cucumber	7.21	11.1	7.21	12.4	Plant Height
Lettuce	3.61	7.10	1.80	3.64	Fresh Weight
Oilseed rape	≥230.8	≥230.8	≥230.8	≥230.8	None
Radish	57.7	>115.4	14.43	28.0	Fresh Weight
Soybean	0.45	1.31	0.45	1.97	Plant Height
Sugar beet	28.85	70.6	28.85	20.1	Fresh Weight

* Units are g a.i./ha

Morphological Observations (negative percent reductions indicate promoted growth)

Barnyard Grass:
The application rate range for barnyard grass included a negative control, 3.61, 7.21, 14.4, 28.9, 57.7, 115.4, and 230.8 g a.i./ha. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 729, 715, 727, 743, 704, 727, 718, and 714 mm respectively, which indicated a 2, 0, -2, 3, 0, 2, and 2% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 26.7, 25.6, 24.8, 25.8, 25.1, 27.2, 25.5, and 26.3 g, respectively, which indicated a 4, 7, 3, 6, -2, 4, and 2% inhibition for the respective treatment levels, when compared to the control.No visual injury was observed for any species at any treatment level.

Corn:
The application rate range for corn included a negative control, 3.61, 7.21, 14.4, 28.9, 57.7, 115.4, and 230.8 g a.i./ha. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 1160, 1110, 1130, 1150, 1120, 1150, 1130, and 1140 mm respectively, which indicated a 4, 3, 1, 3, 1, 3, and 2% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 186, 176, 180, 187, 182, 181, 179, and 175 g, respectively, which indicated a 5, 3, 0, 2, 3, 4, and 6% inhibition for the respective treatment levels, when compared to the control. No visual injury was observed for any species at any treatment level.

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Onion:
The application rate range for onion included a negative control, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, 57.7, 115.4, and 230.8 g a.i./ha. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 268, 247, 274, 258, 233, 259, 236, 279, 268, 213, and 234 mm respectively, which indicated a 8, -2, 4, 13, 3, 12-4, 0, 21, and 13% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 11.4, 8.41, 11.5, 9.33, 7.68, 8.97, 7.72, 12.0, 10.2, 6.04, and 7.09 g, respectively, which indicated a 26, -1 ,18, 32, 21, 32, -5, 11, 47, and 38% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 3, 0, 0, 5, 0, 2, 0, 2, 12, and 8% respectively.

Wheat:
The application rate range for wheat included a negative control, 3.61, 7.21, 14.4, 28.9, 57.7, 115.4, and 230.8 g a.i./ha. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 366, 355, 365, 381, 358, 359, 374, and 387 mm respectively, which indicated a 3, 0, -4, 2, 2, -2, and -6% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 6.24, 6.29, 6.48, 6.44, 6.09, 5.89, 6.30, and 6.73 g, respectively, which indicated a -1, -4, -3, 2, 6, -1, and -8% inhibition for the respective treatment levels, when compared to the control. Visual injury was only observed in the 28.85 and 230.8 g a.i./ha treatment levels with ratings of 3 and 2%, respectively.

Cucumber:
The application rate range for cucumber included a negative control, 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, and 57.7 g a.i./ha. The percent survival was 100% for the control and all treatment levels except the 0.056, 14.4, and 57.7 g a.i./ha treatment levels which had survival percentages of 97, 97, and 28%, respectively. The mean shoot length for the control and treatment levels was 416, 352, 385, 408, 393, 394, 409, 406, 397, 427, 205, 189, and 52.2 mm respectively, which indicated a 15, 7, 2, 6, 5, 2, 2, 5, -3, 50, 55, and 87% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 147, 127, 134, 141, 140, 140, 142, 141, 138, 135, 88.9, 90.9, and 6.09 g, respectively, which indicated a 14, 9, 4, 5, 5, 3, 4, 6, 8, 40, 38, and 96% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 0, 3, 0, 0, 0, 0, 8, 12, 28, 42, 47, and 88% respectively.

Lettuce:
The application rate range for lettuce included a negative control, 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, and 57.7 g a.i./ha. The percent survival was

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100% for the control and all treatment levels except the 14.4, 28.9, and 57.7 g a.i./ha treatment levels which had survival percentages of 92, 97, and 14%, respectively. The mean shoot length for the control and treatment levels was 157, 156, 157, 157, 159, 162, 156, 166, 166, 122, 52.1, 61.5, and 33.8 mm respectively, which indicated a 0, 0, 0, -1, -3, 0, -6, -6, 23, 67, 61, and 79% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 56.3, 56.1, 56.5, 55.7, 58.0, 58.5, 57.3, 56.4, 47.7, 22.8, 2.89, and 4.62 g, respectively, which indicated a 0, 0, 1, -3, -4, -2, 0, 15, 59, 95, and 92% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 0, 0, 0, 0, 0, 0, 2, 12, 38, 67, 65, and 92% respectively.

Soybean:
The application rate range for soybean included a negative control, 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, and 57.7 g a.i./ha. The percent survival was 100% for the control and all treatment levels except the 57.7 g a.i./ha treatment level which had a survival percentage of 70%. The mean shoot length for the control and treatment levels was 278, 296, 285, 279, 292, 268, 211, 193, 181, 121, 106, 121, and 103 mm respectively, which indicated a -7, -3, 0, -5, 4, 24, 31, 35, 57, 62, 56, and 63% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 64.9, 64.2, 62.9, 62.9, 65.8, 65.8, 54.6, 50.2, 41.4, 19.3, 15.8, 18.4, and 6.46 g, respectively, which indicated a 1, 3, 3, -1, -1, 16, 23, 36, 70, 76, 72, and 90% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 0, 0, 0, 0, 0, 0, 2, 12, 38, 67, 65, and 92% respectively.

Sugar beet:
The application rate range for sugar beet included a negative control, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, and 57.7 g a.i./ha. The percent survival was 100% for all treatment levels except the control, 7.21, 14.4, and 57.7 g a.i./ha treatment level which had survival percentages of 97, 97, 92, and 44%, respectively. The mean shoot length for the control and treatment levels was 181, 183, 175, 170, 183, 182, 180, 188, 194, 169, 178, and 132 mm respectively, which indicated a -1, 3, 6, -1, 0, 1, -4, -7, 7, 2, and 28% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 70.6, 71.7, 67.9, 66.2, 69.4, 67.9, 73.1, 70.4, 69.4, 44.1, 57.3, and 5.57 g, respectively, which indicated a -2, 4, 6, 2, 4, -4, 0, 2, 37, 19, and 92% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 2, 0, 2, 2, 2, 3, 8, 18, 35, 50, 47, and 87% respectively.

Oilseed rape:
The application rate range for oilseed rape included a negative control, 0.11, 0.23, 0.45,

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0.90, 1.80, 3.61, 7.21, 14.4, 28.9, 57.7, 115.4, and 230.8 g a.i./ha. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 292, 279, 287, 275, 284, 292, 291, 289, 283, 294, 293, 289, and 292 mm respectively, which indicated a 5, 2, 6, 3, 0, 0, 1, 3, -1, -1, 1, and 0% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 128, 125, 126, 121, 125, 129, 128, 129, 131, 131, 131, 126, and 129 g, respectively, which indicated a 2, 2, 6, 2, -1, 0, -1, -2, -2, -2, 1, and -1% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 0, 0, 0, 0, 0, 0, 0, 2, 10, 8, 20, 30, and 38% respectively.

Radish:
The application rate range for radish included a negative control, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.4, 28.9, 57.7, 115.4, and 230.8 g a.i./ha. The percent survival was 100% for the control and all treatment levels. The mean shoot length for the control and treatment levels was 179, 177, 179, 179, 189, 182, 180, 186, 193, 190, 191, 186, 180, and 162 mm respectively, which indicated a 1, 0, 0, -6, -1, 0, -4, -8, -6, -7, -10, -1, and 9% inhibition for the respective treatment levels, when compared to the control. The mean fresh weight for the control and the treatment levels was 161, 156, 133, 147, 157, 147, 154, 153, 144, 149, 141, 122, 114, and 91.7 g, respectively, which indicated a 3, 17, 9, 3, 9, 4, 5, 11, 7, 12, 24, 29, and 43% inhibition for the respective treatment levels, when compared to the control. Visual injury ratings for the control and treatment levels were 20, 0, 10, 0, 0, 3, 0, 10, 10, 12, 17, 22, 28, and 37% respectively.

Statistical Results

Statistical Method: The means and standard deviations were calculated for the percent emergence, phytotoxicity ratings, shoot length, and dry weight data. Statistical analysis of the concentration versus effect data was performed using SAS for Windows or Minitab software.

Most sensitive monocot: Onion
Most sensitive parameter: Fresh Weight
NOEC: 57.7 g a.i./ha
EC_{25}: 78.2 g a.i./ha 95% C.I.: 54.4-121 g a.i./ha
EC_{50}: >230.8 g a.i./ha 95% C.I.: N/A
Slope: Not reported

Most sensitive dicot: Soybean
Most sensitive parameter: Shoot Length
NOEC: 0.45 g a.i./ha
EC_{25}: 1.31 g a.i./ha 95% C.I.: 0.960-1.79 g a.i./ha

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EC_{50}: 7.40 g a.i./ha 95% C.I.: 4.66-14.1 g a.i./ha
Slope: Not reported

13. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Shoot length and dry weight data were analyzed to determine if they satisfied the assumptions of ANOVA (i.e., normal distribution and homogeneity of variances). If they did, the NOEC was determined using ANOVA, followed by Bonferroni's t-test (unequal replicates, non-monotonic response), Dunnett's test (equal replicates, non-monotonic response), or William's test (monotonic response). If the data did not meet these assumptions, transformations (e.g., square-root, inverse square-root, or natural log) were attempted. If these transformations were successful, the NOEC was determined using a method described above. If the transformations were not successful, the NOEC was determined using the non-parametric Kruskal-Wallis test. These analyses were conducted using TOXSTAT statistical software. The EC_{05} and EC_{25} values and their 95% confidence intervals and slopes were determined using the Probit method via Nuthatch statistical software. Toxicity values were visually estimated for species and endpoints which exhibited reductions equal to or less than 5% from the control.

Results Synopsis

Crop	Shoot Length*			Fresh Weight*			Most Sensitive Parameter
	NOEC	EC_{05}	EC_{25}	NOEC	EC_{05}	EC_{25}	
Barnyard Grass	230.8	>230.8	>230.8	230.8	ND	>230.8	None
Corn	230.8	>230.8	>230.8	230.8	>230.8	>230.8	None
Onion	1.8[a]	78	>230.8	1.8[a]	0.012	53[a]	Fresh weight
Wheat	230.8	>230.8	>230.8	230.8	>230.8	>230.8	None

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Crop	Shoot Length*			Fresh Weight*			Most Sensitive Parameter
	NOEC	EC_{05}	EC_{25}	NOEC	EC_{05}	EC_{25}	
Cucumber	7.21	5.2	12[b]	7.21	19	26[b]	Shoot length
Soybean	0.45	0.027	0.75[a]	0.45	0.22	1.4[a]	Shoot length
Sugar beet	28.9	36	56[a]	3.61[a]	0.15	8.4[a]	Fresh weight
Lettuce	3.61	1.7	6.4[a]	28.9[b]	1.4	3.3[a]	Fresh weight
Rape	230.8	>230.8	>230.8	230.8	>230.8	>230.8	None
Radish	115.4[b]	76	>115.4	7.21[a]	8.7	54[b]	Fresh weight

[a]The reviewer's estimate was lower than the study authors'.
[b] The reviewer's estimate was higher than the study authors'.
*units are g a.i./ha
ND=could not determine using the Probit method.

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EC_x Values, Confidence Intervals, and Slopes

Species	Shoot Length*					Fresh Weight*				
	EC_{05}	Confidence Interval	EC_{25}	Confidence Interval	Slope	EC_{05}	Confidence Interval	EC_{25}	Confidence Interval	Slope
Barnyard Grass	>230.8	N/A	>230.8	N/A	N/A	ND	N/A	>230.8	N/A	N/A
Corn	>230.8	N/A	>230.8	N/A	N/A	>230.8	N/A	>230.8	N/A	N/A
Onion	78	15-420	>230.8	N/A	1.06±0.813	0.012	$2.0e^{-8}$-$7.4e^{3}$	53[a]	0.4-7200	0.266±0.17
Wheat	>230.8	N/A	>230.8	N/A	N/A	>230.8	N/A	>230.8	N/A	N/A
Cucumber	5.2	3.0-9.1	12[b]	9.0-17	2.58±0.376	19	16-23	26[b]	23-30	6.82±0.74
Soybean	0.027	0.0053-0.14	0.75[a]	0.29-1.9	0.676±0.08	0.22	0.11-0.46	1.4[a]	0.92-2.2	1.20±0.1
Sugar beet	36	22-59	56[a]	51-61	4.95±2.53	0.15	0.00033-64	8.4[a]	0.75-93	0.553±0.3
Lettuce	1.7	0.99-2.8	6.4[a]	4.7-8.6	1.67±0.155	1.4	0.97-2.0	3.3[a]	2.5-4.2	2.66±0.224
Rape	>230.8	N/A	>230.8	N/A	N/A	>230.8	N/A	>230.8	N/A	N/A
Radish	76	24-240	>115.4	N/A	2.93±4.0	8.7	3.0-25	54[b]	37-79	1.22±0.28

[a] The reviewer's estimate was lower than the study authors'.
[b] The reviewer's estimate was higher than the study authors'.
*units are g a.i./ha
ND=could not determine using the Probit method.

Most sensitive dicot: Soybean
Most sensitive parameter: Shoot length
NOEC: 0.45 g a.i./ha ($4.0e^{-4}$ lb a.i./A)
EC_{05}: 0.027 g a.i./ha ($2.4e^{-5}$ lb a.i./A)
95% C.I.: 0.0053-0.14 g a.i./ha ($4.7e^{-6}$-$1.2e^{-4}$ lb a.i./A)
EC_{25}: 0.75 g a.i./ha ($6.6e^{-4}$ lb a.i./A)
95% C.I.: 0.29-1.9 g a.i./ha ($2.6e^{-4}$-$1.7e^{-3}$ lb a.i./A)
Slope: 0.676±0.0759

Most sensitive monocot: Onion
Most sensitive parameter: Fresh weight
NOEC: 1.8 g a.i./ha ($1.6e^{-3}$ lb a.i./A)
EC_{05}: 0.012 g a.i./ha ($1.0e^{-5}$ lb a.i./A)
95% C.I.: $2.0e^{-8}$-$7.4e^{3}$ g a.i./ha ($1.8e^{-11}$-6.51 lb a.i./A)
EC_{25}: 53 g a.i./ha (0.05 lb a.i./A)
95% C.I.: 0.40-7200 g a.i./ha ($3.5e^{-4}$-6.3 lb a.i./A)
Slope: 0.266±0.167

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study authors'. Soybean was the most sensitive species, based on shoot length and onion was the most sensitive monocot species based on fresh shoot weight. Differences between the reviewer's and the study authors' estimates can be attributed to the different statistical methods which were used to derive these estimates. The NOEC for onion was determined by Dunnett's test, but as evident by the confidence intervals, there was large variability in the data. Because the reviewer's analysis provided EC_{05} values and slopes for all estimates, the reviewer's values are reported in the Conclusions section. The reviewer has also provided the toxicity values for the most sensitive monocot and dicot species in units of lb a.i./A.

The definitive study for all species was conducted from August 8 to 29, 2003. The temperatures in Greenhouse 3 ranged from 19.8 to 34.9°C and the humidity ranged from 39 to 94%. The temperatures in Greenhouse 5 ranged from 19.4 to 36.1°C and the humidity ranged from 43 to 94%. The temperatures in Greenhouse 7 ranged from 17.5 to 35.7°C and the humidity ranged from 45 to 94%. The temperatures in Greenhouse 8 ranged from 18.3 to 32.8°C and the humidity ranged from 50 to 94%. Natural sunlight was supplemented with high-pressure sodium (Greenhouses 7 and 8) and metal halide (Greenhouses 3 and 5) light during the treatment exposures.

After the seeds were established, the plants were moved to an open-air propagation area and exposed to direct ambient sunlight. The open air propagation area was covered prior to and during rain events to prevent plant damage and washout of the soil.

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EAD Comments:

After review of the study data and the US EPA DER, the reviewer is in agreement with the conclusion reached by the US EPA, with the recommendation that the results for the sugar beet be omitted due to possible interference from Thiram use.

15. REFERENCES:

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APPENDIX I. OUTPUT FROM REVIEWER'S STATISTICAL VERIFICATION:

onion vv length
File: 5825il Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	25340.240	2815.582	4.886
Within (Error)	50	28811.040	576.221	
Total	59	54151.280		

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

onion vv length
File: 5825il Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	268.333	268.333		
2	0.9	274.100	274.100	-0.416	
3	1.8	257.967	257.967	0.748	
4	3.61	232.833	232.833	2.562	*
5	7.21	259.400	259.400	0.645	
6	14.43	236.200	236.200	2.319	
7	28.9	279.067	279.067	-0.774	
8	57.7	267.767	267.767	0.041	
9	115.4	213.400	213.400	3.964	*
10	230.8	234.933	234.933	2.410	

Dunnett table value = 2.51 (1 Tailed Value, P=0.05, df=40,9)

onion vv length
File: 5825il Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.9	6	34.786	13.0	-5.767
3	1.8	6	34.786	13.0	10.367
4	3.61	6	34.786	13.0	35.500
5	7.21	6	34.786	13.0	8.933
6	14.43	6	34.786	13.0	32.133
7	28.9	6	34.786	13.0	-10.733
8	57.7	6	34.786	13.0	0.567
9	115.4	6	34.786	13.0	54.933

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10	230.8	6	34.786	13.0	33.400

onion vv length
File: 5825il Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	268.333	268.333	271.217
2	0.9	6	274.100	274.100	271.217
3	1.8	6	257.967	257.967	257.967
4	3.61	6	232.833	232.833	255.053
5	7.21	6	259.400	259.400	255.053
6	14.43	6	236.200	236.200	255.053
7	28.9	6	279.067	279.067	255.053
8	57.7	6	267.767	267.767	255.053
9	115.4	6	213.400	213.400	224.167
10	230.8	6	234.933	234.933	224.167

onion vv length
File: 5825il Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	271.217				
0.9	271.217	0.208		1.68	k= 1, v=50
1.8	257.967	0.748		1.76	k= 2, v=50
3.61	255.053	0.958		1.79	k= 3, v=50
7.21	255.053	0.958		1.80	k= 4, v=50
14.43	255.053	0.958		1.80	k= 5, v=50
28.9	255.053	0.958		1.81	k= 6, v=50
57.7	255.053	0.958		1.81	k= 7, v=50
115.4	224.167	3.187	*	1.81	k= 8, v=50
230.8	224.167	3.187	*	1.82	k= 9, v=50

s = 24.005
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	78.	15.	4.2E+02	0.37	0.19
EC10	1.7E+02	69.	4.3E+02	0.20	0.40
EC25	6.5E+02	86.	4.9E+03	0.44	0.13
EC50	2.8E+03	44.	1.8E+05	0.90	0.016

Slope = 1.06 Std.Err. = 0.813

!!!Poor fit: p < 0.001 based on DF= 7.00 50.0

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5825IL : onion vv length

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	268.	259.	9.11	100.	0.00
0.900	6.00	274.	259.	14.9	100.	0.0108
1.80	6.00	258.	259.	-1.17	100.	0.0361
3.61	6.00	233.	259.	-26.1	99.9	0.110
7.21	6.00	259.	258.	0.961	99.7	0.304
14.4	6.00	236.	257.	-21.0	99.2	0.768
28.9	6.00	279.	255.	24.4	98.2	1.77
57.7	6.00	268.	250.	18.1	96.3	3.70
115.	6.00	213.	241.	-27.4	92.9	7.11
231.	6.00	235.	227.	8.19	87.5	12.5

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

Cucumber vv length
File: 5825cl Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	847242.818	94138.091	19.630
Within (Error)	49	234987.825	4795.670	
Total	58	1082230.643		

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

Cucumber vv length
File: 5825cl Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	415.556	415.556		
2	0.23	392.583	392.583	0.575	
3	0.45	394.417	394.417	0.529	
4	0.9	409.222	409.222	0.158	
5	1.8	405.639	405.639	0.248	
6	3.61	397.556	397.556	0.450	
7	7.21	426.611	426.611	-0.277	
8	14.43	204.556	204.556	5.277	*
9	28.9	188.500	188.500	5.679	*
10	57.7	52.267	52.267	8.663	*

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Bonferroni T table value = 2.66 (1 Tailed Value, P=0.05, df=40,9)

Cucumber vv length
File: 5825cl Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.23	6	106.472	25.6	22.972
3	0.45	6	106.472	25.6	21.139
4	0.9	6	106.472	25.6	6.333
5	1.8	6	106.472	25.6	9.917
6	3.61	6	106.472	25.6	18.000
7	7.21	6	106.472	25.6	-11.056
8	14.43	6	106.472	25.6	211.000
9	28.9	6	106.472	25.6	227.056
10	57.7	5	111.669	26.9	363.289

Cucumber vv length
File: 5825cl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	415.556	415.556	415.556
2	0.23	6	392.583	392.583	404.338
3	0.45	6	394.417	394.417	404.338
4	0.9	6	409.222	409.222	404.338
5	1.8	6	405.639	405.639	404.338
6	3.61	6	397.556	397.556	404.338
7	7.21	6	426.611	426.611	404.338
8	14.43	6	204.556	204.556	204.556
9	28.9	6	188.500	188.500	188.500
10	57.7	5	52.267	52.267	52.267

Cucumber vv length
File: 5825cl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	415.556				
0.23	404.338	0.281		1.68	k= 1, v=49
0.45	404.338	0.281		1.76	k= 2, v=49
0.9	404.338	0.281		1.79	k= 3, v=49
1.8	404.338	0.281		1.80	k= 4, v=49
3.61	404.338	0.281		1.80	k= 5, v=49

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7.21	404.338	0.281		1.81	k= 6, v=49
14.43	204.556	5.277	*	1.81	k= 7, v=49
28.9	188.500	5.679	*	1.81	k= 8, v=49
57.7	52.267	8.663	*	1.82	k= 9, v=49

s = 69.251
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	5.2	3.0	9.1	0.12	0.57
EC10	7.2	4.5	12.	0.10	0.63
EC25	12.	9.0	17.	0.071	0.72
EC50	23.	19.	28.	0.044	0.82

Slope = 2.58 Std.Err. = 0.376

Goodness of fit: p = 0.095 based on DF= 10. 64.

5825CLN.TXT : Cucumber vv length

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	416.	399.	16.5	100.	0.00
0.0280	6.00	352.	399.	-46.8	100.	3.11e-12
0.0560	6.00	385.	399.	-14.1	100.	8.68e-10
0.110	6.00	408.	399.	9.37	100.	1.19e-07
0.230	6.00	393.	399.	-6.46	100.	1.35e-05
0.450	6.00	394.	399.	-4.63	100.	0.000564
0.900	6.00	409.	399.	10.2	100.	0.0151
1.80	6.00	406.	398.	7.50	99.8	0.227
3.61	6.00	398.	391.	6.42	98.0	1.98
7.21	6.00	427.	359.	67.4	90.0	9.99
14.4	6.00	205.	277.	-72.0	69.3	30.7
28.9	6.00	189.	157.	32.0	39.2	60.8
57.7	5.00	52.3	58.7	-6.48	14.7	85.3

soybean vv height
File: 5825sl Transform: NATURAL LOG(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	8.629	0.959	53.278
Within (Error)	49	0.884	0.018	
Total	58	9.512		

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

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soybean vv height
File: 5825sl Transform: NATURAL LOG(Y)

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	5.620	277.500		
2	0.23	5.659	291.944	-0.500	
3	0.45	5.578	267.611	0.549	
4	0.9	5.344	210.833	3.565	*
5	1.8	5.259	193.194	4.664	*
6	3.61	5.190	181.472	5.554	*
7	7.21	4.791	120.722	10.707	*
8	14.43	4.662	105.917	12.364	*
9	28.9	4.789	121.417	10.735	*
10	57.7	4.627	102.427	12.226	*

Bonferroni T table value = 2.66 (1 Tailed Value, P=0.05, df=40,9)

soybean vv height
File: 5825sl Transform: NATURAL LOG(Y)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.23	6	51.430	18.5	-14.444
3	0.45	6	51.430	18.5	9.889
4	0.9	6	51.430	18.5	66.667
5	1.8	6	51.430	18.5	84.306
6	3.61	6	51.430	18.5	96.028
7	7.21	6	51.430	18.5	156.778
8	14.43	6	51.430	18.5	171.583
9	28.9	6	51.430	18.5	156.083
10	57.7	5	53.679	19.3	175.073

soybean vv height
File: 5825sl Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	277.500	5.620	5.640
2	0.23	6	291.944	5.659	5.640
3	0.45	6	267.611	5.578	5.578
4	0.9	6	210.833	5.344	5.344
5	1.8	6	193.194	5.259	5.259
6	3.61	6	181.472	5.190	5.190
7	7.21	6	120.722	4.791	4.791
8	14.43	6	105.917	4.662	4.726

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9	28.9	6	121.417	4.789	4.726
10	57.7	5	102.427	4.627	4.627

soybean vv height
File: 5825sl Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	5.640				
0.23	5.640	0.250		1.68	k= 1, v=49
0.45	5.578	0.549		1.76	k= 2, v=49
0.9	5.344	3.562	*	1.79	k= 3, v=49
1.8	5.259	4.660	*	1.80	k= 4, v=49
3.61	5.190	5.549	*	1.80	k= 5, v=49
7.21	4.791	10.698	*	1.81	k= 6, v=49
14.43	4.726	11.540	*	1.81	k= 7, v=49
28.9	4.726	11.540	*	1.81	k= 8, v=49
57.7	4.627	12.215	*	1.82	k= 9, v=49

s = 0.134
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.027	0.0053	0.14	0.36	0.20
EC10	0.094	0.024	0.37	0.30	0.25
EC25	0.75	0.29	1.9	0.20	0.39
EC50	7.4	4.4	13.	0.11	0.59

Slope = 0.676 Std.Err. = 0.0759

!!!Poor fit: p < 0.001 based on DF= 10.0 64.0

5825SLN.TXT : soybean vv height

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	277.	306.	-28.3	100.	0.00
0.0280	6.00	296.	290.	5.28	94.9	5.07
0.0560	6.00	285.	283.	2.81	92.4	7.57
0.110	6.00	279.	273.	5.91	89.2	10.8
0.230	6.00	292.	259.	33.2	84.6	15.4
0.450	6.00	268.	243.	24.6	79.5	20.5
0.900	6.00	211.	224.	-13.0	73.2	26.8
1.80	6.00	193.	202.	-8.96	66.1	33.9
3.61	6.00	181.	178.	3.02	58.4	41.6
7.21	6.00	121.	154.	-33.1	50.3	49.7
14.4	6.00	106.	129.	-23.2	42.2	57.8
28.9	6.00	121.	105.	16.0	34.5	65.5
57.7	5.00	102.	83.6	18.9	27.3	72.7

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!!!Warning: EC5 not bracketed by doses evaluated.

sugarbeet vv height
File: 5825bl Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	181.394	181.394	211.500
2	0.23	169.917	169.917	133.000
3	0.45	183.389	183.389	222.000
4	0.9	181.944	181.944	221.000
5	1.8	179.583	179.583	199.000
6	3.61	187.694	187.694	257.500
7	7.21	193.600	193.600	245.000
8	14.43	169.361	169.361	129.000
9	28.9	177.833	177.833	191.000
10	57.7	131.739	131.739	21.000

Calculated H Value = 24.688 Critical H Value Table = 16.920
Since Calc H > Crit H REJECT Ho:All groups are equal.

sugarbeet vv height
File: 5825bl Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON-- KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 1 0 0 0 0 0 0 0 0 0 0 / 0 8 2 9 5 1 4 3 6 7
10	57.7	131.739	131.739	\
8	14.43	169.361	169.361	. \
2	0.23	169.917	169.917	. . \
9	28.9	177.833	177.833	. . . \
5	1.8	179.583	179.583	. . . . \
1	control	181.394	181.394	. . . . . \
4	0.9	181.944	181.944	* . . . . . \
3	0.45	183.389	183.389	* . . . . . \
6	3.61	187.694	187.694	* . . . . . \
7	7.21	193.600	193.600	* . . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,10) = 3.261 SE = 10.083

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	36.	22.	59.	0.11	0.61
EC10	42.	30.	59.	0.071	0.72
EC25	56.	51.	61.	0.019	0.92
EC50	77.	57.	1.0E+02	0.066	0.74

Slope = 4.95 Std.Err. = 2.53

333

Goodness of fit: p = 0.10 based on DF= 9.0 60.

