

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

<b>Data Requirement:</b>	PMRA DATA CODE	{.....}
	EPA DP Barcode	367525
	OECD Data Point	{.....}
	EPA MRID	47807005
	EPA Guideline	OPPTS 850.5400 (158.660)

**Test material:** Trifluralin Metabolite TR-15 **Purity:** 99%  
**Common name:** Trifluralin Metabolite TR-15  
**Chemical name:** IUPAC Not reported  
CAS name 1H-Benzimidazole, 2-ethyl-4-nitro-6-(trifluoromethyl)  
CAS No. Not reported  
Synonyms Not reported

**Primary Reviewer:** Moncie Wright  
**Staff Scientist, Cambridge Environmental**

**Signature:** *Moncie V Wright*  
**Date:** 10/30/09

**Secondary Reviewer:** Teri S. Myers  
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**Signature:** *Teri S Myers*  
**Date:** 12/02/09

**Primary Reviewer:** Christine Hartless  
{EPA/OPP/EFED/ERB1}

*Christine Hartless* **Date:** 4/20/10  
4-20-10

**Secondary Reviewer(s):** {.....}  
{EPA/OECD/PMRA}

**Date:** {.....}

**Reference/Submission No.:** {.....}

**Company Code** {.....} [For PMRA]  
**Active Code** {.....} [For PMRA]  
**Use Site Category:** {.....} [For PMRA]  
**EPA PC Code** 036101

**Date Evaluation Completed:** 4/20/10

**CITATION:** Marino, T.A., Staley, J.L., Gilles, M.M., and McClymont, E.L. 2001. Trifluralin metabolite TR-15: Growth inhibition test with the freshwater green alga, *Selenastrum capricornutum* PRINTZ. Unpublished study performed by The Dow Chemical Company, Toxicology and Environmental Research and Consulting, Midland, Michigan. Laboratory Study ID: 011102. Study sponsored by Dow AgroSciences LLC, Indianapolis, Indiana. Study completed December 10, 2001.



**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
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PMRA Submission Number {.....}

EPA MRID Number 47807005

**EXECUTIVE SUMMARY:**

In a 96-hour acute toxicity study, cultures of the freshwater green algae (*Selenastrum capricornutum*) were exposed to Trifluralin metabolite TR-15 at nominal concentrations of 0 (negative and solvent control), 0.0782, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10 mg ai/L under static conditions. The arithmetic mean-measured concentrations were <0.03 (<LLQ, negative and solvent control), 0.0509, 0.101, 0.254, 0.451, 0.908, 1.86, 5.28, and 9.15 mg ai/