5825BLN.TXT : sugarbeet vv height

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	181.	181.	0.663	100.	0.00
0.0560	6.00	183.	181.	2.24	100.	1.57e-14
0.110	6.00	177.	181.	-3.65	100.	1.57e-14
0.230	6.00	170.	181.	-10.8	100.	1.57e-14
0.450	6.00	183.	181.	2.66	100.	1.57e-14
0.900	6.00	182.	181.	1.21	100.	1.57e-14
1.80	6.00	180.	181.	-1.15	100.	3.15e-14
3.61	6.00	188.	181.	6.96	100.	2.62e-09
7.21	6.00	194.	181.	12.9	100.	1.91e-05
14.4	6.00	169.	181.	-11.3	100.	0.0167
28.9	6.00	178.	177.	0.368	98.2	1.81
57.7	6.00	132.	132.	-0.0190	72.9	27.1

!!!Warning: EC50 not bracketed by doses evaluated.

lettuce vv height
File: 582511 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	144732.182	16081.354	99.360
Within (Error)	49	7930.633	161.850	
Total	58	152662.815		

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

lettuce vv height
File: 582511 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	157.083	157.083		
2	0.23	158.778	158.778	-0.231	
3	0.45	162.444	162.444	-0.730	
4	0.9	155.583	155.583	0.204	
5	1.8	165.556	165.556	-1.153	
6	3.61	166.083	166.083	-1.225	
7	7.21	121.722	121.722	4.814	*
8	14.43	52.128	52.128	14.289	*
9	28.9	61.522	61.522	13.010	*
10	57.7	33.800	33.800	16.003	*

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Bonferroni T table value = 2.66 (1 Tailed Value, P=0.05, df=40,9)

lettuce vv height
File: 582511 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.23	6	19.560	12.5	-1.694
3	0.45	6	19.560	12.5	-5.361
4	0.9	6	19.560	12.5	1.500
5	1.8	6	19.560	12.5	-8.472
6	3.61	6	19.560	12.5	-9.000
7	7.21	6	19.560	12.5	35.361
8	14.43	6	19.560	12.5	104.956
9	28.9	6	19.560	12.5	95.561
10	57.7	5	20.515	13.1	123.283

lettuce vv height
File: 582511 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	157.083	157.083	160.921
2	0.23	6	158.778	158.778	160.921
3	0.45	6	162.444	162.444	160.921
4	0.9	6	155.583	155.583	160.921
5	1.8	6	165.556	165.556	160.921
6	3.61	6	166.083	166.083	160.921
7	7.21	6	121.722	121.722	121.722
8	14.43	6	52.128	52.128	56.825
9	28.9	6	61.522	61.522	56.825
10	57.7	5	33.800	33.800	33.800

lettuce vv height
File: 582511 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	160.921				
0.23	160.921	0.523		1.68	k= 1, v=49
0.45	160.921	0.523		1.76	k= 2, v=49
0.9	160.921	0.523		1.79	k= 3, v=49
1.8	160.921	0.523		1.80	k= 4, v=49

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3.61	160.921	0.523		1.80	k= 5, v=49
7.21	121.722	4.814	*	1.81	k= 6, v=49
14.43	56.825	13.650	*	1.81	k= 7, v=49
28.9	56.825	13.650	*	1.81	k= 8, v=49
57.7	33.800	16.003	*	1.82	k= 9, v=49

s = 12.722
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.7	0.99	2.8	0.12	0.59
EC10	2.8	1.8	4.3	0.097	0.64
EC25	6.4	4.7	8.6	0.066	0.74
EC50	16.	13.	19.	0.040	0.83

Slope = 1.67 Std.Err. = 0.155

!!!Poor fit: p < 0.001 based on DF= 10.0 64.0

5825LLN.TXT : lettuce vv height

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	157.	162.	-4.49	100.	0.00
0.0280	6.00	156.	162.	-5.10	100.	0.000192
0.0560	6.00	157.	162.	-4.38	100.	0.00192
0.110	6.00	157.	162.	-4.89	100.	0.0144
0.230	6.00	159.	161.	-2.64	99.9	0.100
0.450	6.00	162.	161.	1.62	99.5	0.465
0.900	6.00	156.	159.	-3.08	98.2	1.80
1.80	6.00	166.	153.	13.0	94.4	5.56
3.61	6.00	166.	139.	26.9	86.2	13.8
7.21	6.00	122.	116.	5.32	72.0	28.0
14.4	6.00	52.1	85.9	-33.8	53.2	46.8
28.9	6.00	61.5	54.2	7.35	33.5	66.5
57.7	5.00	33.8	28.5	5.25	17.7	82.3

radish vv height
File: 5825rl Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	179.111	179.111	155.000
2	0.45	179.917	179.917	149.000
3	0.9	179.889	179.889	150.000
4	1.8	185.611	185.611	193.000
5	3.61	193.278	193.278	249.000
6	7.21	189.556	189.556	227.000
7	14.43	190.667	190.667	242.000
8	28.9	196.417	196.417	267.000
9	57.7	179.944	179.944	156.000
10	115.4	162.361	162.361	42.000

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Calculated H Value = 22.210 Critical H Value Table = 16.920
Since Calc H > Crit H REJECT Ho:All groups are equal.

radish vv height
File: 5825rl Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 1 0 0 0 0 0 0 0 0 0 0 / 0 1 3 2 9 4 6 7 5 8
10	115.4	162.361	162.361	\
1	control	179.111	179.111	. \
3	0.9	179.889	179.889	. . \
2	0.45	179.917	179.917	. . . \
9	57.7	179.944	179.944	. . . . \
4	1.8	185.611	185.611	. . . . . \
6	7.21	189.556	189.556	. . . . . \
7	14.43	190.667	190.667	* . . . . . \
5	3.61	193.278	193.278	* . . . . . \
8	28.9	196.417	196.417	* . . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,10) = 3.261 SE = 10.082

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	76.	24.	2.4E+02	0.25	0.32
EC10	1.0E+02	61.	1.7E+02	0.11	0.59
EC25	1.6E+02	54.	5.0E+02	0.24	0.33
EC50	2.8E+02	23.	3.4E+03	0.54	0.083

Slope = 2.93 Std.Err. = 4.00

Goodness of fit: p = 0.14 based on DF= 11. 70.

5825RLN.TXT : radish vv height

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	179.	187.	-7.42	100.	0.00
0.0280	6.00	177.	187.	-9.31	100.	1.52e-14
0.0560	6.00	167.	187.	-19.1	100.	1.52e-14
0.110	6.00	179.	187.	-7.97	100.	1.52e-14
0.230	6.00	222.	187.	35.9	100.	1.52e-14
0.450	6.00	180.	187.	-6.61	100.	1.52e-14
0.900	6.00	180.	187.	-6.64	100.	1.45e-11
1.80	6.00	186.	187.	-0.918	100.	6.94e-09
3.61	6.00	193.	187.	6.75	100.	1.59e-06
7.21	6.00	190.	187.	3.03	100.	0.000165

337

14.4	6.00	191.	187.	4.15	100.	0.00823
28.9	6.00	196.	186.	10.3	99.8	0.196
57.7	6.00	180.	182.	-2.38	97.7	2.25
115.	6.00	162.	162.	0.272	86.9	13.1

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

barnyard grass vv weight
File: 5825gw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	26.587	3.798	0.600
Within (Error)	40	253.210	6.330	
Total	47	279.797		

Critical F value = 2.25 (0.05,7,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

barnyard grass vv weight
File: 5825gw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	26.721	26.721		
2	3.61	25.629	25.629	0.752	
3	7.21	24.798	24.798	1.324	
4	14.43	25.849	25.849	0.600	
5	28.9	25.109	25.109	1.110	
6	57.7	27.161	27.161	-0.303	
7	115.8	25.540	25.540	0.813	
8	230.8	26.251	26.251	0.323	

Dunnett table value = 2.42 (1 Tailed Value, P=0.05, df=40,7)

barnyard grass vv weight
File: 5825gw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	3.61	6	3.515	13.2	1.092
3	7.21	6	3.515	13.2	1.923
4	14.43	6	3.515	13.2	0.872

338

5	28.9	6	3.515	13.2	1.612
6	57.7	6	3.515	13.2	-0.440
7	115.8	6	3.515	13.2	1.181
8	230.8	6	3.515	13.2	0.470

barnyard grass vv weight
File: 5825gw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	26.721	26.721	26.721
2	3.61	6	25.629	25.629	25.762
3	7.21	6	24.798	24.798	25.762
4	14.43	6	25.849	25.849	25.762
5	28.9	6	25.109	25.109	25.762
6	57.7	6	27.161	27.161	25.762
7	115.8	6	25.540	25.540	25.762
8	230.8	6	26.251	26.251	25.762

barnyard grass vv weight
File: 5825gw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	26.721				
3.61	25.762	0.660		1.68	k= 1, v=40
7.21	25.762	0.660		1.76	k= 2, v=40
14.43	25.762	0.660		1.79	k= 3, v=40
28.9	25.762	0.660		1.80	k= 4, v=40
57.7	25.762	0.660		1.80	k= 5, v=40
115.8	25.762	0.660		1.81	k= 6, v=40
230.8	25.762	0.660		1.81	k= 7, v=40

s = 2.516
Note: df used for table values are approximate when v > 20.

ECx

!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.

corn vv weight
File: 5825cw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F

339

Between	7	732.253	104.608	0.964
Within (Error)	40	4339.698	108.492	
Total	47	5071.951		

Critical F value = 2.25 (0.05,7,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

corn vv weight
File: 5825cw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	186.432	186.432		
2	3.61	176.290	176.290	1.686	
3	7.21	180.101	180.101	1.053	
4	14.43	186.791	186.791	-0.060	
5	28.9	181.643	181.643	0.796	
6	57.7	180.568	180.568	0.975	
7	115.8	179.246	179.246	1.195	
8	230.8	175.285	175.285	1.854	

Dunnett table value = 2.42 (1 Tailed Value, P=0.05, df=40,7)

corn vv weight
File: 5825cw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	3.61	6	14.553	7.8	10.142
3	7.21	6	14.553	7.8	6.330
4	14.43	6	14.553	7.8	-0.360
5	28.9	6	14.553	7.8	4.788
6	57.7	6	14.553	7.8	5.864
7	115.8	6	14.553	7.8	7.186
8	230.8	6	14.553	7.8	11.147

corn vv weight
File: 5825cw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	186.432	186.432	186.432

340

2	3.61	6	176.290	176.290	181.206
3	7.21	6	180.101	180.101	181.206
4	14.43	6	186.791	186.791	181.206
5	28.9	6	181.643	181.643	181.206
6	57.7	6	180.568	180.568	180.568
7	115.8	6	179.246	179.246	179.246
8	230.8	6	175.285	175.285	175.285

corn vv weight
File: 5825cw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	186.432				
3.61	181.206	0.869		1.68	k= 1, v=40
7.21	181.206	0.869		1.76	k= 2, v=40
14.43	181.206	0.869		1.79	k= 3, v=40
28.9	181.206	0.869		1.80	k= 4, v=40
57.7	180.568	0.975		1.80	k= 5, v=40
115.8	179.246	1.195		1.81	k= 6, v=40
230.8	175.285	1.854	*	1.81	k= 7, v=40

s = 10.416
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	7.0E+02	0.024	2.0E+07	2.2	3.5E-05
EC10	2.0E+05	1.8E-05	2.2E+15	5.0	9.1E-11
EC25	2.5E+09	4.6E-13	1.4E+31	11.	1.8E-22
EC50	9.2E+13	7.0E-22	1.2E+49	17.	7.7E-36

Slope = 0.148 Std.Err. = 0.218

Goodness of fit: p = 0.50 based on DF= 5.0 40.

5825CW : corn vv weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	186.	186.	0.311	100.	0.00
3.61	6.00	176.	182.	-5.43	97.6	2.37
7.21	6.00	180.	181.	-1.13	97.4	2.63
14.4	6.00	187.	181.	6.08	97.1	2.91
28.9	6.00	182.	180.	1.51	96.8	3.22
57.7	6.00	181.	180.	1.06	96.4	3.55
116.	6.00	179.	179.	0.414	96.1	3.92
231.	6.00	175.	178.	-2.82	95.7	4.31

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

onion vv weight
File: 5825iw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	203.112	22.568	4.637
Within (Error)	50	243.365	4.867	
Total	59	446.477		

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

onion vv weight
File: 5825iw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	11.367	11.367		
2	0.45	8.412	8.412	2.321	
3	0.9	11.493	11.493	-0.099	
4	1.8	9.326	9.326	1.602	
5	3.61	7.676	7.676	2.898	*
6	7.21	8.969	8.969	1.883	
7	14.43	7.717	7.717	2.866	*
8	28.9	11.987	11.987	-0.487	
9	57.7	10.235	10.235	0.889	
10	115.8	6.039	6.039	4.184	*

Dunnett table value = 2.51 (1 Tailed Value, P=0.05, df=40,9)

onion vv weight
File: 5825iw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.45	6	3.197	28.1	2.956
3	0.9	6	3.197	28.1	-0.126
4	1.8	6	3.197	28.1	2.041

5	3.61	6	3.197	28.1	3.691
6	7.21	6	3.197	28.1	2.399
7	14.43	6	3.197	28.1	3.650
8	28.9	6	3.197	28.1	-0.620
9	57.7	6	3.197	28.1	1.132
10	115.8	6	3.197	28.1	5.329

onion vv weight
File: 5825iw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	11.367	11.367	11.367
2	0.45	6	8.412	8.412	9.952
3	0.9	6	11.493	11.493	9.952
4	1.8	6	9.326	9.326	9.326
5	3.61	6	7.676	7.676	9.317
6	7.21	6	8.969	8.969	9.317
7	14.43	6	7.717	7.717	9.317
8	28.9	6	11.987	11.987	9.317
9	57.7	6	10.235	10.235	9.317
10	115.8	6	6.039	6.039	6.039

onion vv weight
File: 5825iw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	11.367				
0.45	9.952	1.111		1.68	k= 1, v=50
0.9	9.952	1.111		1.76	k= 2, v=50
1.8	9.326	1.602		1.79	k= 3, v=50
3.61	9.317	1.610		1.80	k= 4, v=50
7.21	9.317	1.610		1.80	k= 5, v=50
14.43	9.317	1.610		1.81	k= 6, v=50
28.9	9.317	1.610		1.81	k= 7, v=50
57.7	9.317	1.610		1.81	k= 8, v=50
115.8	6.039	4.183	*	1.82	k= 9, v=50

s = 2.206
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.012	2.0E-08	7.4E+03	2.9	1.7E-06
EC10	0.28	1.7E-05	4.5E+03	2.1	6.2E-05
EC25	53.	0.40	7.2E+03	1.1	0.0074
EC50	1.8E+04	18.	1.9E+07	1.5	0.00097

Slope = 0.266 Std.Err. = 0.167

!!!Poor fit: p < 0.001 based on DF= 8.00 55.0

5825IW : onion vv weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	11.4	11.1	0.228	100.	0.00
0.450	6.00	8.41	9.92	-1.50	89.0	11.0
0.900	6.00	11.5	9.74	1.75	87.4	12.6
1.80	6.00	9.33	9.55	-0.220	85.7	14.3
3.61	6.00	7.68	9.33	-1.66	83.8	16.2
7.21	6.00	8.97	9.11	-0.139	81.8	18.2
14.4	6.00	7.72	8.86	-1.14	79.6	20.4
28.9	6.00	12.0	8.60	3.39	77.2	22.8
57.7	6.00	10.2	8.32	1.91	74.7	25.3
116.	6.00	6.04	8.03	-1.99	72.1	27.9
231.	6.00	7.09	7.72	-0.628	69.3	30.7

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

cucumber vv weight
File: 5825uw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	93655.747	10406.194	35.826
Within (Error)	49	14232.836	290.466	
Total	58	107888.583		

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

cucumber vv weight
File: 5825uw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	147.173	147.173		
2	0.23	139.916	139.916	0.737	
3	0.45	140.076	140.076	0.721	
4	0.9	141.975	141.975	0.528	

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5	1.8	140.666	140.666	0.661	
6	3.61	138.162	138.162	0.916	
7	7.21	134.860	134.860	1.251	
8	14.43	88.889	88.889	5.923	*
9	28.9	90.939	90.939	5.715	*
10	57.7	6.087	6.087	13.671	*

Bonferroni T table value = 2.66 (1 Tailed Value, P=0.05, df=40,9)

cucumber vv weight
File: 5825uw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.23	6	26.203	17.8	7.257
3	0.45	6	26.203	17.8	7.097
4	0.9	6	26.203	17.8	5.198
5	1.8	6	26.203	17.8	6.507
6	3.61	6	26.203	17.8	9.011
7	7.21	6	26.203	17.8	12.313
8	14.43	6	26.203	17.8	58.284
9	28.9	6	26.203	17.8	56.234
10	57.7	5	27.482	18.7	141.086

cucumber vv weight
File: 5825uw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	147.173	147.173	147.173
2	0.23	6	139.916	139.916	140.658
3	0.45	6	140.076	140.076	140.658
4	0.9	6	141.975	141.975	140.658
5	1.8	6	140.666	140.666	140.658
6	3.61	6	138.162	138.162	138.162
7	7.21	6	134.860	134.860	134.860
8	14.43	6	88.889	88.889	89.914
9	28.9	6	90.939	90.939	89.914
10	57.7	5	6.087	6.087	6.087

cucumber vv weight
File: 5825uw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

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control	147.173				
0.23	140.658	0.662		1.68	k= 1, v=49
0.45	140.658	0.662		1.76	k= 2, v=49
0.9	140.658	0.662		1.79	k= 3, v=49
1.8	140.658	0.662		1.80	k= 4, v=49
3.61	138.162	0.916		1.80	k= 5, v=49
7.21	134.860	1.251		1.81	k= 6, v=49
14.43	89.914	5.819	*	1.81	k= 7, v=49
28.9	89.914	5.819	*	1.81	k= 8, v=49
57.7	6.087	13.671	*	1.82	k= 9, v=49

s = 17.043
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	19.	16.	23.	0.039	0.84
EC10	21.	18.	25.	0.034	0.86
EC25	26.	23.	30.	0.026	0.89
EC50	33.	30.	36.	0.019	0.92

Slope = 6.82 Std.Err. = 0.740

!!!Poor fit: p < 0.001 based on DF= 10.0 64.0

5825UW : cucumber vv weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	147.	134.	12.9	100.	0.00
0.0280	6.00	127.	134.	-7.52	100.	2.12e-14
0.0560	6.00	134.	134.	-0.261	100.	2.12e-14
0.110	6.00	141.	134.	6.83	100.	2.12e-14
0.230	6.00	140.	134.	5.61	100.	2.12e-14
0.450	6.00	140.	134.	5.77	100.	2.12e-14
0.900	6.00	142.	134.	7.67	100.	2.12e-14
1.80	6.00	141.	134.	6.36	100.	2.12e-14
3.61	6.00	138.	134.	3.86	100.	2.78e-09
7.21	6.00	135.	134.	0.554	100.	0.000330
14.4	6.00	88.9	133.	-44.5	99.3	0.711
28.9	6.00	90.9	87.8	3.17	65.4	34.6
57.7	5.00	6.09	6.61	-0.521	4.92	95.1

soybean vv weight
File: 5825sw Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	229.595	25.511	114.399
Within (Error)	50	11.147	0.223	

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Total 59 240.741

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

soybean vv weight
File: 5825sw Transform: SQUARE ROOT(Y)

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	8.055	64.939		
2	0.23	8.109	65.842	-0.197	
3	0.45	8.109	65.820	-0.200	
4	0.9	7.377	54.638	2.488	
5	1.8	7.072	50.248	3.606	*
6	3.61	6.402	41.372	6.062	*
7	7.21	4.373	19.282	13.505	*
8	14.43	3.971	15.789	14.981	*
9	28.9	4.222	18.417	14.058	*
10	57.7	2.533	6.457	20.252	*

Dunnett table value = 2.51 (1 Tailed Value, P=0.05, df=40,9)

soybean vv weight
File: 5825sw Transform: SQUARE ROOT(Y)

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.23	6	10.556	16.3	-0.903
3	0.45	6	10.556	16.3	-0.880
4	0.9	6	10.556	16.3	10.302
5	1.8	6	10.556	16.3	14.692
6	3.61	6	10.556	16.3	23.568
7	7.21	6	10.556	16.3	45.658
8	14.43	6	10.556	16.3	49.151
9	28.9	6	10.556	16.3	46.523
10	57.7	6	10.556	16.3	58.482

soybean vv weight
File: 5825sw Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	64.939	8.055	8.091

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2	0.23	6	65.842	8.109	8.091
3	0.45	6	65.820	8.109	8.091
4	0.9	6	54.638	7.377	7.377
5	1.8	6	50.248	7.072	7.072
6	3.61	6	41.372	6.402	6.402
7	7.21	6	19.282	4.373	4.373
8	14.43	6	15.789	3.971	4.096
9	28.9	6	18.417	4.222	4.096
10	57.7	6	6.457	2.533	2.533

soybean vv weight
File: 5825sw Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	8.091				
0.23	8.091	0.132		1.68	k= 1, v=50
0.45	8.091	0.132		1.76	k= 2, v=50
0.9	7.377	2.488	*	1.79	k= 3, v=50
1.8	7.072	3.606	*	1.80	k= 4, v=50
3.61	6.402	6.063	*	1.80	k= 5, v=50
7.21	4.373	13.507	*	1.81	k= 6, v=50
14.43	4.096	14.522	*	1.81	k= 7, v=50
28.9	4.096	14.522	*	1.81	k= 8, v=50
57.7	2.533	20.256	*	1.82	k= 9, v=50

s = 0.472
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.22	0.11	0.46	0.16	0.49
EC10	0.45	0.24	0.83	0.13	0.54
EC25	1.4	0.92	2.2	0.097	0.64
EC50	5.2	3.9	6.9	0.062	0.75

Slope = 1.20 Std.Err. = 0.0950

!!!Poor fit: p < 0.001 based on DF= 10.0 65.0

5825SW : soybean vv weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	64.9	66.3	-1.35	100.	0.00
0.0280	6.00	64.2	66.1	-1.86	99.7	0.314
0.0560	6.00	62.9	65.7	-2.78	99.1	0.888
0.110	6.00	62.9	64.8	-1.97	97.8	2.18
0.230	6.00	65.8	62.9	2.95	94.9	5.14
0.450	6.00	65.8	59.7	6.16	90.0	10.0

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0.900	6.00	54.6	54.4	0.220	82.1	17.9
1.80	6.00	50.2	47.1	3.11	71.1	28.9
3.61	6.00	41.4	38.2	3.17	57.6	42.4
7.21	6.00	19.3	28.7	-9.41	43.3	56.7
14.4	6.00	15.8	19.7	-3.93	29.7	70.3
28.9	6.00	18.4	12.3	6.13	18.5	81.5
57.7	6.00	6.46	6.93	-0.470	10.4	89.6

sugarbeet vv weight
File: 5825bw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	23769.733	2641.081	22.967
Within (Error)	50	5749.785	114.996	
Total	59	29519.519		

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

sugarbeet vv weight
File: 5825bw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	70.550	70.550		
2	0.23	69.397	69.397	0.186	
3	0.45	67.853	67.853	0.436	
4	0.9	73.059	73.059	-0.405	
5	1.8	70.368	70.368	0.029	
6	3.61	69.370	69.370	0.191	
7	7.21	44.062	44.062	4.278	*
8	14.43	57.295	57.295	2.141	
9	28.9	5.573	5.573	10.495	*
10	57.7	70.550	70.550	0.000	

Dunnett table value = 2.51 (1 Tailed Value, P=0.05, df=40,9)

sugarbeet vv weight
File: 5825bw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.23	6	15.540	22.0	1.153
3	0.45	6	15.540	22.0	2.698

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4	0.9	6	15.540	22.0	-2.509
5	1.8	6	15.540	22.0	0.182
6	3.61	6	15.540	22.0	1.180
7	7.21	6	15.540	22.0	26.488
8	14.43	6	15.540	22.0	13.255
9	28.9	6	15.540	22.0	64.977
10	57.7	6	15.540	22.0	0.000

sugarbeet vv weight
File: 5825bw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	70.550	70.550	58.614
2	0.23	6	69.397	69.397	58.614
3	0.45	6	67.853	67.853	58.614
4	0.9	6	73.059	73.059	58.614
5	1.8	6	70.368	70.368	58.614
6	3.61	6	69.370	69.370	58.614
7	7.21	6	44.062	44.062	58.614
8	14.43	6	57.295	57.295	58.614
9	28.9	6	5.573	5.573	58.614
10	57.7	6	70.550	70.550	70.550

sugarbeet vv weight
File: 5825bw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	58.614				
0.23	58.614	1.928	*	1.68	k= 1, v=50
0.45	58.614	1.928	*	1.76	k= 2, v=50
0.9	58.614	1.928	*	1.79	k= 3, v=50
1.8	58.614	1.928	*	1.80	k= 4, v=50
3.61	58.614	1.928	*	1.80	k= 5, v=50
7.21	58.614	1.928	*	1.81	k= 6, v=50
14.43	58.614	1.928	*	1.81	k= 7, v=50
28.9	58.614	1.928	*	1.81	k= 8, v=50
57.7	70.550	0.000		1.82	k= 9, v=50

s = 10.724
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.15	0.00033	64.	1.3	0.0023
EC10	0.67	0.0065	69.	1.0	0.0097
EC25	8.4	0.75	93.	0.53	0.090

350

EC50 1.4E+02 21. 9.2E+02 0.41 0.15

Slope = 0.553 Std.Err. = 0.279

!!!Poor fit: p < 0.001 based on DF= 10.0 65.0

5825BW : sugarbeet vv weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	70.6	73.0	-2.49	100.	0.00
0.0280	6.00	71.7	71.5	0.126	97.9	2.06
0.0560	6.00	67.9	70.8	-2.94	97.0	3.03
0.110	6.00	66.2	69.9	-3.70	95.7	4.33
0.230	6.00	69.4	68.5	0.895	93.8	6.22
0.450	6.00	67.9	66.9	0.978	91.6	8.44
0.900	6.00	73.1	64.8	8.29	88.7	11.3
1.80	6.00	70.4	62.2	8.17	85.2	14.8
3.61	6.00	69.4	59.1	10.2	80.9	19.1
7.21	6.00	44.1	55.6	-11.5	76.1	23.9
14.4	6.00	57.3	51.6	5.69	70.7	29.3
28.9	6.00	5.57	47.2	-41.7	64.7	35.3
57.7	6.00	70.6	42.6	27.9	58.3	41.7

!!!Warning: EC50 not bracketed by doses evaluated.

lettuce vv weight
File: 58251w Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	56.280	56.280	253.000
2	0.23	58.044	58.044	259.000
3	0.45	58.534	58.534	278.000
4	0.9	57.282	57.282	257.000
5	1.8	55.370	55.370	248.000
6	3.61	47.653	47.653	140.000
7	7.21	22.784	22.784	123.000
8	14.43	2.887	2.887	63.000
9	28.9	4.624	4.624	75.000
10	57.7	0.214	0.214	15.000

Calculated H Value = 47.687 Critical H Value Table = 16.920
Since Calc H > Crit H REJECT Ho:All groups are equal.

lettuce vv weight
File: 58251w Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

		GROUP
TRANSFORMED	ORIGINAL	1 0 0 0 0 0 0 0 0 0

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GROUP	IDENTIFICATION	MEAN	MEAN	0 8 9 7 6 5 1 4 2 3
10	57.7	0.214	0.214	\
8	14.43	2.887	2.887	. \
9	28.9	4.624	4.624	. . \
7	7.21	22.784	22.784	. . . \
6	3.61	47.653	47.653	. . . . \
5	1.8	55.370	55.370	* . . . . \
1	control	56.280	56.280	* . . . . . \
4	0.9	57.282	57.282	* * . . . . . \
2	0.23	58.044	58.044	* * . . . . . \
3	0.45	58.534	58.534	* * * . . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,10) = 3.261 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.4	0.97	2.0	0.082	0.69
EC10	1.9	1.4	2.7	0.071	0.72
EC25	3.3	2.5	4.2	0.054	0.78
EC50	5.8	4.9	6.9	0.037	0.84

Slope = 2.66 Std.Err. = 0.224

!!!Poor fit: p < 0.001 based on DF= 10.0 63.0

5825LW : lettuce vv weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	56.3	57.8	-1.47	100.	0.00
0.0280	6.00	56.1	57.8	-1.66	100.	3.43e-08
0.0560	6.00	56.5	57.8	-1.23	100.	3.98e-06
0.110	6.00	55.7	57.8	-2.03	100.	0.000224
0.230	6.00	58.0	57.7	0.299	100.	0.00936
0.450	6.00	58.5	57.7	0.872	99.8	0.154
0.900	6.00	57.3	56.9	0.422	98.5	1.54
1.80	6.00	55.4	52.7	2.65	91.3	8.72
3.61	5.00	47.7	41.0	6.64	71.0	29.0
7.21	6.00	22.8	23.3	-0.497	40.3	59.7
14.4	6.00	2.89	8.52	-5.63	14.8	85.2
28.9	6.00	4.62	1.86	2.77	3.22	96.8
57.7	5.00	0.214	0.233	-0.0194	0.404	99.6

radish vv weight
File: 5825rw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	25673.684	2852.632	8.443

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Within (Error)	50	16893.235	337.865
Total	59	42566.919	

Critical F value = 2.12 (0.05,9,40)
Since F > Critical F REJECT Ho:All groups equal

radish vv weight
File: 5825rw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	161.468	161.468		
2	0.45	147.492	147.492	1.317	
3	0.9	154.476	154.476	0.659	
4	1.8	153.103	153.103	0.788	
5	3.61	144.359	144.359	1.612	
6	7.21	149.282	149.282	1.148	
7	14.43	140.995	140.995	1.929	
8	28.9	122.042	122.042	3.715	*
9	57.7	114.170	114.170	4.457	*
10	115.4	91.747	91.747	6.570	*

Dunnett table value = 2.51 (1 Tailed Value, P=0.05, df=40,9)

radish vv weight
File: 5825rw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	0.45	6	26.637	16.5	13.975
3	0.9	6	26.637	16.5	6.991
4	1.8	6	26.637	16.5	8.364
5	3.61	6	26.637	16.5	17.109
6	7.21	6	26.637	16.5	12.186
7	14.43	6	26.637	16.5	20.473
8	28.9	6	26.637	16.5	39.426
9	57.7	6	26.637	16.5	47.298
10	115.4	6	26.637	16.5	69.721

radish vv weight
File: 5825rw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

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1	control	6	161.468	161.468	161.468
2	0.45	6	147.492	147.492	151.691
3	0.9	6	154.476	154.476	151.691
4	1.8	6	153.103	153.103	151.691
5	3.61	6	144.359	144.359	146.820
6	7.21	6	149.282	149.282	146.820
7	14.43	6	140.995	140.995	140.995
8	28.9	6	122.042	122.042	122.042
9	57.7	6	114.170	114.170	114.170
10	115.4	6	91.747	91.747	91.747

radish vv weight
File: 5825rw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	161.468				
0.45	151.691	0.921		1.68	k= 1, v=50
0.9	151.691	0.921		1.76	k= 2, v=50
1.8	151.691	0.921		1.79	k= 3, v=50
3.61	146.820	1.380		1.80	k= 4, v=50
7.21	146.820	1.380		1.80	k= 5, v=50
14.43	140.995	1.929	*	1.81	k= 6, v=50
28.9	122.042	3.715	*	1.81	k= 7, v=50
57.7	114.170	4.457	*	1.81	k= 8, v=50
115.4	91.747	6.570	*	1.82	k= 9, v=50

s = 18.381
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	8.7	3.0	25.	0.23	0.35
EC10	17.	8.0	37.	0.17	0.46
EC25	54.	37.	79.	0.082	0.69
EC50	1.9E+02	1.1E+02	3.3E+02	0.12	0.59

Slope = 1.22 Std.Err. = 0.280

Goodness of fit: p = 0.62 based on DF= 11. 70.

5825RW : radish vv weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	161.	151.	10.1	100.	0.00
0.0280	6.00	156.	151.	5.06	100.	0.000135
0.0560	6.00	133.	151.	-18.0	100.	0.000764
0.110	6.00	147.	151.	-4.04	100.	0.00365

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0.230	6.00	157.	151.	5.71	100.	0.0176
0.450	6.00	147.	151.	-3.76	99.9	0.0646
0.900	6.00	154.	151.	3.45	99.8	0.219
1.80	6.00	153.	150.	2.74	99.3	0.654
3.61	6.00	144.	149.	-4.37	98.3	1.73
7.21	6.00	149.	145.	4.06	95.9	4.05
14.4	6.00	141.	139.	2.41	91.6	8.44
28.9	6.00	122.	128.	-5.56	84.3	15.7
57.7	6.00	114.	112.	2.33	73.9	26.1
115.	6.00	91.7	91.9	-0.144	60.7	39.3

!!!Warning: EC50 not bracketed by doses evaluated.

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Data Evaluation Report on the acute toxicity of Aminopyralid (XDE-750) to aquatic vascular plants *Lemna gibba*

PMRA Submission #: 2004-0789 EPA MRID#: 462358-26

Data Requirement:	PMRA Data Code:	9.8.5
	EPA DP Barcode:	D301682
	OECD Data Point:	IIA 8.6.1
	EPA MRID:	462358-26
	EPA Guideline:	123-2

Test material: Aminopyralid **Purity:** 94.5%
Common name: XDE-750
Chemical name: IUPAC: 4-amino-3,6-dichloro-picolinic acid
CAS name: Not reported
CAS No.: 150114-71-9
Synonyms: XR-750

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/17/04

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/4/04

Primary Reviewer: Brian D. Kiernan
Biologist, OPP/EFED/ERBIV
Signature: 6/16/05
Date: 12/13/2004

Secondary Revi ewer(s): Monika Engel
PMRA-EAD
Signature:
Date: February 7, 2005

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
EPA PC Code	005100	

Date Evaluation Completed: June 08, 2005

CITATION: Hoberg, J.R. 2003. XDE-750 - Toxicity to Duckweed, *Lemna gibba*. Unpublished study performed by Springborn Smithers Laboratories, Inc., Wareham, Massachusetts. Laboratory Project Identification No. 12550.6160. Study submitted by The Dow Chemical Company for Dow AgroSciences LLC, Midland, Michigan. Study initiated December 14, 2001 and completed October 10, 2003.

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EXECUTIVE SUMMARY:

In a 14-day acute toxicity study, freshwater aquatic vascular plants duckweed, *Lemna gibba* G3, were exposed to aminopyralid (XDE-750) at mean measured concentrations <1.3-1.4 (LOQ, controls), 5.2, 11, 21, 44, and 88 ppm a.i. under static conditions. The nominal test concentrations were 0 (negative and solvent controls), 6.3, 13, 25, 50, and 100 ppm a.i. After 14 days, the frond number percent inhibitions were 2.1, 3.0, 4.0, 1.3, and 13% in the 5.2, 11, 21, 44, and 88 ppm a.i. treatment groups, respectively, compared to the solvent control. The growth rate percent inhibitions were 2, 4, 2, -2, and 2% in the 5.2, 11, 21, 44, and 88 ppm a.i. treatment groups, respectively, compared to the pooled control. The growth rate percent inhibitions were 0, 8, 16, 2, and 12% in the 5.2, 11, 21, 44, and 88 ppm a.i. treatment groups, respectively, compared to the pooled control. Only the frond number endpoint was sensitive to treatment with aminopyralid; the EC_{50} was >88 ppm a.i. for all endpoints and the NOEC was 44 ppm a.i.

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as **Acceptable.**

EAD Conclusion:

The EAD is in agreement with the conclusions reported by the study author and the EPA reviewer. The NOEC and EC50 for frond number were 44 ppm a.i. and > 88 ppm a.i. respectively. The NOEC and EC50 for both growth rate and dry weight were 88 ppm a.i. and > 88 ppm a.i. respectively.

Results Synopsis

Test Organism: *Lemna gibba* G3
Test Type: Static

Number of fronds:
NOEC: 44 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: 7.7 ppm a.i. 95% C.I.: 0.41-140 ppm a.i.
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: 0.515±0.293

Growth rates:
NOEC: 88 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: >88 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: N/A

Plant biomass (dry weight):
NOEC: 88 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: 4.3 ppm a.i. 95% C.I.: $1.4e^{-5}$-$1.3e^{6}$
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: 0.316±0.663

Endpoint(s) Affected: Frond number

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test protocol was based on the following guidelines: OECD Proposed Guideline 221 and U.S. EPA-FIFRA Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants Guidelines 122-2 and 123-2. The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The pretest health of the test organism was not reported.
2. The test conditions were static and test solution renewal is recommended. However, the mean measured concentrations were within an acceptable range of nominal concentrations (83-88%).

These deviations do not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice with the exception of the collection of samples for routine water contaminant screening analyses.

A. MATERIALS:

1. Test Material Aminopyralid, XDE-750

Description: Not reported

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound
Under Test Conditions: The Day 0 measured concentrations ranged from 96 to 100% of nominal concentrations and day 14 measured concentrations ranged from 70 to 81% of nominal concentrations . The mean measured concentrations were 83-88% of nominal.

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)
OECD requirements were not reported.

Storage conditions of test chemicals: The test substance was stored at room temperature in the dark.

2. Test organism:

Name: Duckweed, *Lemna gibba* *(EPA requires a vascular species: Lemna gibba.)*
Strain, if provided: G3
Source: Laboratory cultures (original supplier: University of Toronto, Toronto, Canada)
Age of inoculum: 2 days old
Method of cultivation: 20X Algal Assay Procedure (AAP) Medium

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B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: Definitive test concentrations were based upon results of a range-finding test. The 14-day test concentrations were 0.0010, 0.010, 0.10, 1.0, and 10 ppm a.i with dilution water and solvent controls. The frond densities were 126, 822, 764, 856, and 786 fronds/replicate in the 0.0010, 0.010, 0.10, 1.0, and 10 ppm a.i, respectively. The pooled control cell density was 143 fronds/replicate. The fronds in the 0.010, 0.10, 1.0, and 10 ppm a.i treatment groups were smaller than the controls. The 0.0010 ppm a.i. treatment group and control fronds were normal. Green algae (*Pseudokirchneriella subcapitata*) was observed in the controls and 0.0010 ppm a.i. treatment group, and could be responsible for the low frond numbers.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	Continuous culture 20X Algal Assay Procedure (AAP) Medium (Table 1, p. 23); same as test. Not reported	
Test system static/static renewal/ renewal rate for static renewal:	Static	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Environmental chamber	
Duration of the test	14 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel material: (glass/polystyrene) size: fill volume:	 Sterile crystallizing dishes 270 mL 100 mL	

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Parameter	Details	Remarks *Criteria*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X Algal Assay Procedure (AAP) Medium 7.4-8.0 (Table 2, p. 24) 8.3-8.8 disodium EDTA $NaHCO_3$	*EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Sterile deionized water 7.5 ± 0.1 pH adjusted using 0.1 *N* NaOH or 0.1 *N* HCl 1.0 mg/L (December 2001) N/A Not detected Not detected N/A	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Not reported.	
Sediment used **(for rooted aquatic vascular plants)** origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable	
Number of replicates control: solvent control: treatments:	 3 3 3	
Number of plants/replicate	5 plants per replicate	*EPA requires 5 plants.*

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Parameter	Details	Remarks *Criteria*
Number of fronds/plant	3 fronds per plant (15 total fronds per replicate)	*EPA requires 3 fronds per plant.*
Test concentrations nominal: measured:	0 (negative and solvent controls), 6.3, 13, 25, 50, and 100 ppm a.i. <1.3-1.4 (LOQ, controls), 5.2, 11, 21, 44, and 88 ppm a.i.	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	Dimethylformamide, 0.10 mL/L	
Method and interval of analytical verification	HPLC; days 0 and 14.	
Test conditions temperature: photoperiod: light intensity and quality:	23-26°C continuous light 7500-9700 lux	*EPA temperature: 25°C* *EPA photoperiod: continuous* *EPA light: 5.0 Klux (±15%)*
Reference chemical (if used) name: concentrations:	None	
Other parameters, if any	None	

2. Observations

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Frond density, growth rates, and dry weight (biomass).	
Measurement technique for frond number and other end points	Direct counts.	
Observation intervals	Days 7 and 14.	
Other observations, if any	N/A	

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Indicate whether there was an exponential growth in the control	Yes	
Were raw data included?	Replicate data provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 14 days, the frond number percent inhibitions were 2.1, 3.0, 4.0, 1.3, and 13%% in the 5.2, 11, 21, 44, and 88 ppm a.i. treatment groups, respectively, compared to the solvent control. The difference in frond number was significant in the 88 ppm a.i. treatment group. The growth rate percent inhibitions were 2, 4, 2, -2, and 2% in the 5.2, 11, 21, 44, and 88 ppm a.i. treatment groups, respectively, compared to the pooled control. The growth rate percent inhibitions were 0, 8, 16, 2, and 12% in the 5.2, 11, 21, 44, and 88 ppm a.i. treatment groups, respectively, compared to the pooled control.

By day 14, the effect of less root formation was observed in the 21, 44, and 88 ppm a.i. treatment groups and the fronds were slightly chlorotic in the 44 and 88 ppm a.i. treatment groups.

Table 3: Effect of Aminopyralid (XDE-750) on frond number of Duckweed, *Lemna gibba*

Treatment mean measured (and nominal) concentrations, ppm a.i.	Initial frond number/test solution	Mean frond number at			Mean Growth Rate ($days^{-1}$)	Mean Biomass (dry weights, g)
		7 days	14 days	% inhibition at 14 days[b]		
Negative control (dilution water)	15	446	863	---	0.49	0.1442
Solvent control	15	361	793	---	0.46	0.1322
5.2 (6.3)	15	360	776	2.1	0.46	0.379
11 (13)	15	348	769	3.0	0.45	0.1266
21 (25)	15	367	761	4.0	0.46	0.1157
44 (50)	15	414	782	1.3	0.48	0.1358
88 (100)	15	372	688	13*	0.46	0.1214
Reference chemical (if used)	Not applicable					

[a] Nominal concentrations are in parentheses.
[b] The % frond number inhibition was based on solvent control.
* Significantly reduced compared to the solvent control (Williams' Test).

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Table 4: Statistical endpoint values.

Statistical Endpoint[a]	frond No.	growth rate (day 14)	dry weight
NOEC or EC_{05} (ppm a.i.)	44	88	88
LOEC (ppm a.i.)	88	>88	>88
EC_{50} (ppm a.i.) (95% C.I.)	>88	>88	>88
EC_{25} (ppm a.i.) (95% C.I.)	>88	>88	>88
Reference chemical NOAEC IC_{50}/EC_{50}	Not applicable	Not applicable	Not reported

[a] Statistical data based on mean measured test concentrations.

B. REPORTED STATISTICS: A t-test was used to compare the dilution water (negative) and solvent controls. The controls were pooled for growth rate and dry weight statistical analyses and the solvent control was used for the frond number analysis. The NOEC was estimated based on significance data (William's test) and the EC_{50} were empirically estimated to be greater than the highest concentration tested (no concentrations with >50% inhibition). The reported statistics were based on the mean measured test concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Frond number and dry weight data were determined to satisfy the assumptions of ANOVA, so the NOEC and LOEC were determined using William's test. The EC_{05} values for these endpoints was determined using the Probit method via Nuthatch software. The EC_{50} values were determined visually, as inhibition did not exceed 50% for any endpoint.

Number of fronds:
NOEC: 44 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: 7.7 ppm a.i. 95% C.I.: 0.41-140 ppm a.i.
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: 0.515±0.293

Growth rates:
NOEC: 88 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: >88 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: N/A

Plant biomass (dry weight):
NOEC: 88 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: 4.3 ppm a.i. 95% C.I.: $1.4e^{-5}$-$1.3e^{6}$
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: 0.316±0.663

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Endpoint(s) Affected: Frond number

D. STUDY DEFICIENCIES:

The deviations did not affect the acceptability or the validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer agreed with the study author's conclusions. In addition, the reviewer provided EC_{05} estimates for frond number and dry weight endpoints. The reviewer's results are reported in the Executive Summary and Conclusions sections.

EAD Comments:
After review of the study data and the US EPA DER, the reviewer is in agreement with the conclusion reached by the US EPA. No amendments to the DER are recommended.

F. CONCLUSIONS: This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Acceptable.

Number of fronds:
NOEC: 44 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: 7.7 ppm a.i. 95% C.I.: 0.41-140 ppm a.i.
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: 0.515±0.293

Growth rates:
NOEC: 88 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: >88 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: N/A

Plant biomass (dry weight):
NOEC: 88 ppm a.i.
LOEC: >88 ppm a.i.
EC_{05}: 4.3 ppm a.i. 95% C.I.: $1.4e^{-5}$-$1.3e^{6}$
EC_{50}/IC_{50}: >88 ppm a.i. 95% C.I.: N/A
Slope: 0.316±0.663

Endpoint(s) Affected: Frond number

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III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

frond production
File: 5826f Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	21061.833	4212.367	1.768
Within (Error)	12	28594.667	2382.889	
Total	17	49656.500		

Critical F value = 3.11 (0.05,5,12)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

frond production
File: 5826f Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	792.667	792.667		
2	5.2	776.000	776.000	0.418	
3	11	768.667	768.667	0.602	
4	21	761.000	761.000	0.795	
5	44	782.333	782.333	0.259	
6	88	688.333	688.333	2.618	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

frond production
File: 5826f Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	3			
2	5.2	3	99.643	12.6	16.667
3	11	3	99.643	12.6	24.000
4	21	3	99.643	12.6	31.667
5	44	3	99.643	12.6	10.333
6	88	3	99.643	12.6	104.333

frond production
File: 5826f Transform: NO TRANSFORMATION

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	3	792.667	792.667	792.667
2	5.2	3	776.000	776.000	776.000
3	11	3	768.667	768.667	770.667
4	21	3	761.000	761.000	770.667
5	44	3	782.333	782.333	770.667
6	88	3	688.333	688.333	688.333

frond production
File: 5826f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
solvent control	792.667				
5.2	776.000	0.418		1.78	k= 1, v=12
11	770.667	0.552		1.87	k= 2, v=12
21	770.667	0.552		1.90	k= 3, v=12
44	770.667	0.552		1.92	k= 4, v=12
88	688.333	2.618	*	1.93	k= 5, v=12

s = 48.815
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	7.7	0.41	1.4E+02	0.61	0.054
EC10	39.	7.8	2.0E+02	0.33	0.20
EC25	5.9E+02	27.	1.3E+04	0.64	0.046
EC50	1.2E+04	19.	7.9E+06	1.3	0.0015

Slope = 0.515 Std.Err. = 0.293

Goodness of fit: p = 0.35 based on DF= 3.0 15.

5826F : frond production

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	828.	825.	2.60	100.	0.00
5.20	3.00	776.	791.	-15.0	95.8	4.15
11.0	3.00	769.	777.	-8.15	94.1	5.87
21.0	3.00	761.	761.	-0.215	92.2	7.76
44.0	3.00	782.	739.	43.3	89.5	10.5
88.0	3.00	688.	714.	-25.2	86.5	13.5

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!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

dry weight
File: 5826w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.0016	0.0003	0.600
Within (Error)	15	0.0070	0.0005	
Total	20	0.0086		

Critical F value = 2.90 (0.05,5,15)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

dry weight
File: 5826w Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.138	0.138		
2	5.2	0.138	0.138	0.020	
3	11	0.127	0.127	0.737	
4	21	0.116	0.116	1.422	
5	44	0.136	0.136	0.153	
6	88	0.121	0.121	1.061	

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

dry weight
File: 5826w Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	5.2	3	0.041	29.8	0.000
3	11	3	0.041	29.8	0.012
4	21	3	0.041	29.8	0.022
5	44	3	0.041	29.8	0.002
6	88	3	0.041	29.8	0.017

dry weight
File: 5826w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

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1	GRPS 1&2 POOLED	6	0.138	0.138	0.138
2	5.2	3	0.138	0.138	0.138
3	11	3	0.127	0.127	0.127
4	21	3	0.116	0.116	0.126
5	44	3	0.136	0.136	0.126
6	88	3	0.121	0.121	0.121

dry weight
File: 5826w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.138				
5.2	0.138	0.021		1.75	k= 1, v=15
11	0.127	0.760		1.84	k= 2, v=15
21	0.126	0.812		1.87	k= 3, v=15
44	0.126	0.812		1.88	k= 4, v=15
88	0.121	1.095		1.89	k= 5, v=15

s = 0.022
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	4.3	1.4E-05	1.3E+06	2.6	3.3E-06
EC10	60.	0.059	6.1E+04	1.4	0.00099
EC25	5.0E+03	2.0E-06	1.3E+13	4.5	4.0E-10
EC50	6.8E+05	1.9E-13	2.5E+24	8.8	2.7E-19

Slope = 0.316 Std.Err. = 0.663

Goodness of fit: p = 0.58 based on DF= 3.0 15.

5826W : dry weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	0.138	0.138	-0.000182	100.	0.00
5.20	3.00	0.138	0.131	0.00682	94.7	5.29
11.0	3.00	0.127	0.129	-0.00285	93.5	6.49
21.0	3.00	0.116	0.128	-0.0120	92.3	7.69
44.0	3.00	0.136	0.126	0.0102	90.7	9.27
88.0	3.00	0.121	0.123	-0.00182	89.1	10.9

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

Data Evaluation Report on the acute toxicity of Aminopyralid on the Freshwater Diatom, *Navicula pelliculosa*

PMRA Submission #:2004-0789 EPA MRID #: 462358-27

Data Requirement:		
	PMRA DATA CODE	9.8.2-1
	EPA DP Barcode	D301682
	OECD Data Point	II A8.4
	EPA MRID	462358-27
	EPA Guideline	123-2

Test material: Aminopyralid **Purity:** 94.5%
Common name: XDE-750 Technical Grade
Chemical name: IUPAC: 2-pyridinecarboxylic acid, 4-amino-3,6-dichloro
CAS name: Not reported
CAS No.: Not reported
Synonyms: XR-750 Technical Grade

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/17/04

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation
Signature:
Date: 9/29/04

Primary Reviewer: Brian D. Kiernan
Biologist, OPP/EFED/ERBIV
Signature: 8/16/05
Date: 12/13/2004

Secondary Reviewer(s): #1615
EAD, PMRA
Signature:
Date: February 4, 2005

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 005100

Date Evaluation Completed: June 12, 2005

CITATION: Hoberg, J.R. 2002. XDE-750 - Toxicity to the Freshwater Diatom (*Navicula pelliculosa*). Unpublished study performed by Springborn Smithers Laboratories, Inc., Wareham, Massachusetts. Laboratory Project Identification No. 12550.6198. Study submitted by The Dow Chemical Company for Dow AgroSciences LLC, Midland, Michigan. Experimental start date February 28, 2002 and experimental termination date March 5, 2002. The final report issued May 17, 2002.

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EXECUTIVE SUMMARY:

In a 120-hour acute toxicity study, cultures of *Navicula pelliculosa* were exposed to Aminopyralid, as XDE-750, under static conditions. The nominal test concentrations were 6.3, 13, 25, 50, and 100 ppm a.i., compared to negative and solvent controls. The mean measured concentrations were <0.61 and <0.64 (LOQ, negative and solvent controls), 6.0, 12, 23, 48, and 100 ppm a.i.

By 120 hours, the cell density percent inhibitions were 15, 28, 21, 100, and 100% for the 6.0, 12, 23, 48, and 100 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour biomass were 15, 38, 32, 106, and 109% in the 6.0, 12, 23, 48, and 100 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour growth rates were 4, 19, 4, 133, and 144% in the 6.0, 12, 23, 48, and 100 ppm a.i. treatment groups, respectively, compared to the solvent control. The cell density and biomass were significantly reduced in the 12, 23, 48, and 100 ppm a.i. treatment groups, and the growth rates were significantly reduced in the 48 and 100 ppm a.i. treatment groups. However, the pH at these two highest levels was very acidic (3.6-4.2) at test initiation due to the addition of test substance to the test dilution water. The pH was still very acidic after 120 hours. As a result, it is not clear if the endpoints measured were affected by the dosage or by the pH levels at the higher doses. No other signs of toxicity (eg. unusual cell shape and colour) were observed after 120 hours. Biomass was the most sensitive endpoint, with an EC_{50} of 18 ppm; the NOEC for biomass and cell density was 6.0 ppm a.i..

The study is scientifically sound and but does not satisfy the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Navicula pelliculosa* because the pH in the higher doses was too low. This study is classified as Supplemental, and useful for risk assessment purposes.

EAD Conclusion:

The EAD reviewer believes that acidic pH interfered strongly on the toxic response observed in this test. Because of this interference, all EC_{50} values reported in the DER as well as the NOEC value reported for growth rate are not reliable and cannot be used for the purpose of risk assessment. For the cell density and biomass endpoints, the NOEC was 6.0 mg a.i./L because significant inhibition was observed in the next higher treatment levels (12 and 23 mg a.i./L) even though the pH was in an acceptable range.

Because of the low pH issue, this study satisfies only partly the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Navicula pelliculosa*. This study is classified as supplemental.

Results Synopsis

Test Organism: *Navicula pelliculosa*
Test Type: Static

Cell density:
NOEC: 6.0 ppm a.i.
LOEC: 12 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 22 ppm a.i. 95% C.I.: 6.0-81 ppm a.i.

Growth rates:
NOEC: 23 ppm a.i.

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LOEC: 48 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 21 ppm a.i. 95% C.I.: 3.7-140 ppm a.i.

Plant biomass (area under the growth curve):
NOEC: 6.0 ppm a.i.
LOEC: 12 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 18 ppm a.i. 95% C.I.: 5.4-59 ppm a.i.

Endpoint(s) Affected: Cell density , growth rates, and biomass.
Most sensitive endpoint: Biomass

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA FIFRA Subdivision J Guidelines 122-2 and 123-2, OECD Guideline #201, and EC Guideline L383A-C.3.

There were only three replicates per treatment group. Tests with this species (*Navicula pelliculosa*) should be conducted with four replicates per treatment because of the variability historically associated with response by this species. In this study, there did not appear to be excessive variability among replicates within a treatment. The pH in the highest treatment levels was exceedingly low and may have had a deleterious effect on the organisms.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice with the exception of the collection of samples for routine water contaminant screening analyses.

A. MATERIALS:

1. Test Material Aminopyralid, XDE-750

Description: Not reported

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound
Under Test Conditions: The mean measured concentration of Aminopyralid were 92-100% of nominal at hour 0 and 92-96% of nominal at hour 120 (Table 3, p. 27).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test substance was stored at room temperature in the dark.

2. Test organism:

Name: *Navicula pelliculosa*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: 1530.45
Source: Originally from Carolina Biological Supply, Burlington, NC. Current in-house laboratory cultures.
Age of inoculum: 6 days old
Method of cultivation: Algal Assay Procedure (AAP) medium (Table 1, p. 25).

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B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: The definitive nominal test concentration was based on results of a range-finding test. The range-finding test was conducted at concentrations of 0.10, 1.0, 10, and 100 ppm a.i., with dilution water and solvent controls. The 120-hour cell densities were 140 x 10^4 and 199 x 10^4 cells/mL for the dilution water control and solvent control, respectively. The 0.10, 1.0, 10, and 100 ppm a.i. treatment groups had 120-hour cell densities of 136, 171, 194, and 0 x 10^4 cells/mL, respectively.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not)	Continuous Algal Assay Procedure (AAP) medium (Table 1, p. 25); same as test.	Inoculum used in test was taken from stock culture and transferred to fresh medium six days before testing.
health: (any toxicity observed)	Not reported	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber	
Duration of the test	120 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*

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Parameter	Details	Remarks *Criteria*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks with stainless steel caps 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	Algal Assay Procedure (AAP) medium 3.6-7.1 3.6-9.7 disodium EDTA $NaHCO_3$ N/A	The pH was exceedingly low in the highest treatment levels *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Dilution water Sterilized and deionized 7.5 ± 0.1 N/A pH adjusted using 0.1 *N* NaOH or 0.1 *N* HCl 0.62-0.74 mg/L (February-March 2002) Not reported Not detected Not detected Not reported	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 100 ± 10 rpm	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*

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Parameter	Details	Remarks *Criteria*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	 3 3 3	One additional replicate of the 25 ppm a.i. treatment group was not inoculated with algae and used for analytical determination. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*

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Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	0 (negative and solvent controls), 6.3, 13, 25, 50, and 100 ppm a.i. <0.61-0.64 (LOQ, negative and solvent controls), 6.0, 12, 23, 48, and 100 ppm a.i.	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	Dimethylformamide, 0.100 mL/L	
Method and interval of analytical verification	HPLC; 0 and 120 hours	
Test conditions temperature: photoperiod: light intensity and quality:	23-24°C Continuous 4000-5400 lux	*EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical {if used) name: concentrations:	N/A	
Other parameters, if any	None	

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2. Observations

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell densities, biomass (area under the growth curve), and growth rates.	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Haemocytometer and a compound microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water and solvent control group cell densities at test termination were 237X and 205X greater, respectively, than the dilution water and solvent control group cell densities at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 120 hours, the cell density percent inhibitions were 15, 28, 21, 100, and 100% for the 6.0, 12, 23, 48, and 100 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour biomass were 15, 38, 32, 106, and 109% in the 6.0, 12, 23, 48, and 100 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour growth rates were 4, 19, 4, 133, and 144% in the 6.0, 12, 23, 48, and 100 ppm a.i. treatment groups, respectively, compared to the solvent control. The cell density and biomass were significantly reduced in the 12, 23, 48, and 100 ppm a.i. treatment groups, and the growth rates were significantly reduced in the 48 and 100 ppm a.i. treatment groups.

Table 3: Effect of Aminopyralid, XDE-750, on freshwater diatom (*Navicula pelliculosa*)

Treatment mean measured and nominal concentrations [a] (ppm a.i.)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	120 hours	
			cell count	% inhibition[b]
Dilution water control	10,000	20,000	2,370,000	--
Solvent control	10,000	18,300	2,050,000	--
6.0 (6.3)	10,000	24,200	1,880,000	15
12 (13)	10,000	23,300	1,600,000	28*
23 (25)	10,000	13,300	1,740,000	21*
48 (50)	10,000	6,700	0	100*
100 (100)	10,000	2,500	0	100*
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.
[b] The % inhibition was based on pooled control.
* Significantly reduced compared to the pooled control (Williams test).

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Table 4: Effect of Aminopyralid, XDE-750, on freshwater diatom *(Navicula pelliculosa)*

Mean Measured and Nominal Treatment Concentrations [a] (ppm a.i.)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
Dilution water control	10,000	1.12	--	214,000	--
Solvent control	10,000	1.01	--	171,000	--
6.0 (6.3)	10,000	1.03	4	163,000	15
12 (13)	10,000	0.87	19	119,000	38**
23 (25)	10,000	1.03	4	131,000	32**
48 (50)	10,000	-0.35	133*	-11,000	106**
100 (100)	10,000	-0.47	144*	-18,000	109**
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

[a] The nominal test concentrations are presented in parentheses.
[b] The data was based on the 0-72 hours of the test.
* Significantly reduced compared to the solvent control (Williams test).
** Significantly reduced compared to the pooled control (Williams test).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass [a]	Growth rate[a]	Cell density
NOEC or EC_{05} (ppm a.i.)	6.0	23	6.0
EC_{50} (ppm a.i.)	18	21	22
IC_{50} or EC_{50} (ppm a.i.) (95% C.I.)	5.4-59	3.7-140	6.0-8.1
IC_{25}/EC_{25} (ppm a.i.) (and 95% C.I.)	Not reported	Not reported	11 (2.6-38)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

[a] Based on 0-72 hour data.
N/A = Not applicable

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B. REPORTED STATISTICS:

Statistical Method: The growth rate and biomass equations are presented on page 18. A t-test was used to compare the dilution water (negative) and solvent controls. The controls were pooled for cell density and biomass, and the growth rates were compared to the solvent control. The 120-hour data passed the tests for normality (Chi-square) and homogeneity of variance (Bartlett's). The 120-hour NOEC and LOEC values were determined using the Williams test. The EC_{50} values were determined by linear regression of the response using a computer program. The reported statistics were based on the mean measured test concentrations.

Cell density:
NOEC: 6.0 ppm a.i.
LOEC: 12 ppm a.i.
EC_{25}: 11 ppm a.i. 95% C.I.: 2.6-38 ppm a.i.
EC_{50}/IC_{50}: 22 ppm a.i. 95% C.I.: 6.0-81 ppm a.i.
Slope: 80

Growth rates:
NOEC: 23 ppm a.i.
LOEC: 48 ppm a.i.
EC_{05}: not determined 95% C.I.: N/A
EC_{50}/IC_{50}: 21 ppm a.i. 95% C.I.: 3.7-140 ppm a.i.
Slope: 120

Plant biomass (area under the growth curve):
NOEC: 6.0 ppm a.i.
LOEC: 12 ppm a.i.
EC_{05}: not determined 95% C.I.: N/A
EC_{50}/IC_{50}: 18 ppm a.i. 95% C.I.: 5.4-59 ppm a.i.
Slope: 85

Endpoint(s) Affected: Cell density , growth rates, and biomass.
Most sensitive endpoint: Biomass

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density and biomass data did not satisfy the assumptions of ANOVA (homogeneity of variances and normality), so the non-parametric Kruskal-Wallis test was used to determine the NOEC. Prior to this determination, the solvent control group was compared to the negative control group using a t-test and, upon finding no differences, the two were pooled for comparison to treatment. Growth rate data satisfied the assumptions of ANOVA, so the NOEC for this endpoint was determined using Dunnett's test; a difference was detected between the two control groups for this endpoint, so treatment groups were compared to the solvent control group. These analyses were conducted using TOXSTAT statistical software. The Toxanal was used to verify EC_{50} values because the distribution of the data precluded the use of Nuthatch. Therefore, probit slopes are not reported. The author's calculated endpoints are reported.

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D. STUDY DEFICIENCIES:

There were only three replicates per treatment group. Tests with this species (*Navicula pelliculosa*) should be conducted with four replicates per treatment because of the variability historically associated with response by this species. In this study, there did not appear to be excessive variability among replicates within a treatment. The pH in the highest treatment levels may have adversely affected the response of organisms in those treatments.

E. REVIEWER'S COMMENTS:

The reviewer verified the EC_{50} estimates using Toxanal software rather than the favored Nuthatch because of the distribution of the data, so they could not be verified. Furthermore, the reviewer's NOEC estimates for cell density and biomass data were higher than the study author's because the reviewer relied on non-parametric methods to determine these values. As a result, the study author's results are reported in the Executive Summary and Conclusions sections.

EAD Comments:

After review of the study data and the U.S. EPA DER, the EAD reviewer is in disagreement with part of the conclusions reached by the U.S. EPA. Indeed, because algae are sensitive to acidic pH, the EAD reviewer believes that there is a strong possibility that this factor had an inhibitory effect on their growth. The cut-off observed in the concentration-response curve, especially for cell density and growth rate inhibition, also suggests that toxicity was pH-related. The study author should have ajdusted the pH prior to testing (before adding algae) as it is suggested in the U.S. EPA OPPTS 850.5400 guideline for algal toxicity.

F. CONCLUSIONS: The study is scientifically sound but does not satisfy the guidelines for an aquatic nonvascular plant study with *Navicula pelliculosa* [§123-2]. This study is classified as Supplemental. The low pH in the highest test concentrations require assumption of chemical toxicity, when effects may be due to acidity. Biomass was the most sensitive endpoint.

EAD classifies this study as Supplemental.

Cell density:
NOEC: 6.0 ppm a.i.
LOEC: 12 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 22 ppm a.i. 95% C.I.: 6.0-81 ppm a.i.
Slope: 80

Growth rates:
NOEC: 23 ppm a.i.
LOEC: 48 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 21 ppm a.i. 95% C.I.: 3.7-140 ppm a.i.
Slope: 120

Plant biomass (area under the growth curve):
NOEC: 6.0 ppm a.i.

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LOEC: 12 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 18 ppm a.i. 95% C.I.: 5.4-59 ppm a.i.
Slope: 85

Endpoint(s) Affected: Cell density , growth rates, and biomass.
Most sensitive endpoint: Biomass

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III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 5827cd Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	221.667	221.667	104.000
2	6.0	188.333	188.333	44.000
3	12	160.333	160.333	27.000
4	23	174.333	174.333	35.000
5	48	0.000	0.000	10.500
6	100	0.000	0.000	10.500

Calculated H Value = 16.793 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

cell density
File: 5827cd Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 5 6 3 4 2 1
5	48	0.000	0.000	\
6	100	0.000	0.000	. \
3	12	160.333	160.333	. . \
4	23	174.333	174.333	. . . \
2	6.0	188.333	188.333	. . . . \
1	GRPS 1&2 POOLED	221.667	221.667	* * . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

biomass
File: 5827b Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	19.250	19.250	101.500
2	6	16.333	16.333	45.000
3	12	11.933	11.933	30.000
4	23	13.100	13.100	33.500
5	48	-1.100	-1.100	15.000
6	100	-1.800	-1.800	6.000

Calculated H Value = 15.920 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

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biomass
File: 5827b Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 3 4 2 1
6	100	-1.800	-1.800	\
5	48	-1.100	-1.100	. \
3	12	11.933	11.933	. . \
4	23	13.100	13.100	. . . \
2	6	16.333	16.333	. . . . \
1	GRPS 1&2 POOLED	19.250	19.250	* . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

growth rate (0-72)
File: 5827g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	3.138	0.784	112.000
Within (Error)	9	0.063	0.007	
Total	13	3.201		

Critical F value = 3.63 (0.05,4,9)
Since F > Critical F REJECT Ho:All groups equal

growth rate (0-72)
File: 5827g Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.013	1.013		
2	6.0	1.033	1.033	-0.293	
3	12	0.873	0.873	2.049	
4	23	1.027	1.027	-0.195	
5	48	-0.355	-0.355	17.916	*

Bonferroni T table value = 2.69 (1 Tailed Value, P=0.05, df=9,4)

growth rate (0-72)
File: 5827g Transform: NO TRANSFORMATION

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BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	3			
2	6.0	3	0.183	18.1	-0.020
3	12	3	0.183	18.1	0.140
4	23	3	0.183	18.1	-0.013
5	48	2	0.205	20.2	1.368

growth rate (0-72)
File: 5827g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	3	1.013	1.013	1.023
2	6.0	3	1.033	1.033	1.023
3	12	3	0.873	0.873	0.950
4	23	3	1.027	1.027	0.950
5	48	2	-0.355	-0.355	-0.355

growth rate (0-72)
File: 5827g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
solvent control	1.023				
6.0	1.023	0.146		1.83	k= 1, v= 9
12	0.950	0.928		1.93	k= 2, v= 9
23	0.950	0.928		1.96	k= 3, v= 9
48	-0.355	17.928	*	1.98	k= 4, v= 9

s = 0.084
Note: df used for table values are approximate when v > 20.

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Data Evaluation Report on the acute toxicity of Aminopyralid on the Marine Diatom, *Skeletonema costatum*
PMRA Submission #: 2004-0789 EPA MRID #: 462358-28

Data Requirement:		
	PMRA DATA CODE	9.8.3
	EPA DP Barcode	D301682
	OECD Data Point	II A 8.4
	EPA MRID	462358-28
	EPA Guideline	123-2

Test material: Aminopyralid **Purity:** 94.5%
Common name: XDE-750 Technical Grade
Chemical name: IUPAC: 2-pyridinecarboxylic acid, 4-amino-3,6-dichloro
CAS name: Not reported
CAS No.: Not reported
Synonyms: XR-750 Technical Grade

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/17/04

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation
Signature:
Date: 9/29/04

Primary Reviewer: Brian D. Kiernan
EPA/OPP/EFED/ERBIV
Signature:
Date: 12/10/2004 6/16/05

Secondary Reviewer(s): #1615, EAD
PMRA
Signature:
Date: 07-Feb-05

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 005100

Date Evaluation Completed: 06/16/05

CITATION: Hoberg, J.R. 2002. XDE-750 - Growth inhibition test with marine diatom (*Skeletonema costatum*). Unpublished study performed by Springborn Smithers Laboratories, Inc., Wareham, Massachusetts. Laboratory Project Identification No. 12550.6200. Study submitted by The Dow Chemical Company for Dow AgroSciences LLC, Midland, Michigan. Experimental start date March 14, 2002 and experimental termination date March 19, 2002. The final report issued May 17, 2002.

EXECUTIVE SUMMARY:

In a 120-hour acute toxicity study, cultures of *Skeletonema costatum* were exposed to Aminopyralid, as XDE-750, under static conditions. The nominal test concentrations were 6.3, 13, 25, 50, and 100 mg a.i./L a.i., compared to negative and solvent controls. The mean measured concentrations were <0.70-0.71 (LOQ, negative and solvent controls), 6.2, 13, 25, 50, and 100 mg a.i./L a.i.

By 120 hours, the cell density percent inhibitions were 40, -9, 9, 0, and 6% for the 6.2, 13, 25, 50, and 100 mg a.i./L a.i. treatment groups, respectively, compared to the pooled control. **The pooled control was the mean of the data obtained for both the negative and solvent controls, as there was no statistical difference between these two controls (verified by a t-test).** The reduction in cell density at the 6.2 mg a.i./L level was due to an unexplained drop in one replicate between 96 and 120 hours. The percent inhibitions for 0-72 hour biomass were -15, 1, 32, 33, and 58% in the 6.2, 13, 25, 50, and 100 mg a.i./L a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour growth rates were 0, 2, 14, 15, and 26% in the 6.2, 13, 25, 50, and 100 mg a.i./L a.i. treatment groups, respectively, compared to the pooled control. The growth rates and biomass were significantly reduced in the 25, 50, and 100 mg a.i./L a.i. treatment groups. Biomass was the most sensitive endpoint, with an EC_{50} of 70 mg a.i./L a.i.; the NOEC and EC_{05} for biomass was 13 and 7.7 mg a.i./L a.i.. The NOEC for growth rate and cell density were 13 and 100 mg a.i./L (the highest concentration tested), respectively, and the EC_{50} was >100 mg a.i./L for both endpoints.

The study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Skeletonema costatum*. This study is classified as Acceptable by both the USEPA and PMRA.

Results Synopsis

Test Organism: *Skeletonema costatum*
Test Type: Static

Cell density:
NOEC: 100 mg a.i./L a.i.
LOEC: >100 mg a.i./L a.i.
EC_{05}: could not be determined 95% C.I.:
EC_{50}/IC_{50}: >100 mg a.i./L a.i. 95% C.I.: N/A
Slope: N/A

Growth rates:
NOEC: 13 mg a.i./L a.i.
LOEC: 25 mg a.i./L a.i.
EC_{05}: 12 mg a.i./L a.i. 95% C.I.: 1.7-82 mg a.i./L a.i.
EC_{50}/IC_{50}: >100 mg a.i./L a.i. 95% C.I.: N/A
Slope: 1.12±0.485

Plant biomass (area under the growth curve):
NOEC: 13 mg a.i./L a.i.
LOEC: 25 mg a.i./L a.i.
EC_{05}: 7.7 mg a.i./L a.i. 95% C.I.: 1.2-49 mg a.i./L a.i.
EC_{50}/IC_{50}: 70 mg a.i./L a.i. 95% C.I.: 41-120 mg a.i./L a.i.
Slope: 1.71±0.627

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Endpoint(s) Affected: Growth rates and biomass.
Most sensitive endpoint: Biomass

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA FIFRA Subdivision J Guidelines 122-2 and 123-2, OECD Guideline #201, and EC Guideline L383A-C.3. No deviations were observed.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice with the exception of the collection of samples for routine water contaminant screening analyses.

A. MATERIALS:

1. Test Material Aminopyralid, XDE-750

Description: Not reported

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound
Under Test Conditions: The measured concentrations of Aminopyralid were 100% of nominal at hour 0 and 92-100% of nominal at hour 120 (Table 3, p. 27).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test substance was stored at room temperature in the dark.

2. Test organism:
Name: *Skeletonema costatum*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: CCMP 1332
Source: Originally from Bigelow Laboratories, West Boothbay Harbor, Maine. Current in-house laboratory cultures.
Age of inoculum: 6 days old
Method of cultivation: Artificially Enriched Seawater (AES) medium (Table 1, p. 25).

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B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: The definitive nominal test concentration was based on results of a range-finding test. The range-finding test was conducted at concentrations of 0.10, 1.0, 10, and 100 mg a.i./L a.i., with dilution water and solvent controls. The 120-hour cell densities were 103 x 10^4 and 135 x 10^4 cells/mL for the dilution water control and solvent control, respectively. The 0.10, 1.0, 10, and 100 mg a.i./L a.i. treatment groups had 120-hour cell densities of 136, 128, 98, and 115 x 10^4 cells/mL, respectively.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	Continuous Artificially Enriched Seawater (AES) medium (Table 1, p. 25); same as test. Not reported	Inoculum used in test was taken from stock culture and transferred to fresh medium six days before testing. *EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber	
Duration of the test	120 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*

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Parameter	Details	Remarks *Criteria*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks with stainless steel caps 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	Artificially Enriched Seawater (AES) medium 7.0-8.0 8.5-8.7 disodium EDTA Not reported 30 ± 2 g/L	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Natural seawater Sterilized and filtered 8.0 ± 0.1 30 ± 2 g/L pH adjusted, if necessary <2.0 mg/L (March 2002) Not reported Not detected Not detected Not reported	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 60 ± 10 rpm	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*

Parameter	Details	Remarks *Criteria*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	 3 3 3	One additional replicate of the 25 mg a.i./L a.i. treatment group was not inoculated with algae and used for analytical determination. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	0 (negative and solvent controls), 6.3, 13, 25, 50, and 100 mg a.i./L a.i. <0.70-0.71 (LOQ, negative and solvent controls), 6.2, 13, 25, 50, and 100 mg a.i./L a.i.	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	Dimethylformamide, 0.100 mL/L	
Method and interval of analytical verification	HPLC; 0 and 120 hours	
Test conditions temperature: photoperiod: light intensity and quality:	 20-21°C Continuous 3200-4300 lux	EPA requires a photoperiod of 14 hr light:10 hr dark for *S. costatum*. *EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical {if used) name: concentrations:	 N/A	
Other parameters, if any	None	

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2. Observations

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell densities, biomass (area under the growth curve), and growth rates.	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Hemacytometer and a compound microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water and solvent control group cell densities at test termination were 94X and 112X greater, respectively, than the dilution water and solvent control group cell densities at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 120 hours, the cell density percent inhibitions were 40, -9, 9, 0, and 6% for the 6.2, 13, 25, 50, and 100 mg a.i./L a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour biomass were -15, 1, 32, 33, and 58% in the 6.2, 13, 25, 50, and 100 mg a.i./L a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour growth rates were 0, 2, 14, 15, and 26% in the 6.2, 13, 25, 50, and 100 mg a.i./L a.i. treatment groups, respectively, compared to the pooled control. The growth rates and biomass were significantly reduced in the 25, 50, and 100 mg a.i./L a.i. treatment groups.

Table 3: Effect of Aminopyralid, XDE-750, on marine diatom (*Skeletonema costatum*)

Treatment mean measured and nominal concentrations [a] (mg a.i./L a.i.)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	120 hours	
			cell count	% inhibition[b]
Dilution water control	10,000	34,200	940,000	--
Solvent control	10,000	24,200	1,120,000	--
6.2 (6.3)	10,000	35,000	620,000	40
13 (13)	10,000	21,700	1,120,000	-9
25 (25)	10,000	20,800	940,000	9
50 (50)	10,000	26,700	1,030,000	0
100 (100)	10,000	18,300	970,000	6
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.
[b] The % inhibition was based on pooled control.
* Significantly reduced compared to the pooled control (Williams test).

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Table 4: Effect of Aminopyralid, XDE-750, on marine diatom *(Skeletonema costatum)*

Mean Measured and Nominal Treatment Concentrations [a] (mg a.i./L a.i.)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
Dilution water control	10,000	1.01	--	185,000	--
Solvent control	10,000	1.10	--	233,000	--
6.2 (6.3)	10,000	1.06	0	241,000	-15
13 (13)	10,000	1.04	2	206,000	1
25 (25)	10,000	0.91	14*	143,000	32*
50 (50)	10,000	0.9	15*	141,000	33*
100 (100)	10,000	0.78	26*	87,000	58*
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

[a] The nominal test concentrations are presented in parentheses.
[b] The data was based on the 0-72 hours of the test.
* Significantly reduced compared to the pooled control (Williams test).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass [a]	Growth rate[a]	Cell density
NOEC or EC_{05} (mg a.i./L a.i.)	13	13	100
EC_{50} (mg a.i./L a.i.)	77	>100	>100
IC_{50} or EC_{50} (mg a.i./L a.i.) (95% C.I.)	13-1000	N/A	N/A
IC_{25}/EC_{25} (mg a.i./L a.i.) (and 95% C.I.)	Not reported	Not reported	>100
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

[a] Based on 0-72 hour data.
N/A = Not applicable

B. REPORTED STATISTICS:

Statistical Method: The growth rate and biomass equations are presented on page 18. A t-test was used to compare

the dilution water (negative) and solvent controls. The controls were pooled for all endpoints. The 120-hour data passed the tests for normality (Shapiro-Wilks') and homogeneity of variance (Bartlett's). The 120-hour NOEC and LOEC values were determined using the Williams test. The biomass EC_{50} value was determined by linear regression of the response using an unspecified computer program. The reported statistics were based on the mean measured test concentrations.

Cell density:
NOEC: 100 mg a.i./L a.i.
LOEC: >100 mg a.i./L a.i.
EC_{25}: >100 mg a.i./L a.i. 95% C.I.: N/A
EC_{50}/IC_{50}: >100 mg a.i./L a.i. 95% C.I.: N/A
Slope: N/A

Growth rates:
NOEC: 13 mg a.i./L a.i.
LOEC: 25 mg a.i./L a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50}: >100 mg a.i./L a.i. 95% C.I.: N/A
Slope: N/A

Plant biomass (area under the growth curve):
NOEC: 13 mg a.i./L a.i.
LOEC: 25 mg a.i./L a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 77 mg a.i./L a.i. 95% C.I.: 13-1000 mg a.i./L a.i.
Slope: 58

Endpoint(s) Affected: Growth rates and biomass.
Most sensitive endpoint: Biomass

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density, biomass, and growth rate data satisfied the assumptions of ANOVA (homogeneity of variances and normality), so the NOEC and LOEC values were determined using ANOVA, followed by William's test (if necessary). Prior to this determination, the solvent control group was compared to the negative control group using a t-test and, upon finding no differences, the two were pooled for comparison to treatment. These analyses were conducted using TOXSTAT statistical software. The Probit method was used to determine EC_x values for biomass and growth rate; these values were not determined for cell density because of the lack of effect.

Cell density:
NOEC: 100 mg a.i./L a.i.
LOEC: >100 mg a.i./L a.i.
EC_{05}: could not be determined 95% C.I.:
EC_{50}/IC_{50}: >100 mg a.i./L a.i. 95% C.I.: N/A
Slope: N/A

Growth rates:
NOEC: 13 mg a.i./L a.i.
LOEC: 25 mg a.i./L a.i.
EC_{05}: 12 mg a.i./L a.i. 95% C.I.: 1.7-82 mg a.i./L a.i.

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EC_{50}/IC_{50}: >100 mg a.i./L a.i. 95% C.I.: N/A
Slope: 1.12±0.485

Plant biomass (area under the growth curve):
NOEC: 13 mg a.i./L a.i.
LOEC: 25 mg a.i./L a.i.
EC_{05}: 7.7 mg a.i./L a.i. 95% C.I.: 1.2-49 mg a.i./L a.i.
EC_{50}/IC_{50}: 70 mg a.i./L a.i. 95% C.I.: 41-120 mg a.i./L a.i.
Slope: 1.71±0.627

Endpoint(s) Affected: Growth rates and biomass.
Most sensitive endpoint: Biomass

D. STUDY DEFICIENCIES:

No deviations were observed.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study author's; biomass was the most sensitive endpoint. Because the reviewer's EC_{50} estimate was associated with a narrower 95% confidence interval and EC_{05} estimates were provided for all endpoints, the reviewer's conclusions are reported in the Executive Summary and Conclusions sections.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Skeletonema costatum* [§123-2]. This study is classified as Core. Biomass was the most sensitive endpoint.

Cell density:
NOEC: 100 mg a.i./L a.i.
LOEC: >100 mg a.i./L a.i.
EC_{05}: could not be determined 95% C.I.:
EC_{50}/IC_{50}: >100 mg a.i./L a.i. 95% C.I.: N/A
Slope: N/A

Growth rates:
NOEC: 13 mg a.i./L a.i.
LOEC: 25 mg a.i./L a.i.
EC_{05}: 12 mg a.i./L a.i. 95% C.I.: 1.7-82 mg a.i./L a.i.
EC_{50}/IC_{50}: >100 mg a.i./L a.i. 95% C.I.: N/A
Slope: 1.12±0.485

Plant biomass (area under the growth curve):
NOEC: 13 mg a.i./L a.i.
LOEC: 25 mg a.i./L a.i.
EC_{05}: 7.7 mg a.i./L a.i. 95% C.I.: 1.2-49 mg a.i./L a.i.
EC_{50}/IC_{50}: 70 mg a.i./L a.i. 95% C.I.: 41-120 mg a.i./L a.i.
Slope: 1.71±0.627

Endpoint(s) Affected: Growth rates and biomass.

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Most sensitive endpoint: Biomass

402

III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 5828cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	4657.167	931.433	1.684
Within (Error)	15	8297.500	553.167	
Total	20	12954.667		

Critical F value = 2.90 (0.05,5,15)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

cell density
File: 5828cd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	103.167	103.167		
2	6.2	62.000	62.000	2.475	
3	13	111.667	111.667	-0.511	
4	25	94.333	94.333	0.531	
5	50	102.667	102.667	0.030	
6	100	97.333	97.333	0.351	

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

cell density
File: 5828cd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	6.2	3	43.290	42.0	41.167
3	13	3	43.290	42.0	-8.500
4	25	3	43.290	42.0	8.833
5	50	3	43.290	42.0	0.500
6	100	3	43.290	42.0	5.833

cell density
File: 5828cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

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GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	103.167	103.167	103.167
2	6.2	3	62.000	62.000	93.600
3	13	3	111.667	111.667	93.600
4	25	3	94.333	94.333	93.600
5	50	3	102.667	102.667	93.600
6	100	3	97.333	97.333	93.600

cell density
File: 5828cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	103.167				
6.2	93.600	0.575		1.75	k= 1, v=15
13	93.600	0.575		1.84	k= 2, v=15
25	93.600	0.575		1.87	k= 3, v=15
50	93.600	0.575		1.88	k= 4, v=15
100	93.600	0.575		1.89	k= 5, v=15

s = 23.519
Note: df used for table values are approximate when v > 20.

ECx

!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.

biomass
File: 5828b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	523.148	104.630	4.984
Within (Error)	15	314.875	20.992	
Total	20	838.023		

Critical F value = 2.90 (0.05,5,15)
Since F > Critical F REJECT Ho:All groups equal

biomass
File: 5828b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

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GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	20.917	20.917		
2	6.2	24.100	24.100	-0.983	
3	13	20.600	20.600	0.098	
4	25	14.300	14.300	2.042	
5	50	14.100	14.100	2.104	
6	100	8.767	8.767	3.750	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

biomass
File: 5828b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	6.2	3	8.433	40.3	-3.183
3	13	3	8.433	40.3	0.317
4	25	3	8.433	40.3	6.617
5	50	3	8.433	40.3	6.817
6	100	3	8.433	40.3	12.150

biomass
File: 5828b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	20.917	20.917	21.978
2	6.2	3	24.100	24.100	21.978
3	13	3	20.600	20.600	20.600
4	25	3	14.300	14.300	14.300
5	50	3	14.100	14.100	14.100
6	100	3	8.767	8.767	8.767

biomass
File: 5828b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	21.978				
6.2	21.978	0.328		1.75	k= 1, v=15
13	20.600	0.098		1.84	k= 2, v=15
25	14.300	2.042	*	1.87	k= 3, v=15
50	14.100	2.104	*	1.88	k= 4, v=15

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100	8.767	3.750	*	1.89	k= 5, v=15

s = 4.582
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	7.7	1.2	49.	0.38	0.16
EC10	13.	2.8	56.	0.31	0.22
EC25	28.	11.	72.	0.19	0.39
EC50	70.	41.	1.2E+02	0.11	0.59

Slope = 1.71 Std.Err. = 0.627

Goodness of fit: p = 0.42 based on DF= 3.0 15.

5828B : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	20.9	21.9	-1.03	100.	0.00
6.20	3.00	24.1	21.2	2.94	96.5	3.55
13.0	3.00	20.6	19.6	0.952	89.5	10.5
25.0	3.00	14.3	17.1	-2.80	77.9	22.1
50.0	3.00	14.1	13.2	0.919	60.1	39.9
100.	3.00	8.77	8.72	0.0437	39.8	60.2

growth rate
File: 5828g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.221	0.044	3.667
Within (Error)	15	0.182	0.012	
Total	20	0.403		

Critical F value = 2.90 (0.05,5,15)
Since F > Critical F REJECT Ho:All groups equal

growth rate
File: 5828g Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.060	1.060		
2	6.2	1.057	1.057	0.043	

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3	13	1.043	1.043	0.215
4	25	0.907	0.907	1.980
5	50	0.903	0.903	2.023
6	100	0.780	0.780	3.615 *

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

growth rate
File: 5828g Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	6.2	3	0.202	19.0	0.003
3	13	3	0.202	19.0	0.017
4	25	3	0.202	19.0	0.153
5	50	3	0.202	19.0	0.157
6	100	3	0.202	19.0	0.280

growth rate
File: 5828g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	1.060	1.060	1.060
2	6.2	3	1.057	1.057	1.057
3	13	3	1.043	1.043	1.043
4	25	3	0.907	0.907	0.907
5	50	3	0.903	0.903	0.903
6	100	3	0.780	0.780	0.780

growth rate
File: 5828g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	1.060				
6.2	1.057	0.043		1.75	k= 1, v=15
13	1.043	0.214		1.84	k= 2, v=15
25	0.907	1.970	*	1.87	k= 3, v=15
50	0.903	2.013	*	1.88	k= 4, v=15
100	0.780	3.597	*	1.89	k= 5, v=15

s = 0.110
Note: df used for table values are approximate when v > 20.

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Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	12.	1.7	82.	0.40	0.15
EC10	25.	6.8	94.	0.27	0.27
EC25	89.	45.	1.7E+02	0.14	0.51
EC50	3.6E+02	84.	1.5E+03	0.30	0.24

Slope = 1.12 Std.Err. = 0.485

Goodness of fit: p = 0.75 based on DF= 3.0 15.

5828G : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	1.06	1.07	-0.00691	100.	0.00
6.20	3.00	1.06	1.04	0.0161	97.5	2.47
13.0	3.00	1.04	1.01	0.0342	94.6	5.41
25.0	3.00	0.907	0.962	-0.0549	90.1	9.87
50.0	3.00	0.903	0.885	0.0183	83.0	17.0
100.	3.00	0.780	0.780	0.000132	73.1	26.9

!!!Warning: EC50 not bracketed by doses evaluated.

Data Evaluation Report on the acute toxicity of Aminopyralid on the Cyanobacteria, *Anabaena flos-aquae*
PMRA Submission #:{.....} EPA MRID #: 462358-29

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D301682
	OECD Data Point	{.....}
	EPA MRID	462358-29
	EPA Guideline	123-2

Test material: Aminopyralid **Purity:** 94.5%
Common name: XDE-750 Technical Grade
Chemical name: IUPAC: 2-pyridinecarboxylic acid, 4-amino-3,6-dichloro
CAS name: Not reported
CAS No.: Not reported
Synonyms: XR-750 Technical Grade

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 8/17/04

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation

Signature:
Date: 9/30/04

Primary Reviewer: Brian D. Kiernan
Biologist, OPP/EFED/ERBIV

Signature:
Date: 12/13/2004 6/16/05

Secondary Reviewer(s):
PMRA

Signature:
Date:

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 005100

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Hoberg, J.R. 2002. XDE-750 - Toxicity to the Blue-green Alga (*Anabaena flos-aquae)*. Unpublished study performed by Springborn Smithers Laboratories, Inc., Wareham, Massachusetts. Laboratory Project Identification No. 12550.6199. Study submitted by The Dow Chemical Company for Dow AgroSciences LLC, Midland, Michigan. Experimental start date March 7, 2002 and experimental termination date March 12, 2002. The final report issued May 17, 2002.

2030250

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EXECUTIVE SUMMARY:

In a 120-hour acute toxicity study, cultures of *Anabaena flos-aquae* were exposed to aminopyralid, as XDE-750, under static conditions. The study followed U.S. EPA FIFRA Guideline Section J, §123-2, OECD Guideline No. 201 and EC Guideline L383A-C.3. The nominal test concentrations were 0.40, 1.0, 2.6, 6.4, 16, 40, and 100 mg a.i./L and there were negative and solvent controls. The mean measured concentrations were <0.059-0.063 (LOQ, negative and solvent controls), 0.39, 1.0, 2.5, 6.2, 16, 38, and 100 mg a.i./L.

By 120 hours, the cell density percent inhibition was -6, -16, 0, -2, 6, 79, and 100% for the 0.39, 1.0, 2.5, 6.2, 16, 38, and 100 mg a.i./L treatment groups, respectively, compared to the pooled control. The pooled control was the mean of the data obtained for both the negative and solvent controls, as there was no statistical difference between these two controls (verified by a t-test). The cell density was significantly reduced in the 38 and 100 mg a.i./L treatment groups. The cell density EC_{50} was 27 mg a.i./L and the NOEC was 16 mg a.i./L. However, cell density was observed to be zero in at least two control replicates at each observation interval through 96 hours, compromising confidence in the study's ability to detect a dose response. Moreover, the pH was very acidic (3.5-4.9) in the two highest treatment levels. This factor is believed to have had a deleterious effect on algal growth.

The percent inhibition for 0-72 hour biomass was 47, 5, 71, 66, 43, 58, and 105% in the 0.39, 1.0, 2.5, 6.2, 16, 38, and 100 mg a.i./L treatment groups, respectively, compared to the pooled control. Due to coefficients of variation ranging from 55-346%, no statistically significant differences were detected among the treatments. Also, a well-defined concentration-response relationship was not observed. However, the consistent and appreciable reduction in biomass at all treatment levels, except the 1.0 mg a.i./L level, indicates there may be a treatment effect at the lowest level tested. Therefore this study should be repeated.

Due to the variability in the controls and the low pH in two treatment levels, the ability of this study to detect treatment effects is compromised, and therefore is inconsistent with the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Anabaena flos-aquae*. This study is classified as unacceptable.

Results Synopsis

Test Organism: *Anabaena flos-aquae*
Test Type: Static

This study is unacceptable.

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA FIFRA Subdivision J Guidelines 122-2 and 123-2, OECD Guideline #201, and EC Guideline L383A-C.3.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice with the exception of the collection of samples for routine water contaminant screening analyses.

A. MATERIALS:

1. Test Material Aminopyralid, XDE-750 Technical Grade

Description: Not reported

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound
Under Test Conditions: The mean measured concentration of Aminopyralid were 100% of nominal at hour 0 and 90-100% of nominal at hour 120 (Table 3, p. 26).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test substance was stored at room temperature in the dark.

2. Test organism:

Name: *Anabaena flos-aquae*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: LB 2557
Source: Originally from University of Texas. Current in-house laboratory cultures.
Age of inoculum: 3 days old
Method of cultivation: Algal Assay Procedure (AAP) medium (Table 1, p. 24).

B. STUDY DESIGN:

1. Experimental Conditions

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a) Range-finding Study: The definitive nominal test concentration was based on results of a range-finding test. The range-finding test was conducted at concentrations of 0.10, 1.0, 10, and 100 ppm a.i., with dilution water and solvent controls. The 120-hour cell densities were 50 x 10^4 and 26 x 10^4 cells/mL for the dilution water control and solvent control, respectively. The 0.10, 1.0, 10, and 100 ppm a.i. treatment groups had 120-hour cell densities of 30, 33, 24, and 0 x 10^4 cells/mL, respectively.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	Continuous Algal Assay Procedure (AAP) medium (Table 1, p. 24); same as test. Not reported	Inoculum used in test was taken from stock culture and transferred to fresh medium three days before testing. *EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system static/static renewal: renewal rate for static renewal:	Static	—
Incubation facility	Environmental chamber	
Duration of the test	120 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks with stainless steel caps 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*

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Parameter	Details	Remarks *Criteria*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	Algal Assay Procedure (AAP) medium 3.5-7.2 3.6-7.5 disodium EDTA $NaHCO_3$ N/A	The pH was too low in the higher concentration treatment levels. *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Dilution water Sterilized and deionized 7.5 ± 0.1 N/A pH adjusted using 0.1 *N* NaOH or 0.1 *N* HCl 0.74 mg/L (March 2002) Not reported Not detected Not detected Not reported	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 100 ± 10 rpm	Sonification is preferred for *Anabaena* spp. *EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*

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Parameter	Details	Remarks *Criteria*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	 3 3 3	One additional replicate of the 6.4 ppm a.i. treatment group was not inoculated with algae and used for analytical determination. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	0 (negative and solvent controls), 0.40, 1.0, 2.6, 6.4, 16, 40, and 100 ppm a.i. <0.059-0.063 (LOQ, negative and solvent controls), 0.39, 1.0, 2.5, 6.2, 16, 38, and 100 ppm a.i.	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	Dimethylformamide, 0.10 mL/L	
Method and interval of analytical verification	HPLC; 0 and 120 hours	
Test conditions temperature: photoperiod: light intensity and quality:	23-24°C Continuous 1800-2500 lux	*EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical {if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell densities and biomass (area under the growth curve).	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Haemocytomcter and a compound microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water and solvent control group cell densities at test termination were 63X and 63X greater, respectively, than the dilution water and solvent control group cell densities at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 120 hours, the cell density percent inhibitions were -6, -16, 0, -2, 6, 79, and 100% for the 0.39, 1.0, 2.5, 6.2, 16, 38, and 100 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour biomass were 47, 5, 71, 66, 43, 58, and 105% in the 0.39, 1.0, 2.5, 6.2, 16, 38, and 100 ppm a.i. treatment groups, respectively, compared to the pooled control. The cell density was significantly reduced in the 38 and 100 ppm a.i. treatment groups. The lack of statistically significant effects on biomass is due to highly variability in the data. Reduction in biomass of 47% in the lowest test dose indicates there may be biologically significant effects at very low doses. The inconsistency between the increased cell density and the reduction in biomass is not explained. This study must be repeated.

Table 3: Effect of Aminopyralid, XDE-750, on algae (*Anabaena flos-aquae*)

Treatment mean measured and nominal concentrations [a] (ppm a.i.)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	120 hours	
			cell count	% inhibition[b]
Dilution water control	10,000	7,500	630,000	--
Solvent control	10,000	45,000	630,000	--
0.39 (0.40)	10,000	11,700	670,000	-6
1.0 (1.0)	10,000	0	730,000	-16
2.5 (2.6)	10,000	2,500	630,000	0
6.2 (6.4)	10,000	21,700	640,000	-2
16 (17)	10,000	0	590,000	6
38 (40)	10,000	60,000	130,000	79*
100 (100)	10,000	0	0	100*
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.
[b] The % inhibition was based on pooled control.
* Significantly reduced compared to the pooled control (Williams test).

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Table 4: Effect of Aminopyralid, XDE-750, on algae *(Anabaena flos-aquae)*

Mean Measured and Nominal Treatment Concentrations [a] (ppm a.i.)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
Dilution water control	10,000	ND	--	169,000	--
Solvent control	10,000	ND	--	122,000	--
0.39 (0.40)	10,000	ND	ND	77,000	47
1.0 (1.0)	10,000	ND	ND	139,000	5
2.5 (2.6)	10,000	ND	ND	42,000	71
6.2 (6.4)	10,000	ND	ND	50,000	66
16 (17)	10,000	ND	ND	83,000	43
38 (40)	10,000	ND	ND	61,000	58
100 (100)	10,000	ND	ND	-8,000	105
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

[a] The nominal test concentrations are presented in parentheses.
[b] The data was based on the 0-72 hours of the test.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass [a]	Growth rate[a]	Cell density
NOEC or EC_{05} (ppm a.i.)	ND	Not reported	16
EC_{50} (ppm a.i.)	ND	Not reported	27
IC_{50} or EC_{50} (ppm a.i.) (95% C.I.)	ND	Not reported	9.9-76
IC_{25}/EC_{25} (ppm a.i.) (and 95% C.I.)	ND	Not reported	15 (5.0-40)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

[a] Based on 0-72 hour data.
N/A = Not applicable
ND = Not determined

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B. REPORTED STATISTICS:

Statistical Method: The biomass equations are presented on page 18. A t-test was used to compare the dilution water (negative) and solvent controls. The controls were pooled for cell density comparisons. The 120-hour data passed the tests for normality (Shapiro-Wilks) and homogeneity of variance (Bartlett's). The 120-hour NOEC and LOEC values were determined using the Williams test. The EC_{50} values were determined by linear regression of the response using a computer program. The reported statistics were based on the mean measured test concentrations. The biomass EC_{50} and NOEC values could not be determined since a well-defined concentration response was not observed.

Cell density:
NOEC: 16 ppm a.i.
LOEC: 38 ppm a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 27 ppm a.i. 95% C.I.: 5.0-40 ppm a.i.
Slope: Not reported

Growth rates: Not reported

Plant biomass (area under the growth curve): Not determined

Endpoint(s) Affected: Cell density

C. VERIFICATION OF STATISTICAL RESULTS:

This study is unacceptable.

D. STUDY DEFICIENCIES:

The high coefficients of variation (55-346%) in the biomass data suggests that the ability of this study to detect effects is inadequate. A 47% reduction in biomass in the lowest dose tested suggests there may be biologically significant effects at low concentrations. Also reducing confidence in the study's ability to detect a dose response is the observed cell density of zero in at least two control replicates at each observation interval through 96 hrs. This study should be repeated.

The pH was too low (3.5- 4.9) in the higher concentration treatment levels (38 and 100 mg a.i./L). The pH should not be less than 5.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions are contrary to the study author's. The consistent reduction in biomass across all treatment levels (except the 1.0 ppm) compromised the ability of the study to adequately assay the toxicity of aminopyralid to the cyanobacteria *Anabaena flos-aquae.*

The low pH in the higher concentration treatment levels may have had a deleterious effect on the organisms in those treatment levels. As suggested in the U.S. EPA OPPTS 850.5400 guideline for algal toxicity, the pH should have been adjusted prior to starting the test, after the addition of the test substance. Also, for the biomass endpoint, the concentration-response relationship was erratic. The study author attributed this lack of a well-defined response to difficulty in homogeneous dispersion of cells prior to counting (cells were dispersed by rapid pipetting of the solution).

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A more suitable technique, such as sonication, should have been used to break down the algal filaments. This would have contributed to more accurate cell counts.

F. CONCLUSIONS: The study does not satisfy the guidelines for an aquatic nonvascular plant study with *Anabaena flos-aquae* [§123-2]. This study is classified as Unacceptable.

III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 5829cdn Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	7329.167	1221.528	17.441
Within (Error)	17	1190.667	70.039	
Total	23	8519.833		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 5829cdn Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	63.000	63.000		
2	0.39	67.000	67.000	-0.676	
3	1.0	73.333	73.333	-1.746	
4	2.5	62.667	62.667	0.056	
5	6.2	63.333	63.333	-0.056	
6	16	59.333	59.333	0.620	
7	38	13.000	13.000	8.449	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

cell density
File: 5829cdn Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	0.39	3	15.712	24.9	-4.000
3	1.0	3	15.712	24.9	-10.333
4	2.5	3	15.712	24.9	0.333
5	6.2	3	15.712	24.9	-0.333
6	16	3	15.712	24.9	3.667
7	38	3	15.712	24.9	50.000

cell density
File: 5829cdn Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	63.000	63.000	66.583
2	0.39	3	67.000	67.000	66.583
3	1.0	3	73.333	73.333	66.583
4	2.5	3	62.667	62.667	63.000
5	6.2	3	63.333	63.333	63.000
6	16	3	59.333	59.333	59.333
7	38	3	13.000	13.000	13.000

cell density
File: 5829cdn Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	66.583				
0.39	66.583	0.606		1.74	k= 1, v=17
1.0	66.583	0.606		1.82	k= 2, v=17
2.5	63.000	0.000		1.85	k= 3, v=17
6.2	63.000	0.000		1.87	k= 4, v=17
16	59.333	0.620		1.87	k= 5, v=17
38	13.000	8.449	*	1.88	k= 6, v=17

s = 8.369
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	14.	9.8	21.	0.081	0.68
EC10	17.	12.	23.	0.070	0.72
EC25	21.	16.	27.	0.051	0.78
EC50	27.	23.	32.	0.032	0.86

Slope = 5.93 Std.Err. = 1.14

Goodness of fit: p = 0.59 based on DF= 5.0 19.

5829CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	63.0	65.3	-2.32	100.	0.00
0.390	3.00	67.0	65.3	1.68	100.	2.18e-14
1.00	3.00	73.3	65.3	8.01	100.	2.18e-14
2.50	3.00	62.7	65.3	-2.66	100.	3.59e-08
6.20	3.00	63.3	65.3	-1.99	100.	0.00665
16.0	3.00	59.3	59.8	-0.489	91.6	8.42

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38.0	3.00	13.0	12.9	0.130	19.7	80.3
100.	3.00	0.00	0.0268	-0.0268	0.0410	100.

biomass
File: 5829b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	676.358	96.623	0.798
Within (Error)	19	2299.608	121.032	
Total	26	2975.967		

Critical F value = 2.54 (0.05,7,19)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

biomass
File: 5829b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	14.550	14.550		
2	0.39	7.733	7.733	0.876	
3	1.0	13.933	13.933	0.079	
4	2.5	4.167	4.167	1.335	
5	6.2	5.033	5.033	1.223	
6	16	8.333	8.333	0.799	
7	38	6.100	6.100	1.086	
8	100	-0.800	-0.800	1.973	

Bonferroni T table value = 2.70 (1 Tailed Value, P=0.05, df=19,7)

biomass
File: 5829b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	0.39	3	20.981	144.2	6.817
3	1.0	3	20.981	144.2	0.617
4	2.5	3	20.981	144.2	10.383
5	6.2	3	20.981	144.2	9.517
6	16	3	20.981	144.2	6.217
7	38	3	20.981	144.2	8.450
8	100	3	20.981	144.2	15.350

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biomass
File: 5829b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	14.550	14.550	14.550
2	0.39	3	7.733	7.733	10.833
3	1.0	3	13.933	13.933	10.833
4	2.5	3	4.167	4.167	5.908
5	6.2	3	5.033	5.033	5.908
6	16	3	8.333	8.333	5.908
7	38	3	6.100	6.100	5.908
8	100	3	-0.800	-0.800	-0.800

biomass
File: 5829b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	14.550				
0.39	10.833	0.478		1.73	k= 1, v=19
1.0	10.833	0.478		1.81	k= 2, v=19
2.5	5.908	1.111		1.84	k= 3, v=19
6.2	5.908	1.111		1.85	k= 4, v=19
16	5.908	1.111		1.86	k= 5, v=19
38	5.908	1.111		1.87	k= 6, v=19
100	-0.800	1.973	*	1.87	k= 7, v=19

s = 11.001
Note: df used for table values are approximate when v > 20.

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Data Evaluation Report on the acute toxicity of Aminopyralid on the Freshwater Algae, *Pseudokirchneriella subcapitata*

PMRA Submission #:{.....} EPA MRID #: 462358-30

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D301682
	OECD Data Point	{.....}
	EPA MRID	462358-30
	EPA Guideline	123-2

Test material: Aminopyralid **Purity:** 94.5%
Common name: XDE-750
Chemical name: IUPAC: 4-amino-3,6-dichloro-picolinic acid
CAS name: Not reported
CAS No.:150114-71-9
Synonyms: XR-750

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/17/04

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/4/04

Primary Reviewer: Brian D. Kiernan
Biologist, OPP/EFED/ERBIV
Signature:
Date: 12/13/2004 6/16/05

Secondary Reviewer(s):
PMRA
Signature:
Date:

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 005100

Date Evaluation Completed: 06/15/05

CITATION: Hoberg, J.R. 2003. XDE-750 - Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. Unpublished study performed by Springborn Smithers Laboratories, Inc., Wareham, Massachusetts. Laboratory Project Identification No. 12550.6161. Study submitted by The Dow Chemical Company for Dow AgroSciences LLC, Midland, Michigan. Experimental start date November 29, 2001 and experimental termination date December 9, 2001. The final report issued October 10, 2003.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed to Aminopyralid, as XDE-750, under static conditions. The nominal test concentrations were 6.3, 13, 25, 50, and 100 ppm a.i., compared to negative and solvent controls. The mean measured concentrations were <1.2 and <1.4 (LOQ, negative and solvent controls), 5.6, 12, 23, 46, and 94 ppm a.i.

By 96 hours, the cell density percent inhibitions were -10, -3, -13, 99, and 99% for the 5.6, 12, 23, 46, and 94 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour biomass were -6, 11, -9, 101, and 103% in the 5.6, 12, 23, 46, and 94 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour growth rates were 1, 3, -4, 104, and 128% in the 5.6, 12, 23, 46, and 94 ppm a.i. treatment groups, respectively, compared to the pooled control. All endpoints were significantly reduced at the 46 and 94 ppm a.i. treatment levels. Growth rate was the most sensitive endpoint, with an EC_{50} of 30 ppm a.i.; the NOEC was 23 ppm a.i. for all endpoints. It is not clear from the study if the endpoints were affected by the dosage or the pH levels at the higher doses. It is assumed here to be due to treatment effect.

The study is scientifically sound but does not satisfy the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata* due to excessive acidity at the higher concentrations. This study is classified as Supplemental.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type: Static

Cell density:
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 32 ppm a.i. 95% C.I.: 9.4-110 ppm a.i.
Slope: N/A

Growth rates:
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 30 ppm a.i. 95% C.I.: 11-79 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve):
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 32 ppm a.i. 95% C.I.: 7.6-130 ppm a.i.
Slope: N/A

Endpoint(s) Affected: Cell density , growth rates, and biomass.
Most sensitive endpoint: Growth rate

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA FIFRA Subdivision J Guidelines 122-2 and 123-2, OECD Guideline #201, and EC Guideline L383A-C.3. However, the pH range was exceedingly large.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice with the exception of the collection of samples for routine water contaminant screening analyses.

A. MATERIALS:

1. Test Material Aminopyralid, XDE-750

Description: Not reported

Lot No./Batch No. : F0031-143

Purity: 94.5%

Stability of Compound
Under Test Conditions: The mean measured concentration of Aminopyralid were 97-100% of nominal at hour 0 and 83-88% of nominal at hour 96 (Table 3, p. 27).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test substance was stored at room temperature in the dark.

2. Test organism:

Name: *Pseudokirchneriella subcapitata*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: 1648
Source: Originally from Carolina Biological Supply, Burlington, NC. Current in-house laboratory cultures.
Age of inoculum: 3 days old
Method of cultivation: Algal Assay Procedure (AAP) medium (Table 1, p. 25).

B. STUDY DESIGN:

1. Experimental Conditions

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a) Range-finding Study: The definitive nominal test concentration was based on results of a range-finding test. The range-finding test was conducted at concentrations of 0.0010, 0.010, 0.10, 1.0, and 10 ppm a.i., with dilution water and solvent controls. The 96-hour cell densities were 249 x 10^4 and 135 x 10^4 cells/mL for the dilution water control and solvent control, respectively. The 0.0010, 0.010, 0.10, 1.0, and 10 ppm a.i. treatment groups had 96-hour cell densities of 268, 250, 185, 284, and 285 x 10^4 cells/mL, respectively.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous	Inoculum used in test was taken from stock culture and transferred to fresh medium three days before testing.
culturing media and conditions: (same as test or not)	Algal Assay Procedure (AAP) medium (Table 1, p. 25); same as test.	*EPA recommends two week acclimation period.*
health: (any toxicity observed)	Not reported	*OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*

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Parameter	Details	Remarks *Criteria*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks with stainless steel caps 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	Algal Assay Procedure (AAP) medium 3.5-7.5 3.5-9.8 disodium EDTA $NaHCO_3$ N/A	Acidity increased greatly with treatment level. *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Dilution water Sterilized and deionized 7.5 ± 0.1 N/A pH adjusted using 0.1 *N* NaOH or 0.1 *N* HCl 1.0 mg a.i./L (December 2001) Not reported Not detected Not detected Not reported	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 100 ± 10 rpm	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*

Parameter	Details	Remarks *Criteria*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	 3 3 3	One additional replicate of the 25 ppm a.i. treatment group was not inoculated with algae and used for analytical determination. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp. tests should be conducted with four replicates.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	0 (negative and solvent controls), 6.3, 13, 25, 50, and 100 ppm a.i. <1.2-1.4 (LOQ, negative and solvent controls), 5.6, 12, 23, 46, and 94 ppm a.i.	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	Dimethylformamide, 0.10 mL/L	
Method and interval of analytical verification	HPLC; 0 and 96 hours	
Test conditions temperature: photoperiod: light intensity and quality:	23-24°C Continuous 3200-4500 lux	*EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical {if used) name: concentrations:	N/A	
Other parameters, if any	None	

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2. Observations

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell densities, biomass (area under the growth curve), and growth rates.	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Haemocytometer and a compound microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water and solvent control group cell densities at test termination were 133X and 136X greater, respectively, than the dilution water and solvent control group cell densities at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 96 hours, the cell density percent inhibitions were -10, -3, -13, 99, and 99% for the 5.6, 12, 23, 46, and 94 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour biomass were -6, 11, -9, 101, and 103% in the 5.6, 12, 23, 46, and 94 ppm a.i. treatment groups, respectively, compared to the pooled control. The percent inhibitions for 0-72 hour growth rates were 1, 3, -4, 104, and 128% in the 5.6, 12, 23, 46, and 94 ppm a.i. treatment groups, respectively, compared to the pooled control. The growth rates were significantly reduced in the 46 and 94 ppm a.i. treatment groups. It is unclear whether the effects were related to treatment level or pH of the media. It is assumed to be a dose response.

Table 3: Effect of Aminopyralid, XDE-750, on freshwater algae (*Pseudokirchneriella subcapitata*)

Treatment mean measured and nominal concentrations [a] (ppm a.i.)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition[b]
Dilution water control	10,000	72,500	1,330,000	--
Solvent control	10,000	45,000	1,360,000	--
5.6 (6.3)	10,000	54,200	1,480,000	-10
12 (13)	10,000	59,200	1,380,000	-3
23 (25)	10,000	56,700	1,510,000	-13
46 (50)	10,000	8,300	10,000	99*
94 (100)	10,000	2,500	10,000	99*
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.
[b] The % inhibition was based on pooled control.
* Significantly reduced compared to the pooled control (Williams test).

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Table 4: Effect of Aminopyralid, XDE-750, on freshwater algae *(Pseudokirchneriella subcapitata)*

Mean Measured and Nominal Treatment Concentrations [a] (ppm a.i.)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
Dilution water control	10,000	1.38	--	506,000	--
Solvent control	10,000	1.45	--	515,000	--
5.6 (6.3)	10,000	1.39	1	541,000	-6
12 (13)	10,000	1.37	3	453,000	11
23 (25)	10,000	1.47	-4	555,000	-9
46 (50)	10,000	-0.05	104*	-3,000*	101*
94 (100)	10,000	-0.40	128*	-17,000*	103*
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

[a] The nominal test concentrations are presented in parentheses.
[b] The data was based on the 0-72 hours of the test.
* Significantly reduced compared to the pooled control (Williams test).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass [a]	Growth rate[a]	Cell density
NOEC or EC_{05} (ppm a.i.)	23	23	23
EC_{50} (ppm a.i.)	32	30	32
IC_{50} or EC_{50} (ppm a.i.) (95% C.I.)	7.6-130	11-79	9.4-110
IC_{25}/EC_{25} (ppm a.i.) (and 95% C.I.)	Not reported	Not reported	Not reported
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

[a] Based on 0-72 hour data.
N/A = Not applicable

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B. REPORTED STATISTICS:

Statistical Method: The growth rate and biomass equations are presented on page 18. A t-test was used to compare the dilution water (negative) and solvent controls. The controls were pooled for all endpoints. The 96-hour NOEC and LOEC values for cell density and biomass were estimated, after the Kruskal -Wallis' test indicated no significant effects. The 96-hour growth rate NOEC and LOEC values were determined using the Williams test. The EC_{50} values were determined by linear regression of the response using a computer program. The reported statistics were based on the mean measured test concentrations.

Cell density:
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 32 ppm a.i. 95% C.I.: 9.4-110 ppm a.i.
Slope: Not reported

Growth rates:
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 30 ppm a.i. 95% C.I.: 11-79 ppm a.i.
Slope: Not reported

Plant biomass (area under the growth curve):
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50}: 32 ppm a.i. 95% C.I.: 7.6-130 ppm a.i.
Slope: Not reported

Endpoint(s) Affected: Cell density , growth rates, and biomass.
Most sensitive endpoint: Growth rates

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: William's test was used to confirm the NOEC. The EC_{50}s were verified using Toxanol, a statistics program available upon request. It was not possible to print out the result, but the program produced similar numbers to those derived by the author. Slopes were determined, but are not reported due to lack of confidence in their veracity.

Cell density:
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: could not determine 95% C.I.: N/A
Slope: N/A

Growth rates:
NOEC: 23 ppm a.i.

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LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50} :could not determine 95% C.I.: N/A
Slope: N/A

Plant biomass (area under the growth curve):
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50} : could not determine 95% C.I.: N/A
Slope: N/A

D. STUDY DEFICIENCIES:

The pH of the higher treatment levels exceeded reasonably expected environmental values and may have had a deleterious affect on the organisms in those treatment levels.

E. REVIEWER'S COMMENTS:

The reviewer could not determine the toxicity values using the usual Nuthatch statistical program; the NOEC could be determined visually and the study author's results for the EC_{50} values are reported in the Executive Summary and Conclusions sections.

F. CONCLUSIONS: The study is scientifically sound but does not satisfy the guidelines for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata* [§123-2] due to excess acidity at the higher concentrations. This study is classified as Supplemental. Growth rate was the most sensitive endpoint, with an EC_{50} of 30 ppm a.i.; the NOEC was 23 ppm a.i. for all endpoints

Cell density:
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 32 ppm a.i. 95% C.I.: 9.4-110 ppm a.i.
Slope: N/A

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Growth rates:
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 30 ppm a.i. 95% C.I.: 11-79 ppm a.i.
Slope: N/A

Plant biomass (area under the growth curve):
NOEC: 23 ppm a.i.
LOEC: 46 ppm a.i.
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}/IC_{50}: 32 ppm a.i. 95% C.I.: 7.6-130 ppm a.i.
Slope: N/A

Endpoint(s) Affected: Cell density , growth rates, and biomass.
Most sensitive endpoint: Growth rate

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III. REFERENCES:

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Horning, W.B. and C.I. Weber, 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-89/014. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

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Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessier, J.R. Menkedick, T.W. Neiheisel, P.A. Lewis, D.J. Kiemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* 27: 103-117.

Williams, D.A. 1972. A comparison of several dose levels with a zero control. *Biometrics* 28: 519-531.

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DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT LC_{50} TEST
§141-1

1. **CHEMICAL:** XDE-750 PC Code No.: 005100

2. **TEST MATERIAL:** XDE-750 Technical Purity: 94.5 ± 0.5%

3. **CITATION:**

Author: J. Aufderheide

Title: XDE-750: Acute Contact Toxicity Test with the Honeybee, *Apis mellifera*

Study Completion Date: September 6, 2001

Laboratory: ABC Laboratories
7200 E. ABC Lane
Columbia, Missouri 65202

Sponsor: The Dow Chemical Company
for Dow AgroSciences LLC
Indianapolis, IN 46268

Laboratory Report ID: ABC Study No. 46595/Dow Study No. 011044

DP Barcode: D301682

MRID No.: 462358-31

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: **Date:** 8/18/04

APPROVED BY: Teri S. Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: **Date:** 10/4/04

5. **APPROVED BY:** Brian D. Kiernan, Biologist, OPP/EFED/ERBIV

Signature: 6/16/05 **Date:** 12/09/2004

PMRA

Signature: **Date:**

2030252

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Apis mellifera*

Age or Size of Test Organism at Test Initiation: Not reported

Type of Concentrations: Nominal

Definitive Study Duration: 48 hours

7. CONCLUSIONS:

The honey bee, *Apis mellifera*, was exposed to Aminopyralid (XDE-750 Technical) for 48 hours at a single nominal concentration of 100 µg a.i./bee. By 48 hours, no mortalities or sublethal effects were observed in the 100 µg a.i./bee treatment group or controls. **The LD_{50} value was >100 µg a.i./bee. As a result, XDE-750 Technical is categorized as practically nontoxic to honeybees on a contact basis.**

This acute contact study is classified as Acceptable. This study is scientifically sound and it satisfies the EFED concerning the guideline requirements for a contact toxicity test with honey bees (Subdivision L, §141-1 or 850.3020).

Reported Statistical Results:

LD_{50}: >100 µg a.i./bee 95% C.I.: N/A

NOEC: 100 µg a.i./bee Probit Slope: N/A

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8. ADEQUACY OF THE STUDY:

A. Classification: This acute contact study is classified as Acceptable. This study is scientifically sound and it satisfies the EFED concerning the guideline requirements for a contact toxicity test with honey bees (Subdivision L, §141-1 or 850.3020).

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS: None

10. SUBMISSION PURPOSE: This study was submitted to provide data on the acute contact toxicity of Aminopyralid (XDE-750 Technical) to honeybees for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	Not reported
Supplier:	Gibbons Honey Farm, Rocheport, Missouri
All bees from the same source?	Yes, from a single, disease-free colony.

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	The cages were plastic and screened. Cages are 14-cm wide x 20-cm long x 10-cm high.
Lighting:	Continuous darkness except at observation periods.

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Guideline Criteria	Reported Information
Temperature:	24.8-25.2°C
Relative humidity:	55-70%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	A range-finding test was conducted at 0.1, 1.0, 10, and 100 µg a.i./bee. There were no mortalities in the control or treatment groups after 48 hours.
Reference toxicant test?	A reference toxicant test was conducted with dimethoate at concentrations of 0.020, 0.20, and 0.40 µg a.i./bee. The 24-hour LD_{50} was 0.063 µg/bee with 95% confidence limits of 0.02 to 0.20 µg/bee (consistent with historical laboratory data).
Method of administration:	The test substance was diluted with acetone, and 1 µL drop of the test solution was applied to the dorsal side of the thorax of each bee.
Nominal doses:	100 µg a.i./bee
Controls: Negative control and/or diluent/solvent control	Negative and Vehicle controls
Number of colonies per group:	3 replicates; 10 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Acetone
Feeding:	500 g/L (w/v) sucrose solution was provided *ad libitum.*
Observations period:	48 hours

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12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	By 48 hours, negative and solvent control mortalities were 0%.
Raw data included:	Replicate data were provided.
Signs of toxicity (if any) were described?	None were observed.

Mortality

Dosage (µg a.i./bee)	No. of bees	Percent Mortality (%)		
		Hour of Study		
		4	24	48
Test Substance (XDE-750 Technical):				
Negative control	30	0	0	0
Vehicle control	30	0	0	0
100	30	0	0	0

Observations: By 48 hours, no mortalities or sublethal effects were observed in the 100 µg a.i./bee treatment group or controls.

Statistical method: The LD_{50} value was estimated based on mortality data. The dimethoate LD_{50} value was calculated using the probit method. The results were based on the nominal test concentration.

Reported Statistical Results:

LD_{50}: >100 µg a.i./bee 95% C.I.: N/A
NOEC: Not reported Probit Slope: N/A

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13. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: The LD_{50} value was estimated visually based on mortality data.

Results:

LD_{50}: >100 µg a.i./bee	95% C.I.: N/A
NOEC: 100 µg a.i./bee	Probit Slope: N/A

14. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study author's.

The test solution used for the contact application was cloudy with a light brown tint.

15. REFERENCES:

U.S. Environmental Protection Agency (U.S. EPA). 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). *Federal Register*.

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ICBPR. Validation Exercise on the Use of Dimethoate as the Toxic Reference Substance in Toxicity Tests on Honeybees (in preparation).

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DATA EVALUATION RECORD
HONEY BEE - ACUTE ORAL LC_{50} TEST
Non-Guideline (OECD 213)

1. **CHEMICAL:** Aminopyralid PC Code No.: 005100

2. **TEST MATERIAL:** XDE-750 Purity: 94.5 ± 0.5%

3. **CITATION:**

Author: J. Aufderheide

Title: XDE-750: Acute Oral Toxicity Test with the Honeybee, *Apis mellifera*

Study Completion Date: September 6, 2001

Laboratory: ABC Laboratories
7200 E. ABC Lane
Columbia, Missouri 65202

Sponsor: The Dow Chemical Company
for Dow AgroSciences LLC
Indianapolis, IN 46268

Laboratory Report ID: ABC Study No. 46596/Dow Study No. 011045

DP Barcode: D301682

MRID No.: 462358-32

PMRA Submission number: 2004-0789

PMRA Data Code: 9.2.4.2

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation **Date:** 8/18/04

THRU: Teri S. Myers, Ph.D., Staff Scientist, Dynamac Corporation **Date:** 10/04/04

5. **APPROVED BY:** Brian D. Kiernan, Biologist, OPP/EFED/ERBIV

Signature: [signature] 6/7/05 **Date:** 12/02/2004

PMRA Reviewer Number: 213; PMRA **Date:** January 24, 2005

Signature:

2030253

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Apis mellifera*

Age or Size of Test Organism at Test Initiation: Not reported

Type of Concentrations: Nominal and actual intake

Definitive Study Duration: 48 hours

7. CONCLUSIONS:

The honey bee, *Apis mellifera* L., was exposed to Aminopyralid (XDE-750) for 48 hours, at test concentrations of 6.0, 15, 30, 60, and 120 µg a.i./bee (actual mean ingested doses were 6.0, 16, 28, 32, and 117 µg a.i./bee, respectively). By 48 hours, there was 3, 7, 0, 0, and 0% mortality observed in the 6.0, 16, 28, 32, and 117 treatment groups µg a.i./bee, respectively, compared to 3% control mortality. No sublethal effects were observed in the control or treatment groups.

This acute oral study is classified as Supplemental. This study is scientifically sound, but it is a non-guideline study and does not fulfill an OPP guideline requirement. However, the results are useful for risk assessment purposes.

EAD Conclusion:

This study is scientifically sound and is classified as **acceptable**. The 48-hour LD_{50} and NOEL of aminopyralid (XDE-750) to the honey bee were >117 µg a.i./bee and 117 µg a.i./bee, respectively.

Results:

LD_{50}: >117 µg a.i./bee 95% C.I.: N/A
NOEL: 117 µg a.i./bee Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: The acute oral study is scientifically sound and is classified as Supplemental.

B. Rationale: This acute oral study is scientifically sound and is classified as Supplemental because the study is a non-guideline study and does not fulfill an OPP guideline requirement.

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C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

N/A

10. SUBMISSION PURPOSE: This study was submitted to provide data on the acute oral toxicity of aminopyralid (XDE-750) to honeybees for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	Not reported
Supplier:	Gibbons Honey Farm, Rocheport, Missouri
All bees from the same source?	Yes, from a single, disease-free colony.

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	The cages were plastic and screened. Cages were 14-cm wide x 20-cm long x 10-cm high.
Lighting:	Continuous darkness except at observation periods.
Temperature:	24.8-25.6°C
Relative humidity:	53-67%

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C. Test Design

Guideline Criteria	Reported Information
Range finding test?	A range-finding test was conducted at 0.10, 1.0, 10, and 100 µg a.i./bee. Food consumption ranged from 74 to 100% with highest consumption rates at the lowest test concentrations. There were no mortalities in the control or treatment groups after 48 hours.
Reference toxicant test?	The reference toxicant, dimethoate, was tested for 24 hours. The test concentrations were 0.020, 0.20, and 0.40 µg/bee (assuming 100% consumption). The 24-hour LD_{50} was 0.083 µg/bee with 95% confidence limits of 0.028 to 0.15 µg/bee. This value was determined by the SAS Probit method (consistent with historical laboratory data).
Method of administration:	The test solutions were mixed with a 500 g/L sucrose solution.
Nominal doses:	6.0, 15, 30, 60, and 120 µg a.i./bee (Actual mean ingested doses were 6.0, 16, 28, 32, and 117 µg a.i./bee, respectively, reviewer-calculated from Table 1, p. 15).
Controls: Negative control and/or diluent/solvent control	Negative control
Number of colonies per group:	3 replicates; 10 bees/replicate

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Guideline Criteria	Reported Information
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	N/A
Feeding:	The test solutions were provided for 6 hours. Then, the bees were supplied with untreated 500 g/L sucrose solution, *ad libitum*.
Observations period:	48 hours

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	0% negative control mortality by 48 hours.
Raw data included:	Replicate data were provided.
Signs of toxicity (if any) were described?	No signs of toxicity were observed.

Mortality

Dosage µg a.i./bee (actual intake: µg a.i./bee)[1]	No. of bees	Percent Mortality (%)[2]		
		Hour of Study		
		4	24	48
Test Substance (XDE-750)				
Control Group	30	3	3	3
6.0 (6.0)	30	0	3	3
15 (16)	30	7	7	7
30 (28)	30	0	0	0

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Dosage µg a.i./bee (actual intake: µg a.i./bee)[1]	No. of bees	Percent Mortality (%)[2]		
		Hour of Study		
		4	24	48
60 (32)	30	0	0	0
120 (117)	30	0	0	0
Toxic Standard (dimethoate, µg/bee):				
Control	30	0	7	N/A
0.020 (0.021)	30	0	0	N/A
0.20 (0.18)	30	80	83	N/A
0.40 (0.24)	30	78	78	N/A

[1] Actual intake concentrations were reviewer-calculated averages from replicate calculated dosages.
[2] Percent mortalities were reviewer-calculated based on replicate data (Table 3-4, pp. 17-18).

Observations: By 48 hours, there was 3, 7, 0, 0, and 0% mortality observed in the 6.0, 16, 28, 32, and 117 treatment groups µg a.i./bee, respectively, compared to 3% control mortality. No sublethal effects were observed in the control or treatment groups.

Statistical method: The LD_{50} values were estimated due to less than 50% mortality. The reported LD_{50} was based on the nominal concentrations.

Reported Statistical Results:

LD_{50}: >120 µg a.i./bee 95% C.I.: N/A
NOEL: Not reported Probit Slope: N/A

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13. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: Values were visually determined due to lack of effects. The reported values were based on the mean measured intake concentrations.

Results:

LD_{50}: >117 µg a.i./bee	95% C.I.: N/A
NOEL: 117 µg a.i./bee	Probit Slope: N/A

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study author's.

The bees were starved for approximately 1.75 hours prior to introduction of the definitive test solution feeders.

The mean actual consumed dosages were reviewer-calculated from replicate calculated dosages (Tables 1 and 2, pp. 15-16). The consumption of the treatment groups ranged from 25 to100% and negative control diets were 100% consumed. The consumption of the reference substance diets ranged from 7 to 100%.

EAD comments:

This study is scientifically sound and is classified as **acceptable**. The study was done using OECD Guideline # 213 without deviations. The EPA reviewer classified this study to be acceptable and supplemental, as it was a non-EPA guideline study and did not fulfill OPP guideline requirement.

No amendments to the DER are required.

15. REFERENCES:

Organization for Economic Cooperation and Development. 1997. Decision of the Council, Revised Principles of GLP [C(97)186/Final].

Finney, D.J. 1971. Probit Analysis. Cambridge University Press.

Gough, H.J., McIndoe, E.C., Lewis, G.B. (1994). The use of dimethoate as a reference compound in laboratory acute toxicity tests on honey bees (*Apis mellifera* L.). 1981-1992. Journal of Apicultural Research 22, 119-125.

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Data Evaluation Report on the Reproductive Effects of XDE-750 (Aminopyralid) on Avian Species *Colinus virginianus*

PMRA Submission Number 2004-0789 EPA MRID Number 462358-12

Data Requirement:	PMRA DATA CODE	9.6.3.1
	EPA DP Barcode	D301682
	OECD Data Point	II A 8.1.4
	EPA MRID	462358-12
	EPA Guideline	§71-4a

Test material: XDE-750 **Purity:** 94.5%
Common name: Aminopyralid
Chemical name: IUPAC: Not reported
CAS name: 3,6-Dichloro-4-amino-2-pyridinecarboxylic acid
CAS No.: Not reported
Synonyms: XDE-750/XR-750

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/01/04

QC Reviewer: Teri S. Myers, PhD
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/10/04

Primary Reviewer: Brian D. Kiernan, Biologist
OPP/EFED/ERB - IV
Signature:
Date: 11/08/04 6/16/05

Secondary Reviewer(s): Brigitte Lavallée
PMRA (1595)
Signature:
Date: February 3, 2005

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 005100

CITATION: Mach, J.J. 2003. Avian Reproduction Study with XDE-750 in Northern Bobwhite (*Colinus virginianus*). Unpublished study performed by Genesis Laboratories, Inc., Wellington, CO. Laboratory Study No. 02001. Study submitted by Dow Chemical Company, Midland, MI for Dow AgroSciences LLC, Indianapolis, IN. Study initiated June 11, 2002 and submitted February 25, 2003.

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EXECUTIVE SUMMARY:

The one-generation reproductive toxicity of XDE-750 (aminopyralid) to groups (20 pens/control group and 15 pens/treatment group) of 1 male and 1 female of 21-week-old Northern Bobwhite quail was assessed over approximately 20 weeks. XDE-750 was administered to the birds in the diet at nominal concentrations of 0 (solvent control; concentration not specified), 675, 1350, and 2700 ppm. Mean-measured concentrations were <1.00 (<LOD, control), 640, 1270, and 2610 ppm a.i., representing 94-97% of nominal concentrations.

There were statistically significant differences found in the lowest dose tested for two survival endpoints (hatchling survival per eggs set and 14-day hatchling survival), but it is unclear whether these were treatment-related effects. Together with apparent downward trends in hatchling per live embryos and hatchlings per pen, it is uncertain that the authors conclusion that these effects are not treatment related can be supported. At the very least, the husbandry during the study can be called into question. Therefore, the study did not determine a NOEC for these endpoints.

This toxicity study is scientifically sound, with the aforementioned uncertainties. Additionally, the quantity and fate of the acetone used in test diet preparation was not specified; and raw data pertaining hatchling weight were not provided. As a result, this study is not consistent with the guideline requirement for an avian reproduction toxicity study using Northern Bobwhite quail (§71-4a) and is classified as SUPPLEMENTAL.

EAD Conclusion:
The status of EAD for this study is acceptable. Therefore, the NOEC for aminopyralid for the bobwhite quail is 2610 mg ai/kg dw of diet, the highest tested concentration, based on reproductive parameters.

Results Synopsis

Test Organism Size/Age: Approximately 21 weeks old at test initiation (225-349 g)

NOEC: not determined
LOEC: not determined
Endpoint(s) Affected: Several hatchling survival endpoints

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures of the U.S. EPA Pesticide Assessment Guidelines, Series 71-4 (1988); and OECD Guidelines for Testing of Chemicals, No. 206 (1984).

Deviations from §71-4 are:

1. The high degree of variability in this study precluded its capacity to detect dose response effects. Therefore, a NOEC was not determined.
2. The concentration of acetone used in preparation of the tests diets was not specified. Also, it was not specified if the acetone was allowed to completely evaporate off the treated feed prior to offering.
3. Raw data on hatchling weight should be submitted for review.
4. Analysis of the stability and homogeneity of XDE-750 in treated feed was not adequately assessed

These deviations did not affect the scientific validity of the study. However, this study is not consistent with guideline requirements.

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COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with United States and OECD standards with the following exception: portions of the sub-batches were not correctly weighed. For each sub-batch, two smaller quantities of feed (≤20 kg) must be weighed to total the sub-batch size. These smaller weights were not recorded, only the total weight of the sub-batch for Batches 3 and 4. Batch 3 was analyzed and found to be within the certified limits. Batch 4 was mixed in the same manner. This will not affect the integrity of the study, as the total weights of the feed were recorded (p. 3).

A. MATERIALS:

1. Test Material XDE-750 (aminopyralid)

Description: White powder

Lot No./Batch No.: F0031-143 (TSN102319)

Purity: 94.5%

Stability of Compound Under Test Conditions: The stability of XDE-750 in avian feed was not assessed.

Storage conditions of test chemical: Ambient

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

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2. **Test organism**: Northern bobwhite (*Colinus virginianus*)

Table 1: Test organism.

Parameter	Details	Remarks *Criteria*
Species (common and scientific names):	Northern Bobwhite quail (*Colinus virginianus*)	*EPA requires: a wild waterfowl species, preferably the mallard,* Anas platyrhynchos, *or an upland game species, preferably the northern bobwhite,* Colinus virginianus.
Age at Study Initiation:	Approximately 21 weeks	It was stated that birds were approaching their first breeding season. *EPA requires: birds should be approaching their first breeding season.*
Body Weight: (mean and range)	Males: Overall range (n=65) 225 to 343 g, with group means of 281 to 291 g. Females: Overall range (n=65) 232 to 349 g, with group means of 278 to 288 g.	Individual body weights were recorded at Weeks 0, 2, 4, 6, 8, and 20 (test termination). *EPA requires that body weights should be recorded at test initiation and at biweekly intervals up to week eight or up to the onset of egg laying and at termination.*
Source:	Barrett's Quail Farm Houston, TX	Birds were from the same hatch, and were phenotypically indistinguishable from wild birds. *EPA requires that all birds should be from the same source.*

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study - None reported.

b. Definitive Study

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Table 2: Experimental Parameters.

Parameter	Details	Remarks *Criteria*
Acclimation period:	14 days	Birds were observed once daily for general physical condition, disease, and abnormalities. Birds were also examined by a veterinarian to assess their general physical condition and suitability for testing.
Conditions (same as test or not):	Same as test	
Feeding:	Dry non-medicated Ranchway 16% Poultry Layer Complete (Ranch-Way, Fort Collins, CO) and municipal water from the Northern Colorado Water Association were provided *ad libitum*.	*EPA recommends a 2-3 week health observation period prior to selection of birds for treatment. Birds must be generally healthy without excess mortality. Feeding should be ad libitum, and sickness, injuries or mortality be noted.*
Health (any mortality observed):	All birds were normal and active (p. 19). No disease or abnormalities were observed and no medication was provided.	
Test duration pre-laying exposure:	Approximately 10 weeks	*EPA requires* ***Pre-laying exposure duration*** *At least 10 weeks prior to the onset of egg-laying.* ***Exposure duration with egg-laying*** *At least 10 weeks.* ***Withdrawal period*** *If reduced reproduction is evident, a withdrawal period of up to 3 weeks should be added to the test phase.*
egg-laying exposure:	Approximately 10 weeks	
withdrawal period, if used:	None	

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Parameter	Details	Remarks *Criteria*
Pen (for parental and offspring) size: construction materials: number:	Parents (one pair) were housed in cages measuring 51 x 25 x 25.5 cm (floor surface of 1275 cm^2). Offspring (by set and group) were housed in 90 x 80 x 25 cm poultry brooders (floor surface of 7200 cm^2). Parental pens were constructed of galvanized steel. Offspring pens were described as box-type (not further specified). 20 parental pens (replicates) for the control group, and 15 parental pens for each toxicant level.	*Pens* *Adequate room and arranged to prevent cross contamination* *Materials* *Nontoxic material and nonbinding material, such as galvanized steel.* *Number* *At least 5 replicate pens are required for mallards housed in groups of 7. For other arrangements, at least 12 pens are required, but considerably more may be needed if birds are kept in pairs. Chicks are to be housed according to parental grouping.*
Number of birds per pen (male:female)	2 birds/pen (1 male:1 female)	*EPA requires one male and 1 female per pen. For quail, 1 male and 2 females is acceptable. For ducks, 2 males and 5 females is acceptable.*
Number of pens per group/treatment negative control: solvent control: treated:	N/A 20 pens 15 pens/treatment	*EPA requires at least 12 pens, but considerably more if birds are kept in pairs. At least 16 is strongly recommended.*
Test concentrations (ppm diet) nominal: measured:	0 (solvent control), 675, 1350, and 2700 ppm diet <1.00 (<LOD, control), 640, 1270, and 2610 ppm a.i.	Mean-measured concentrations were determined from freshly-prepared treated feed collected from Batches 2, 3, and 11 (Table 1, p. 25). Concentrations were corrected for the purity of the test substance (p. 14).

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Parameter	Details	Remarks *Criteria*
		EPA requires at least two concentrations other than the control are required; three or more are recommended.
Maximum labeled field residue anticipated and source of information:	Not specified	However, the Agency is aware that the maximum EEC is 26 ppm based on maximum label rate *EPA requires that the highest test concentrations should show a significant effect or be at or above the actual or expected field residue level. The source [i.e., maximum label rate (in lb ai/A & ppm), label registration no., label date, and site should be cited]*
Solvent/vehicle, if used type: amount:	Acetone Not specified	acetone needs to be addressed in more detail *EPA requires corn oil or other appropriate vehicle not more than 2% of diet by weight*
Was detailed description and nutrient analysis of the basal diet provided? (Yes/No)	Yes. Basal diets contained 16.0% protein, 3.5% fat, 7.0% fiber, and 3.0-4.0% calcium (Appendix D1, p. 106).	Offspring received Ranch-Way Turkey & Game Bird Starter without the addition of test substance (Appendix D2, p. 107). *EPA requires a commercial breeder feed (or its equivalent) that is appropriate for the test species.*

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Parameter	Details	Remarks *Criteria*
Preparation of test diet	The appropriate amount of test material was suspended in acetone, then combined with basal ration and mixed for 15 minutes (p. 14). To facilitate mixing, each test group was split into sub-batches and pooled together after the mix to form a single batch. Treated diets were prepared bi-weekly, and were stored at approximately -17°C until needed.	The final acetone concentration was not reported, and it was not specified if the acetone was allowed to completely evaporate prior to offering. *A premixed containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it must be completely evaporated prior to feeding.*
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes, homogeneity	
Were concentrations in diet verified by chemical analysis?	Yes	Samples were analyzed from feed collected from Batches 2, 3, and 11 (Table 1, p. 25).
Did chemical analysis confirm that diet was stable? and homogeneous?	Stability was not assessed.	However, ancillary data from other studies strongly suggests stability in feed.
Feeding and husbandry	Feeding and husbandry conditions appeared to be adequate, given guideline recommendations.	
Test conditions (pre-laying) temperature: relative humidity: photo-period:	18-27°C, with a mean range of 20-23°C. 31-80%, with a mean range of 49-65%. 7 hours light/day up through Week 8, then increased 2 hours/day for 5 days to 17 hours light/day thereafter.	An average light intensity of 34.1 foot-candles was maintained at bird level until 8/26/02 (2 months after study initiation) and then changed to 17.5 foot-candles to help minimize pecking (p. 13).

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Parameter	Details	Remarks *Criteria*
		EPA Requires *Temperature:* *About 21 °C (70 °F)* *Relative humidity:* *About 55%* *Lighting* *First 8 weeks: 7 h per day.* *Thereafter: 16-17 h per day.* *At least 6 foot candles at bird level.*
Egg Collection and Incubation		
Egg collection and storage collection interval: storage temperature: storage humidity:	Daily 14-22°C, with a mean range of 15-17°C 48-92%, with a mean range of 54-71%	*EPA requires eggs to be collected daily; egg storage temperature approximately 16°C (61°F); humidity approximately 65%.*
Were eggs candled for cracks prior to setting for incubation?	Yes	*EPA requires eggs to be candled on day 0*
Were eggs set weekly?	Yes	
Incubation conditions temperature: humidity:	85-93°F, with a mean range of 89-90°F (wet bulb) 54-77%, with a mean range of 64-66%	Incubation and hatching occurred in the same incubator, in different compartments. Due to the high volume of eggs produced during the last weeks of the egg-laying period, an additional incubator was necessary.
When candling was done for fertility?	Day 11 for fertility and Day 18 for viability.	*EPA requires:* *Quail: approx. day 11* *Ducks: approx. day 14*
When the eggs were transferred to the hatcher?	Day 21	*EPA requires:* *Bobwhite: day 21* *Mallard: day 23*

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Parameter	Details	Remarks *Criteria*
Hatching conditions temperature: humidity: photo-period:	85-93°F, with a mean range of 89-90°F (wet bulb) 54-77%, with a mean range of 64-66% 12 hours light/day (hatchlings)	Incubation and hatching occurred in the same incubator, in different compartments. Due to the high volume of eggs produced during the last weeks of the egg-laying period, an additional incubator was necessary. *EPA requires:* *temperature of 39°C (102°F)* *humidity of 70%*
Day the hatched eggs were removed and counted	Day 24	*EPA requires Bobwhite: day 24* *Mallard: day 27*
Were egg shells washed and dried for at least 48 hrs before measuring?	Yes	
Egg shell thickness no. of eggs used: intervals: mode of measurement:	All eggs laid on one day Day 3 of Weeks 12, 14, 16, 18, and 20. Three points around the equatorial circumference were measured to the nearest 0.001 mm.	*EPA requires newly hatched eggs be collected at least once every two weeks. Thickness of the shell plus membrane should be measured to the nearest 0.01 mm; 3 - 4 measurements per shell.*
Reference chemical, if used	None used	

2. Observations:

Table 3: Observations.

Parameter	Details	Remarks/Criteria
Parameters measured		

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Parameter	Details	Remarks/Criteria
Parental: (mortality, body weight, mean feed consumption)	- mortality - signs of toxicity, injury, or illness - body weight - food consumption - necropsy	At necropsy, specific examination was made on the gastro-intestinal tract, liver, kidneys, bile duct, heart, spleen, and reproductive organs. Other observations were recorded as necessary.
Egg collection and subsequent development: (no. of eggs laid, no. of eggs cracked, shell thickness, no. of eggs set, no. of viable embryos, no. of live 3 week embryos, no. hatched, no. of 14-day survivors, average weight of 14-day-old survivors, mortality, gross pathology, others)	- eggs laid - eggs broken, cracked, small, and soft shelled, etc. - egg shell thickness - eggs set - viable embryos - live 3-week embryos - number of hatchlings - signs of toxicity and physical defects of hatchlings - number of 14-day-old survivors - 14-day-old survivor body weight	*EPA requires:* • *Eggs laid/pen* • *Eggs cracked/pen* • *Eggs set/pen* • *Viable embryos/pen* • *Live 3-week embryos/pen* • *Normal hatchlings/pen* • *14-day-old survivors/pen* • *14-day-old survivors/pen* • *Weights of 14-day-old survivors (mean per pen)* • *Egg shell thickness* • *Food consumption (mean per pen)* • *Initial and final body weight (mean per pen)*
Indicate if the test material was regurgitated	No indications of dietary regurgitation.	
Observation intervals (for various parameters)	Mortality and signs of toxicity were observed daily for adults and hatchlings. Parental body weights were recorded at Weeks 0, 2, 4, 6, 8, and 20 (test termination), and food consumption was determined weekly.	*Body weights and food consumption must be measured at least biweekly.*
Were raw data included?	Yes	except 14 hatching weight, raw mortality and clinical effects for adults

I. RESULTS AND DISCUSSION:

A. MORTALITY:

The author determined that no treatment-related mortality was observed during the study. However, six birds were found dead during the study: one from the control group, one each from the 675 and 2700 ppm groups, and three from the 1350 ppm group (not gender-specific; Table II, p. 26). Only summarized data were provided regarding mortality, clinical effects, and necropsy findings. Therefore, the gender of the decedent animals, clinical effects observed in the decedent animals prior to death, and subsequent necropsy findings could not be differentiated.

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Table 4: Effect of XDE-750 (aminopyralid) on Mortality of *Colinus virginianus*.

Treatment, ppm a.i. measured (and nominal) concentrations	Observation Period		
	Week 7	Week 14	Week 20
	No. Dead	No. Dead	No. Dead
Solvent control	0	0	1
640 (675)	0	1	1
1270 (1350)	0	2	3
2610 (2700)	0	1	1

B. REPRODUCTIVE AND OTHER ENDPOINTS:

Abnormal Effects/Behavior: No treatment-related signs of toxicity were apparent. Effects such as hypo-reactivity, disorientation, immobility, moribundity, ataxia, abnormal head position, and low body carriage were observed in individuals from the 1350 and 2700 ppm groups; however, the study author reported that although these are observations that could be interpreted as toxicosis, the individuals eliciting these responses were not in a dose response, therefore they are considered unrelated to toxicosis (p. 20 and Table II, p. 26). Raw clinical effects data were not provided for review. Other effects observed at all test levels were incidental, and included feather loss, abrasions, healing abrasions, growing feathers, healing toe, and growth on beak.

Food Consumption: No treatment-related effects on food consumption were observed (p. 20 and Table III, p. 27). Overall feed consumption averaged 21-22 g/bird/day for all treatment and control groups. No excess spillage was noted.

Body Weight: No statistically significant treatment-related effects on the differences in body weights were observed (p. 20, and Table IV, p. 28). However, there was a 10% reduction in female body weight at the highest dose tested.

Necropsy: No treatment-related findings were observed at necropsy (p. 21, and Table V, p. 29). Feather loss, lesions, and abrasions were the predominant observation in all groups, including control. Other observations included discolored liver (one bird from the 675 ppm group and two birds in the 2700 ppm group), a lesion or growth on the beak (two birds in the vehicle control group and one bird in the 675 ppm group), growth on the crop (one bird in the vehicle control group), and white milky fluid in intestine and gizzard (unspecified number of birds in the 1350 ppm group).

Reproductive Effects: No treatment-related effects on egg production or quality, fertility, embryonic development, hatchability, or chick survival were observed at any test level (Tables VI-XVII, pp. 30-41). In addition, none of the chicks showed any test substance-related toxicological symptoms during the 14-day maintenance period, and no treatment-related effects on 14-day old chick body weights were observed (p. 23 and Tables XVIII and XIX, pp. 42-43).

A statistically-significant decrease in hatchability (total number of hatchlings as a percentage of viable embryos) was observed between the 1350 ppm and solvent control group (79.5 versus 90.1%, respectively; Table XIV, p. 38). Although the 2700 ppm group had a lower hatchability level (78.2%), it was not statistically different from the solvent control. The statistics were verified for accuracy by the laboratory, and

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no explanation was evident (p. 22). The difference in the 1350 ppm group was reportedly most likely due to the health of the adult birds, which were generally suffering from pecking. The lack of vigor of the hatchlings may be contributed to pecking to as many as 14 adults. The vehicle control and 675 ppm groups had only 5 and 4, respectively, and the 2700 ppm group had 9 adults that were being pecked.

The percent survivability of hatchlings (number of normal 14-day survivors as a percentage of normal hatchlings) was statistically-reduced at the 675 ppm level compared to the solvent control (65.7 versus 89.0%; Table XVI, p. 40). The study author reported that the difference in the hatchling survival may be attributed to the following circumstances. During Week 19, a brooder battery was not turned on, that resulted in the death of 14 hatchlings due to cool temperatures (p. 23). These 14 hatchlings were removed from the calculations. During the same week, in a separate brooder, pecking was attributed to the death of at least 15 hatchlings. A total of 27 hatchlings died in this one brooder, mostly likely attributable to pecking. In addition, during Week 20, another 12 bird deaths were attributed to pecking. Pecking thus may have been the cause for as many as 22 hatchling deaths in this brooder. This total 49 birds that died from causes not common in any of the other brooders. The study author concluded that the statistical difference identified may have been avoided had these hatchlings not suffered these abnormal fates.

Table 5: Reproductive and other parameters (nominal concentrations).

Parameter	Control	675 ppm	1350 ppm	2700 ppm	NOEC/ LOEC
Eggs laid	641	494	444	441	N/A
Eggs laid/hen	32.1	32.9	31.7	31.5	2700 ppm >2700 ppm
Eggs laid/hen/week	3.2	3.3	3.2	3.2	2700 ppm >2700 ppm
Eggs candled	582	447	400	403	N/A
Eggs soft shelled, broken, or damaged	13	11	9	3	N/A
Eggs cracked	2	2	2	2	N/A
Eggs cracked/eggs candled (%)	0.3	0.4	0.5	0.5	2700 ppm >2700 ppm
Shell thickness (mm)	0.198	0.189	0.198	0.194	2700 ppm >2700 ppm
Eggs set	580	445	398	401	N/A
Viable 11-day old embryos	477	399	342	367	N/A
Viable embryos/eggs set (%)	82.2	89.7	85.9	91.5	2700 ppm >2700 ppm
Live 18-day old embryos	471	394	340	363	N/A

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Parameter	Control	675 ppm	1350 ppm	2700 ppm	NOEC/ LOEC
Live 18-day old embryos/viable embryos (%)	98.7	98.7	99.4	98.9	2700 ppm >2700 ppm
No. of total hatchlings	430	340	272	287	N/A
Total hatchlings/viable embryos (%)	90.1	85.2	79.5*	78.2	2700 ppm >2700 ppm
No. of hatchlings to brooders	429	337	271	285	N/A
No. of normal hatchlings	426	335	265	284	N/A
Normal hatchlings/hatchlings to brooders (%)	99.3	99.4	97.8	99.6	2700 ppm >2700 ppm
No. of 14-day old survivors	382	212	231	233	N/A
No. of normal 14-day old survivors	379	211	224	233	N/A
No. of normal 14-day old survivors/No. of normal hatchlings (%)	89.0	65.7*	84.5	82.0	2700 ppm >2700 ppm
No. of 14-day old survivors/eggs laid (%)	59.6	44.2	52.0	52.8	2700 ppm >2700 ppm
14-day old survivors weight (g)	18	18	18	18	2700 ppm >2700 ppm
Mean adult food consumption (g/pen/day)	22	21	21	22	2700 ppm >2700 ppm
Weight of adult males, g at start of treatment: at Week 8: at Week 20 (study termination):	 282 306 316	 286 308 316	 281 302 309	 291 316 325	2700 ppm >2700 ppm
Weight of adult females, g at start of treatment: at Week 8: at Week 20 (study termination):	 274 305 345	 285 312 356	 275 298 349	 285 316 348	2700 ppm >2700 ppm
Gross pathology (proportion of birds with pathological incidents)	No treatment-related abnormalities observed.				

N/A = Not statistically-analyzed.
* Statistically-different from solvent control.

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C. REPORTED STATISTICS:

The following variables were statistically analyzed: adult body weight at each determined interval, weekly mean feed consumption, eggs laid/hen, egg shell thickness, percentage of no. eggs cracked/ no. eggs candled, percentage of no. viable 11-day embryos/no. eggs set, percentage of no. live 18-day embryos/no. viable 11-day embryos, percentage of no. of hatchlings/no. viable 11-day embryos, percentage of no. normal hatchlings/no. hatchlings to brooders, percenttage of no. 14-day normal survivors/no. normal hatchlings, percentage of no. 14-day survivors/no. eggs laid, and 14-day old hatchling body weights (Table XX, p. 44).

Data were assessed for normality using the Chi-square test and for homogeneity of variance using Bartlett's test. If the data set passed the tests for normality and homogeneity, an analysis of variance (ANOVA) was performed to determine statistically-significant differences between groups. If necessary, Dunnett's test (equal replicates) or Bonferroni's test (not equal replicates) was then used to compare the treatment means with the control group mean. If the data set did not pass the tests for normality and homogeneity, they were transformed and re-analyzed. If an appropriate transformation did not succeed in normalizing the distribution, or if the variance was not homogeneous, the original untransformed data were analyzed by Kruskal-Wallis's non-parametric test (H-statistic). Dunn's multiple comparison procedure was used to compare each treatment group with the control. Proportional (percentage) data were arc sine transformed prior to analysis.

All variables were analyzed using TOXSTAT Version 3.4. Sample units were the individual pens within each experimental group, except adult body weights, where the sample unit was the individual bird. Nominal concentrations were used for all estimations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Analysis was conducted using "chicks.sas" (Ver. 3; March 2002), a SAS program written for avian reproductive studies by scientists at EFED/OPP/USEPA. Data for all endpoints were examined graphically using box plots to determine if they exhibited a dose-dependent response, which was ultimately used to select the multiple comparison test to detect LOEC and NOEC. Data for each endpoint were tested to determine if their distributions were normal and if their variances were homogeneous using Shapiro-Wilk's and Levene's tests, respectively. Data that satisfied these assumptions were subjected to Dunnett's and William's tests and data that did not satisfy these assumptions were subjected to the non-parametric Mann-Whitney (with a Bonferroni adjustment) and Jonckheere's tests. Data for dead birds were excluded from the analyses. See Appendix I for output of reviewer's statistical verification to support any reviewer-generated conclusions that may differ from those reported in the study.

Table 6. Reproductive and other parameters (mean-measured concentrations; reviewer-reported).

Parameter	Control	640 ppm	1270 ppm	2610 ppm	NOEC/ LOEC
Eggs laid/pen	33.5	35.0	36.5	31.5	2610 ppm >2610 ppm
Eggs cracked/pen	0.05	0.14	0.17	0.14	2610 ppm >2610 ppm
Eggs not cracked/eggs laid (%)	99.8	99.4	99.6	99.6	2610 ppm >2610 ppm

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Parameter	Control	640 ppm	1270 ppm	2610 ppm	NOEC/ LOEC
Eggs set/pen	30.5	31.5	32.8	28.6	2610 ppm >2610 ppm
Shell thickness	0.20	0.19	0.20	0.20	2610 ppm >2610 ppm
Eggs set/eggs laid (%)	91.3	89.2	89.2	90.8	2610 ppm >2610 ppm
Viable embryo/pen	25.1	28.5	28.3	26.2	2610 ppm >2610 ppm
Viable embryos/eggs set (%)	78.5	88.4	82.8	89.8	2610 ppm >2610 ppm
Live embryos/pen	24.8	28.1	28.2	25.9	2610 ppm >2610 ppm
Live embryo/viable embryo (%)	98.5	98.8	99.1	99.2	2610 ppm >2610 ppm
No. of hatchlings/pen	22.4	24.0	22.0	20.3	2610 ppm >2610 ppm
No. of hatchlings/eggs laid (%)	62.7	66.6	51.5	62.7	2610 ppm >2610 ppm
No. of hatchlings/eggs set (%)	69.0	74.5	57.2	68.9	2610 ppm >2610 ppm
No. of hatchlings/live embryos (%)	88.9	84.8	64.0	77.2	2610 ppm >2610 ppm
Hatchling survival/pen	20.0	15.1	18.6	16.6	2610 ppm >2610 ppm
Hatchling survival/eggs set (%)	60.7	44.4*	47.9	54.9	Not determined
Hatchling survival/no. of hatchlings (%)	87.2	54.1*	85.0	77.4	Not determined
Hatchling weight (g)	NA	NA	NA	NA	NA
Survivor weight (g)	18.5	18.4	18.2	17.9	2610 ppm >2610 ppm
Mean food consumption (g/bird/day)	21.8	21.5	22.0	21.9	2610 ppm >2610 ppm

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Parameter	Control	640 ppm	1270 ppm	2610 ppm	NOEC/ LOEC
Male weight gain (g)	36.2	31.8	25.7	33.8	2610 ppm >2610 ppm
Female weight gain (g)	72.9	72.1	71.8	65.4	2610 ppm >2610 ppm

*Significantly different from the control ($p<0.05$); it is uncertain whether these reductions are related to factors other than treatment (i.e. husbandry issues).
NA=not analyzed; data not provided

E. STUDY DEFICIENCIES:

This study is considered scientifically valid; however, several notable deviations from §71-4 guidance were observed:

* The high degree of variability in this study precluded its capacity to detect dose response effects. Therefore, a NOEC was not determined.
* the stability and homogeneity of the test substance in the treated feed was not assessed;
* a LOEL was not established, and the maximum labeled field residue was not reported, so it is unknown if the highest level tested was an appropriate level to approximate field exposure for this species;
* the volume of acetone used in test diet preparation was not reported, nor was it specified if the acetone was allowed to completely evaporate prior to offering; and
* raw data pertaining to parental mortality, clinical effects, and necropsy were not submitted for review.

As a result, this study is not consistent with the guideline requirement for an avian reproduction study with the Northern Bobwhite quail (§71-4a) and is classified as SUPPLEMENTAL.

F. REVIEWER'S COMMENTS:

Results of the reviewer's statistical analyses were nearly identical to those of the study author. The discrepancies between the reviewer's conclusions and the study author's conclusions were due to the interpretation of the biological significance of the data and that the reviewer is not satisfied that there was no treatment-related effects. Mean-measured concentrations are reported in the Conclusions and Executive Summary sections.

In the analytical report, it was reported that the sensitivity and reproducibility (of the analytical method) were determined by injecting the 2.46 ppm analytical standard six times (p. 112 of Appendix F). The mean, standard deviation, and coefficient of variation were calculated. The standard deviation for the six replicates was multiplied by three in order to determine the limit of detection (LOD) and multiplied by ten in order to determine the limit of quantitation (LOQ). It was then reported that the LOD for the method was 0.050 µg/mL (1.00 ppm) and the LOQ was 0.084 µg/mL (1.68 ppm).

The recovery of the analytical method, determined from analysis of six fortified matrix blanks, averaged 93.7 ± 1.4% (CV = 1.49%; pp. 112-113 of Appendix F). It was not reported if sample results were corrected for the mean procedural recovery.

EAD Comments:
While the US EPA reviewer considered this study as acceptable and supplemental, EAD reviewer considers this study as acceptable and core.

Stability of aminopyralid mixed with acetone was not assessed. Study author did not give a rationale for using a solvent in the preparation of the diet. In previous acute oral and dietary toxicity studies, aminopyralid was mixed with diet preparation without solvent (dietary studies, MRID 4622358-10 and 462358-11) or diluted with water (oral studies, MRID 462358-08, 462358-09). However, results from certain fate studies with aminopyralid suggest that the compound is stable.

Hatchling weigh is an important sub-lethal effect to take for account during a reproductive study.
In the present study, no data were submitted for that endpoint; however, other endpoints resulted in being not affected by exposure to the tested concentrations of aminopyralid. Furthermore, based on the results of acute oral and acute toxicity studies for bobwhite quail and mallard duck (MRID 462358-08 to 462358-11), aminopyralid is not expected to have an effect on bobwhite quail at the tested levels (640, 1270, and 2610 mg ai/kg of diet).

For these reasons, EAD reviewer as classified this study as acceptable.

G. CONCLUSIONS:

This study is scientifically sound, but is not consistent with guideline requirements for an avian reproduction study using the Northern Bobwhite quail (§71-4a) due to the highly variable nature of the data, the statistically significant reductions in important endpoints, and since a NOEC was not established. Additionally, the quantity and fate of the acetone used in test diet preparation was not specified; and raw data pertaining to parental mortality, clinical effects, necropsy, and hatchling weight were not provided. As a result, this study is classified as SUPPLEMENTAL.

NOEC: not determined
LOEC: not determined
Endpoint(s) Affected: hatchling survival

III. REFERENCES:

U.S. Environmental Protection Agency. 1988. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Series 71-4: Avian Reproduction Test. pp. 48-57.

Organization for Economic Cooperation and Development. 1984. OECD Guidelines for Testing of Chemicals, 206, Avian Reproduction Test. 10 pp.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Bobwhite quail repro, Aminopyralid, MRID 46235812
PRINTOUT OF RAW DATA

Obs	TRT	EL	EC	ENC_EL	ES	ES_EL	VE	VE_ES	LE	LE_VE	NH	NH_EL	NH_ES
1	Ctrl	1	0	100.00	1	100.00	0	0.00	0	.	0	0.00	0.00
2	Ctrl	41	0	100.00	38	92.68	0	0.00	0	.	0	0.00	0.00
3	Ctrl	35	1	97.14	31	88.57	25	80.65	24	96.00	21	60.00	67.74
4	Ctrl	42	0	100.00	40	95.24	38	95.00	37	97.37	33	78.57	82.50
5	Ctrl	32	0	100.00	29	90.63	16	55.17	16	100.00	15	46.88	51.72
6	Ctrl	.	.	.	.	.	.	.	.	.	.	.	.
7	Ctrl	28	0	100.00	24	85.71	21	87.50	21	100.00	21	75.00	87.50
8	Ctrl	34	0	100.00	30	88.24	29	96.67	29	100.00	26	76.47	86.67
9	Ctrl	42	0	100.00	39	92.86	37	94.87	37	100.00	36	85.71	92.31
10	Ctrl	17	0	100.00	16	94.12	16	100.00	16	100.00	14	82.35	87.50
11	Ctrl	31	0	100.00	26	83.87	16	61.54	16	100.00	8	25.81	30.77
12	Ctrl	47	0	100.00	44	93.62	41	93.18	40	97.56	37	78.72	84.09
13	Ctrl	41	0	100.00	38	92.68	36	94.74	36	100.00	33	80.49	86.84
14	Ctrl	19	0	100.00	16	84.21	15	93.75	14	93.33	12	63.16	75.00
15	Ctrl	15	0	100.00	14	93.33	14	100.00	13	92.86	10	66.67	71.43
16	Ctrl	49	0	100.00	44	89.80	38	86.36	38	100.00	35	71.43	79.55
17	Ctrl	43	0	100.00	39	90.70	39	100.00	39	100.00	32	74.42	82.05
18	Ctrl	53	0	100.00	49	92.45	47	95.92	46	97.87	45	84.91	91.84
19	Ctrl	26	0	100.00	25	96.15	16	64.00	16	100.00	16	61.54	64.00
20	Ctrl	40	0	100.00	36	90.00	33	91.67	33	100.00	32	80.00	88.89
21	Dose1	6	0	100.00	5	83.33	3	60.00	3	100.00	3	50.00	60.00
22	Dose1	29	0	100.00	23	79.31	23	100.00	23	100.00	20	68.97	86.96
23	Dose1	26	2	92.31	21	80.77	18	85.71	18	100.00	17	65.38	80.95
24	Dose1	37	0	100.00	33	89.19	26	78.79	25	96.15	5	13.51	15.15
25	Dose1	39	0	100.00	37	94.87	37	100.00	37	100.00	37	94.87	100.00
26	Dose1	.	.	.	.	.	.	.	.	.	.	.	.
27	Dose1	47	0	100.00	43	91.49	40	93.02	40	100.00	39	82.98	90.70
28	Dose1	34	0	100.00	32	94.12	30	93.75	30	100.00	30	88.24	93.75
29	Dose1	51	0	100.00	46	90.20	45	97.83	45	100.00	40	78.43	86.96
30	Dose1	30	0	100.00	27	90.00	22	81.48	22	100.00	15	50.00	55.56
31	Dose1	37	0	100.00	33	89.19	27	81.82	26	96.30	25	67.57	75.76
32	Dose1	36	0	100.00	33	91.67	32	96.97	30	93.75	27	75.00	81.82
33	Dose1	39	0	100.00	35	89.74	30	85.71	29	96.67	23	58.97	65.71
34	Dose1	44	0	100.00	40	90.91	34	85.00	34	100.00	31	70.45	77.50
35	Dose1	35	0	100.00	33	94.29	32	96.97	32	100.00	24	68.57	72.73
36	Dose2	27	0	100.00	24	88.89	22	91.67	22	100.00	12	44.44	50.00
37	Dose2	42	0	100.00	40	95.24	31	77.50	30	96.77	21	50.00	52.50
38	Dose2	56	1	98.21	49	87.50	47	95.92	47	100.00	42	75.00	85.71
39	Dose2	36	1	97.22	29	80.56	29	100.00	29	100.00	18	50.00	62.07
40	Dose2	16	0	100.00	15	93.75	14	93.33	13	92.86	4	25.00	26.67
41	Dose2	61	0	100.00	56	91.80	46	82.14	46	100.00	34	55.74	60.71
42	Dose2	15	0	100.00	12	80.00	2	16.67	2	100.00	0	0.00	0.00
43	Dose2	9	0	100.00	8	88.89	6	75.00	6	100.00	1	11.11	12.50
44	Dose2	46	0	100.00	43	93.48	41	95.35	41	100.00	34	73.91	79.07
45	Dose2	43	0	100.00	39	90.70	39	100.00	39	100.00	38	88.37	97.44
46	Dose2	37	0	100.00	34	91.89	34	100.00	34	100.00	34	91.89	100.00
47	Dose2	.	.	.	.	.	.	.	.	.	.	.	.
48	Dose2	.	.	.	.	.	.	.	.	.	.	.	.
49	Dose2	.	.	.	.	.	.	.	.	.	.	.	.
50	Dose2	50	0	100.00	44	88.00	29	65.91	29	100.00	26	52.00	59.09
51	Dose3	20	0	100.00	17	85.00	16	94.12	16	100.00	9	45.00	52.94
52	Dose3	38	0	100.00	35	92.11	33	94.29	32	96.97	26	68.42	74.29
53	Dose3	40	2	95.00	36	90.00	35	97.22	35	100.00	28	70.00	77.78
54	Dose3	45	0	100.00	39	86.67	35	89.74	35	100.00	26	57.78	66.67
55	Dose3	24	0	100.00	22	91.67	18	81.82	18	100.00	7	29.17	31.82
56	Dose3	15	0	100.00	14	93.33	9	64.29	9	100.00	8	53.33	57.14
57	Dose3	52	0	100.00	49	94.23	44	89.80	44	100.00	41	78.85	83.67

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58	Dose3	32	0	100.00	29	90.63	28	96.55	27	96.43	25	78.13	86.21
59	Dose3	.	.	.	.	.	.	.	.	.	.	.	.
60	Dose3	42	0	100.00	39	92.86	37	94.87	37	100.00	31	73.81	79.49
61	Dose3	33	0	100.00	30	90.91	28	93.33	28	100.00	26	78.79	86.67
62	Dose3	12	0	100.00	11	91.67	8	72.73	8	100.00	6	50.00	54.55
63	Dose3	23	0	100.00	20	86.96	19	95.00	19	100.00	14	60.87	70.00
64	Dose3	18	0	100.00	17	94.44	17	100.00	17	100.00	16	88.89	94.12
65	Dose3	47	0	100.00	43	91.49	40	93.02	38	95.00	21	44.68	48.84

Bobwhite quail repro, Aminopyralid, MRID 46235812
PRINTOUT OF RAW DATA (continued)

Obs	TRT	NH_LE	HS	HS_ES	HS_NH	THICK	HATWT	SURVWT	FOOD	WTGAINM	WTGAINF
1	Ctrl	.	0	0.00	.	.	.	.	21	50	43
2	Ctrl	.	0	0.00	.	0.18	.	.	22	53	81
3	Ctrl	87.50	18	58.06	85.71	0.22	.	19	22	28	63
4	Ctrl	89.19	30	75.00	90.91	0.19	.	20	21	21	77
5	Ctrl	93.75	13	44.83	86.67	0.22	.	22	21	15	66
6	Ctrl	.	.	.	.	.	.	.	.	.	.
7	Ctrl	100.00	19	79.17	90.48	0.19	.	18	22	48	73
8	Ctrl	89.66	23	76.67	88.46	0.20	.	19	21	59	88
9	Ctrl	97.30	34	87.18	94.44	0.21	.	19	24	70	74
10	Ctrl	87.50	13	81.25	92.86	0.19	.	18	22	43	116
11	Ctrl	50.00	6	23.08	75.00	0.20	.	14	19	23	83
12	Ctrl	92.50	35	79.55	94.59	0.17	.	21	26	39	69
13	Ctrl	91.67	29	76.32	87.88	0.20	.	17	24	66	104
14	Ctrl	85.71	9	56.25	75.00	0.19	.	16	18	10	86
15	Ctrl	76.92	8	57.14	80.00	.	.	15	21	33	-11
16	Ctrl	92.11	31	70.45	88.57	0.20	.	20	22	12	79
17	Ctrl	82.05	28	71.79	87.50	0.19	.	21	24	43	83
18	Ctrl	97.83	42	85.71	93.33	0.22	.	17	23	27	82
19	Ctrl	100.00	14	56.00	87.50	0.18	.	17	21	14	66
20	Ctrl	96.97	27	75.00	84.38	0.19	.	21	21	34	63
21	Dose1	100.00	0	0.00	0.00	0.23	.	.	20	18	73
22	Dose1	86.96	15	65.22	75.00	0.20	.	19	22	60	55
23	Dose1	94.44	12	57.14	70.59	0.17	.	16	19	6	60
24	Dose1	20.00	0	0.00	0.00	0.21	.	.	19	25	40
25	Dose1	100.00	31	83.78	83.78	0.15	.	20	23	56	82
26	Dose1	.	.	.	.	.	.	.	.	.	.
27	Dose1	97.50	23	53.49	58.97	0.19	.	19	22	39	71
28	Dose1	100.00	17	53.13	56.67	0.20	.	21	22	18	73
29	Dose1	88.89	29	63.04	72.50	0.17	.	18	20	29	46
30	Dose1	68.18	6	22.22	40.00	0.21	.	20	23	23	115
31	Dose1	96.15	9	27.27	36.00	0.19	.	16	21	49	58
32	Dose1	90.00	17	51.52	62.96	0.18	.	19	23	49	99
33	Dose1	79.31	17	48.57	73.91	0.19	.	19	23	2	120
34	Dose1	91.18	20	50.00	64.52	0.21	.	16	23	16	68
35	Dose1	75.00	15	45.45	62.50	0.17	.	18	21	55	50
36	Dose2	54.55	11	45.83	91.67	0.19	.	15	21	-6	41
37	Dose2	70.00	14	35.00	66.67	0.19	.	20	23	85	61
38	Dose2	89.36	35	71.43	83.33	0.20	.	20	24	19	60
39	Dose2	62.07	6	20.69	33.33	0.22	.	13	20	19	105
40	Dose2	30.77	4	26.67	100.00	0.20	.	17	19	37	46
41	Dose2	73.91	31	55.36	91.18	0.21	.	19	23	47	68
42	Dose2	0.00	0	0.00	.	0.19	.	20	22	-11	90
43	Dose2	16.67	1	12.50	100.00	.	.	15	22	33	121
44	Dose2	82.93	31	72.09	91.18	0.20	.	19	24	20	74
45	Dose2	97.44	33	84.62	86.84	0.18	.	20	21	25	36
46	Dose2	100.00	32	94.12	94.12	0.19	.	19	23	44	63
47	Dose2	.	.	.	.	.	.	.	.	.	.
48	Dose2	.	.	.	.	.	.	.	.	.	.
49	Dose2	.	.	.	.	.	.	.	.	.	.
50	Dose2	89.66	25	56.82	96.15	0.22	.	22	22	-4	97
51	Dose3	56.25	6	35.29	66.67	0.20	.	16	21	32	42

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52	Dose3	81.25	26	74.29	100.00	0.20	.	17	22	56	52
53	Dose3	80.00	21	58.33	75.00	0.20	.	18	23	24	70
54	Dose3	74.29	20	51.28	76.92	0.20	.	16	21	18	89
55	Dose3	38.89	3	13.64	42.86	0.21	.	16	25	37	58
56	Dose3	88.89	6	42.86	75.00	0.17	.	19	19	51	19
57	Dose3	93.18	36	73.47	87.80	0.19	.	22	22	61	65
58	Dose3	92.59	18	62.07	72.00	0.20	.	16	22	26	88
59	Dose3	.	.	.	.	.	.	.	.	.	.
60	Dose3	83.78	28	71.79	90.32	0.20	.	22	23	24	83
61	Dose3	92.86	20	66.67	76.92	0.18	.	18	22	25	53
62	Dose3	75.00	3	27.27	50.00	0.24	.	16	21	38	80
63	Dose3	73.68	13	65.00	92.86	0.20	.	18	22	20	45
64	Dose3	94.12	14	82.35	87.50	0.17	.	19	22	32	54
65	Dose3	55.26	19	44.19	90.48	0.20	.	18	21	29	118

Bobwhite quail repro, Aminopyralid, MRID 46235812

ANALYSIS RESULTS FOR VARIABLE EL (Eggs Laid)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.971	0.177	1.382	0.258	USE PARAMETRIC TESTS

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BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	19	33.47	13.27	3.04	39.65	27.08, 39.87
Dose1	14	35.00	10.76	2.88	30.75	28.79, 41.21
Dose2	12	36.50	16.67	4.81	45.66	25.91, 47.09
Dose3	14	31.50	12.91	3.45	40.97	24.05, 38.95

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	35.00	1.00	53.00	.	.
Dose1	36.50	6.00	51.00	104.56	-4.56
Dose2	39.50	9.00	61.00	109.04	-9.04
Dose3	32.50	12.00	52.00	94.10	5.90

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	55	0.34	0.799

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	33.47	.	34.76	.	0.988	0.928	0.975	.	.
Dose1	35.00	0.871	34.76	0.694	.	0.992	0.900	.	.
Dose2	36.50	0.929	34.76	0.725	.	.	0.779	.	.
Dose3	31.50	0.603	31.50	0.445	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

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Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE NEG_EC (Eggs Cracked)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.397	<.001	0.977	0.410	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	19	0.05	0.23	0.05	435.89	0.00, 0.16
Dose1	14	0.14	0.53	0.14	374.17	0.00, 0.45
Dose2	12	0.17	0.39	0.11	233.55	0.00, 0.41
Dose3	14	0.14	0.53	0.14	374.17	0.00, 0.45

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	0.00	0.00	1.00	.	.
Dose1	0.00	0.00	2.00	271.43	-171.43
Dose2	0.00	0.00	1.00	316.67	-216.67
Dose3	0.00	0.00	2.00	271.43	-171.43

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	1.19	0.755

MannWhit(Bon) - testing each trt median signif. greater than control
Jonckheere - test assumes dose-response relationship, testing positive trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	0.00	.	.
Dose1	0.00	1.000	0.396
Dose2	0.00	1.000	0.164
Dose3	0.00	1.000	0.296

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

476

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE ENC_EL ((EL-EC)/EL (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.396	<.001	1.189	0.323	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.	Interval
Ctrl	19	99.85	0.66	0.15	0.66	99.53,	100.00
Dose1	14	99.45	2.06	0.55	2.07	98.26,	100.00
Dose2	12	99.62	0.91	0.26	0.92	99.04,	100.00
Dose3	14	99.64	1.34	0.36	1.34	98.87,	100.00

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	100.00	97.14	100.00	.	.
Dose1	100.00	92.31	100.00	99.60	0.40
Dose2	100.00	97.22	100.00	99.77	0.23
Dose3	100.00	95.00	100.00	99.79	0.21

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	1.10	0.777

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	100.00	.	.
Dose1	100.00	1.000	0.396
Dose2	100.00	1.000	0.175
Dose3	100.00	1.000	0.311

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

477

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE ES (Eggs Set)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.973	0.217	1.358	0.265	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	19	30.47	12.34	2.83	40.51	24.52, 36.42
Dose1	14	31.50	10.27	2.75	32.61	25.57, 37.43
Dose2	12	32.75	15.31	4.42	46.75	23.02, 42.48
Dose3	14	28.64	11.92	3.19	41.62	21.76, 35.53

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	31.00	1.00	49.00	.	.
Dose1	33.00	5.00	46.00	103.37	-3.37
Dose2	36.50	8.00	56.00	107.47	-7.47
Dose3	29.50	11.00	49.00	93.99	6.01

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	55	0.26	0.856

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	30.47	.	31.40	.	0.995	0.960	0.975	.	.
Dose1	31.50	0.848	31.40	0.671	.	0.994	0.930	.	.
Dose2	32.75	0.909	31.40	0.702	.	.	0.836	.	.
Dose3	28.64	0.603	28.64	0.446	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

478

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE ES_EL (EggsSet/EggsLaid (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.944	0.009	0.841	0.477	USE NON-PARAMETRIC TESTS

****
BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	19	91.31	4.07	0.93	4.46	89.35,	93.27
Dose1	14	89.22	4.82	1.29	5.40	86.44,	92.00
Dose2	12	89.22	4.82	1.39	5.40	86.16,	92.29
Dose3	14	90.85	2.85	0.76	3.14	89.21,	92.50

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	92.45	83.87	100.00	.	.
Dose1	90.10	79.31	94.87	97.71	2.29
Dose2	89.79	80.00	95.24	97.72	2.28
Dose3	91.58	85.00	94.44	99.50	0.50

****
NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	1.93	0.586

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	92.45	.	.
Dose1	90.10	0.436	0.137
Dose2	89.79	0.464	0.104
Dose3	91.58	1.000	0.320

SUMMARY	NOEC	LQEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

479

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE VE (Viable Embryo(d14))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.962	0.061	1.275	0.292	USE PARAMETRIC TESTS

****
BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	19	25.11	13.80	3.17	54.98	18.45, 31.76
Dose1	14	28.50	10.29	2.75	36.12	22.56, 34.44
Dose2	12	28.33	14.81	4.28	52.27	18.92, 37.74
Dose3	14	26.21	11.66	3.12	44.47	19.48, 32.94

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	25.00	0.00	47.00	.	.
Dose1	30.00	3.00	45.00	113.52	-13.52
Dose2	30.00	2.00	47.00	112.86	-12.86
Dose3	28.00	8.00	44.00	104.42	-4.42

****
PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	55	0.26	0.853

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	25.11	.	27.02	.	0.875	0.902	0.995	.	.
Dose1	28.50	0.949	27.02	0.751	.	1.000	0.965	.	.
Dose2	28.33	0.940	27.02	0.778	.	.	0.975	.	.
Dose3	26.21	0.851	26.21	0.738	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

480

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE VE_ES (ViableEmbryo/EggsSet (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.752	<.001	3.777	0.015	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	19	78.47	30.70	7.04	39.12	63.68,	93.27
Dose1	14	88.36	10.99	2.94	12.44	82.01,	94.71
Dose2	12	82.79	23.65	6.83	28.57	67.76,	97.82
Dose3	14	89.77	10.10	2.70	11.25	83.94,	95.60

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	93.18	0.00	100.00	.	.
Dose1	89.37	60.00	100.00	112.60	-12.60
Dose2	92.50	16.67	100.00	105.50	-5.50
Dose3	93.73	64.29	100.00	114.39	-14.39

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	0.30	0.960

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	93.18	.	.
Dose1	89.37	1.000	0.608
Dose2	92.50	1.000	0.592
Dose3	93.73	1.000	0.676

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

481

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE LE (Live Embryo(d21))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.962	0.063	1.328	0.275	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	19	24.79	13.69	3.14	55.24	18.19,	31.39
Dose1	14	28.14	10.28	2.75	36.53	22.21,	34.08
Dose2	12	28.17	14.89	4.30	52.85	18.71,	37.63
Dose3	14	25.93	11.43	3.06	44.09	19.33,	32.53

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	24.00	0.00	46.00	.	.
Dose1	29.50	3.00	45.00	113.53	-13.53
Dose2	29.50	2.00	47.00	113.62	-13.62
Dose3	27.50	8.00	44.00	104.60	-4.60

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	55	0.27	0.846

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	24.79	.	26.73	.	0.877	0.888	0.994	.	.
Dose1	28.14	0.949	26.73	0.754	.	1.000	0.967	.	.
Dose2	28.17	0.945	26.73	0.781	.	.	0.970	.	.
Dose3	25.93	0.853	25.93	0.742	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

482

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE LE_VE (LiveEmbryo/ViableEmbryo (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.692	<.001	0.694	0.560	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	17	98.53	2.40	0.58	2.43	97.30, 99.76
Dose1	14	98.78	2.11	0.56	2.13	97.56, 99.99
Dose2	12	99.14	2.18	0.63	2.20	97.75, 100.00
Dose3	14	99.17	1.69	0.45	1.71	98.19, 100.00

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	100.00	92.86	100.00	.	.
Dose1	100.00	93.75	100.00	100.25	-0.25
Dose2	100.00	92.86	100.00	100.62	-0.62
Dose3	100.00	95.00	100.00	100.65	-0.65

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	1.24	0.743

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	100.00	.	.
Dose1	100.00	1.000	0.631
Dose2	100.00	1.000	0.829
Dose3	100.00	1.000	0.845

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

483

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE NH (Number Hatched)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.965	0.088	1.238	0.305	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	19	22.42	13.19	3.03	58.84	16.06,	28.78
Dose1	14	24.00	11.42	3.05	47.56	17.41,	30.59
Dose2	12	22.00	14.98	4.33	68.11	12.48,	31.52
Dose3	14	20.29	10.53	2.81	51.90	14.21,	26.36

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	21.00	0.00	45.00	.	.
Dose1	24.50	3.00	40.00	107.04	-7.04
Dose2	23.50	0.00	42.00	98.12	1.88
Dose3	23.00	6.00	41.00	90.48	9.52

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	55	0.21	0.892

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	22.42	.	23.09	.	0.984	1.000	0.963	.	.
Dose1	24.00	0.879	23.09	0.647	.	0.978	0.863	.	.
Dose2	22.00	0.740	22.00	0.578	.	.	0.986	.	.
Dose3	20.29	0.575	20.29	0.417	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

484

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE NH_EL (NumberHatched/EggsLaid (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.919	<.001	0.868	0.463	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	19	62.74	26.44	6.07	42.15	50.00, 75.49
Dose1	14	66.64	20.00	5.35	30.01	55.09, 78.19
Dose2	12	51.46	28.77	8.30	55.91	33.18, 69.73
Dose3	14	62.69	16.85	4.50	26.88	52.96, 72.42

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	74.42	0.00	85.71	.	.
Dose1	68.77	13.51	94.87	106.21	-6.21
Dose2	51.00	0.00	91.89	82.01	17.99
Dose3	64.65	29.17	88.89	99.92	0.08

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	2.88	0.411

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	74.42	.	.
Dose1	68.77	1.000	0.464
Dose2	51.00	0.306	0.095
Dose3	64.65	0.628	0.137

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

485

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE NH_ES (NumberHatched/EggsSet (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.901	<.001	1.141	0.341	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	19	68.97	28.69	6.58	41.60	55.14, 82.80
Dose1	14	74.54	21.25	5.68	28.51	62.27, 86.81
Dose2	12	57.15	31.71	9.15	55.48	37.00, 77.29
Dose3	14	68.87	17.70	4.73	25.70	58.65, 79.09

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	82.05	0.00	92.31	.	.
Dose1	79.23	15.15	100.00	108.08	-8.08
Dose2	59.90	0.00	100.00	82.86	17.14
Dose3	72.14	31.82	94.12	99.86	0.14

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	3.48	0.323

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	82.05	.	.
Dose1	79.23	1.000	0.587
Dose2	59.90	0.317	0.118
Dose3	72.14	0.526	0.108

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

486

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE NH_LE (NumberHatched/LiveEmbryo (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.874	<.001	4.620	0.006	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	17	88.86	11.82	2.87	13.30	82.79, 94.94
Dose1	14	84.83	21.06	5.63	24.83	72.67, 96.99
Dose2	12	63.95	32.67	9.43	51.09	43.19, 84.70
Dose3	14	77.15	16.74	4.47	21.69	67.48, 86.81

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	91.67	50.00	100.00	.	.
Dose1	90.59	20.00	100.00	95.46	4.54
Dose2	71.96	0.00	100.00	71.96	28.04
Dose3	80.63	38.89	94.12	86.82	13.18

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	8.99	0.029

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	91.67	.	.
Dose1	90.59	1.000	0.453
Dose2	71.96	0.053	0.020
Dose3	80.63	0.053	0.008

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose1	Dose2

487

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE HS (Hatching Survival(d14))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.968	0.128	2.815	0.048	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	19	19.95	12.31	2.82	61.73	14.01, 25.88
Dose1	14	15.07	9.32	2.49	61.83	9.69, 20.45
Dose2	12	18.58	13.85	4.00	74.54	9.78, 27.39
Dose3	14	16.64	9.84	2.63	59.14	10.96, 22.33

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	19.00	0.00	42.00	.	.
Dose1	16.00	0.00	31.00	75.56	24.44
Dose2	19.50	0.00	35.00	93.16	6.84
Dose3	18.50	3.00	36.00	83.43	16.57

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	1.36	0.714

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	19.00	.	.
Dose1	16.00	0.447	0.141
Dose2	19.50	1.000	0.349
Dose3	18.50	0.690	0.317

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	Dose3	>highest dose
Jonckheere	Dose3	>highest dose

488

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE HS_ES (HatchingSurvival/EggsSet (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.943	0.008	0.713	0.549	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf.	Interval
Ctrl	19	60.71	26.53	6.09	43.70	47.92,	73.49
Dose1	14	44.35	23.99	6.41	54.10	30.49,	58.20
Dose2	12	47.93	29.65	8.56	61.86	29.09,	66.76
Dose3	14	54.89	19.97	5.34	36.39	43.36,	66.43

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	71.79	0.00	87.18	.	.
Dose1	50.76	0.00	83.78	73.05	26.95
Dose2	50.60	0.00	94.12	78.95	21.05
Dose3	60.20	13.64	82.35	90.42	9.58

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	5.82	0.121

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	71.79	.	.
Dose1	50.76	0.046	0.011
Dose2	50.60	0.276	0.030
Dose3	60.20	0.298	0.120

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	**<lowest dose**	**Dose1**
Jonckheere	Dose3	>highest dose

499

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE HS_NH (HatchingSurvival/NumberHatched (%))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.855	<.001	4.822	0.005	USE NON-PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	17	87.25	5.94	1.44	6.81	84.20,	90.31
Dose1	14	54.10	26.30	7.03	48.61	38.92,	69.28
Dose2	11	84.95	19.47	5.87	22.92	71.87,	98.03
Dose3	14	77.45	16.17	4.32	20.87	68.12,	86.79

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	87.88	75.00	94.59	.	.
Dose1	62.73	0.00	83.78	62.00	38.00
Dose2	91.18	33.33	100.00	97.36	2.64
Dose3	76.92	42.86	100.00	88.77	11.23

****

NON-PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Kruskal-Wallis test - equality among treatment groups

Degrees of Freedom	TestStat	P-value
3	23.71	<.001

MannWhit(Bon) - testing each trt median signif. less than control
Jonckheere - test assumes dose-response relationship, testing negative trend

Level	Median	MannWhit(Bon adjust)p-value	Jonckheere p-value
Ctrl	87.88	.	.
Dose1	62.73	<.001	<.001
Dose2	91.18	1.000	0.205
Dose3	76.92	0.130	0.245

SUMMARY	NOEC	LOEC
MannWhit (Bonf adjust)	**<lowest dose**	**Dose1**
Jonckheere	Dose3	>highest dose

490

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE THICK (Eggshell thickness)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.987	0.808	0.683	0.567	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	17	0.20	0.01	0.00	7.39	0.19,	0.20
Dose1	14	0.19	0.02	0.01	10.43	0.18,	0.20
Dose2	11	0.20	0.01	0.00	7.03	0.19,	0.21
Dose3	14	0.20	0.02	0.00	8.62	0.19,	0.21

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	0.19	0.17	0.22	.	.
Dose1	0.19	0.15	0.23	96.36	3.64
Dose2	0.20	0.18	0.22	100.98	-0.98
Dose3	0.20	0.17	0.24	100.55	-0.55

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	52	0.86	0.470

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	0.20	.	0.20	.	0.630	0.990	0.998	.	.
Dose1	0.19	0.260	0.19	0.464	.	0.528	0.556	.	.
Dose2	0.20	0.861	0.19	0.503	.	.	0.999	.	.
Dose3	0.20	0.828	0.19	0.511	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

491

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE HATWT (Hatchling Weight)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
.	.	.	.	NO DATA FOR TEST

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval
Ctrl	0	.	.	.	.	. , .
Dose1	0	.	.	.	.	. , .
Dose2	0	.	.	.	.	. , .
Dose3	0	.	.	.	.	. , .

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	.	.	.	.	.
Dose1	.	.	.	.	.
Dose2	.	.	.	.	.
Dose3	.	.	.	.	.

492

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE SURVWT (Survivor Wt (d14))

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.981	0.525	1.089	0.362	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	17	18.47	2.27	0.55	12.27	17.30,	19.64
Dose1	12	18.42	1.68	0.48	9.10	17.35,	19.48
Dose2	12	18.25	2.67	0.77	14.61	16.56,	19.94
Dose3	14	17.93	2.06	0.55	11.47	16.74,	19.12

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	19.00	14.00	22.00	.	.
Dose1	19.00	16.00	21.00	99.71	0.29
Dose2	19.00	13.00	22.00	98.81	1.19
Dose3	18.00	16.00	22.00	97.07	2.93

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	51	0.18	0.911

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	18.47	.	18.47	.	1.000	0.993	0.903	.	.
Dose1	18.42	0.746	18.42	0.555	.	0.998	0.942	.	.
Dose2	18.25	0.666	18.25	0.498	.	.	0.982	.	.
Dose3	17.93	0.478	17.93	0.329	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

493

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE FOOD (Food Consumption)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.977	0.341	0.549	0.651	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	19	21.84	1.83	0.42	8.40	20.96,	22.73
Dose1	14	21.50	1.51	0.40	7.01	20.63,	22.37
Dose2	12	22.00	1.54	0.44	6.99	21.02,	22.98
Dose3	14	21.86	1.35	0.36	6.18	21.08,	22.64

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	22.00	18.00	26.00	.	.
Dose1	22.00	19.00	23.00	98.43	1.57
Dose2	22.00	19.00	24.00	100.72	-0.72
Dose3	22.00	19.00	25.00	100.07	-0.07

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	55	0.24	0.869

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	21.84	.	21.84	.	0.929	0.993	1.000	.	.
Dose1	21.50	0.516	21.78	0.532	.	0.855	0.934	.	.
Dose2	22.00	0.857	21.78	0.567	.	.	0.996	.	.
Dose3	21.86	0.783	21.78	0.583	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

494

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE WTGAINM (Male wt gain)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.976	0.290	1.571	0.207	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	19	36.21	18.33	4.21	50.63	27.37,	45.05
Dose1	14	31.79	19.37	5.18	60.94	20.60,	42.97
Dose2	12	25.67	26.70	7.71	104.03	8.70,	42.63
Dose3	14	33.79	13.46	3.60	39.85	26.01,	41.56

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	34.00	10.00	70.00	.	.
Dose1	27.00	2.00	60.00	87.78	12.22
Dose2	22.50	-11.00	85.00	70.88	29.12
Dose3	30.50	18.00	61.00	93.30	6.70

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	55	0.74	0.533

Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	36.21	.	36.21	.	0.918	0.469	0.985	.	.
Dose1	31.79	0.501	31.79	0.313	.	0.857	0.993	.	.
Dose2	25.67	0.178	30.04	0.254	.	.	0.719	.	.
Dose3	33.79	0.633	30.04	0.248	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

495

Bobwhite quail repro, Aminopyralid, MRID 46235812
ANALYSIS RESULTS FOR VARIABLE WTGAINF (Female wt gain)

TESTS OF ASSUMPTIONS FOR PARAMETRIC ANALYSIS
Shapiro-Wilks test for Normality of Residuals -- alpha-level=0.01
Levenes test for homogeneity of variance(absolute residuals) -- alpha-level=0.05
Use parametric analyses if neither test rejected, otherwise non-parametric analyses.

Shapiro-Wilks Test Stat	Shapiro-Wilks P-value	Levenes Test Stat	Levenes P-value	Conclusion
0.971	0.162	0.279	0.841	USE PARAMETRIC TESTS

****

BASIC SUMMARY STATISTICS

Level	N	Mean	StdDev	StdErr	Coef of Var	95% Conf. Interval	
Ctrl	19	72.89	25.68	5.89	35.23	60.52,	85.27
Dose1	14	72.14	24.52	6.55	33.98	57.99,	86.30
Dose2	12	71.83	26.52	7.66	36.92	54.98,	88.68
Dose3	14	65.43	24.83	6.64	37.95	51.09,	79.76

Level	Median	Min	Max	%of Control(means)	%Reduction(means)
Ctrl	77.00	-11.00	116.00	.	.
Dose1	69.50	40.00	120.00	98.97	1.03
Dose2	65.50	36.00	121.00	98.54	1.46
Dose3	61.50	19.00	118.00	89.76	10.24

****

PARAMETRIC ANALYSES - use alpha-level=0.05 for all tests
Analysis of Variance (ANOVA) - overall F-test

Numerator df	Denominator df	F-stat	P-value
3	55	0.27	0.846

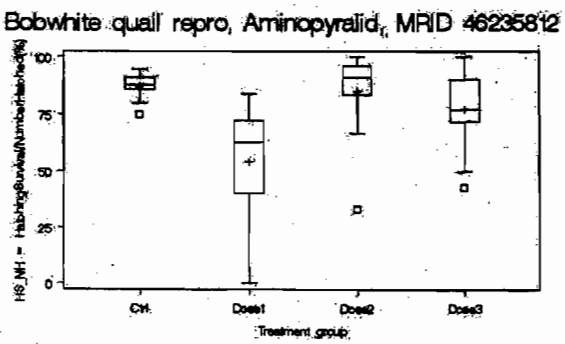
Dunnett - testing each trt mean signif. less than control
Williams - test assumes dose-response relationship, testing negative trend
Tukey - two-sided tests, all possible comparisons, not used for NOEC or LOEC

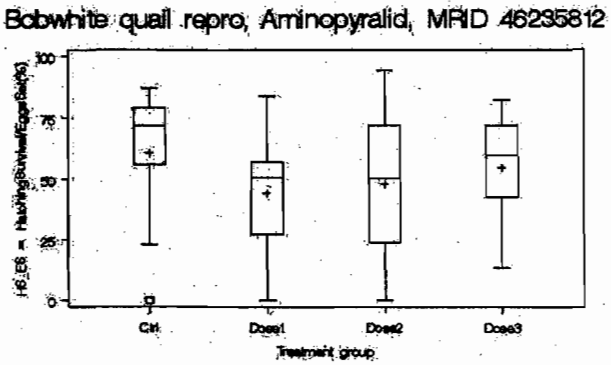
Level	Mean	Dunnett p-value	Isotonic mean	Williams p-value	Tukey p-values Dose1	Dose2	Dose3	Dose4	Dose5
Ctrl	72.89	.	72.89	.	1.000	0.999	0.838	.	.
Dose1	72.14	0.743	72.14	0.547	.	1.000	0.897	.	.
Dose2	71.83	0.732	71.83	0.567	.	.	0.918	.	.
Dose3	65.43	0.412	65.43	0.270	.	.	.	.	.

SUMMARY	NOEC	LOEC
Dunnett	Dose3	>highest dose
Williams	Dose3	>highest dose

496

Box Plots: (graphs for these endpoints are provided for information purposes only; effects were determined to be unrelated to treatment)





497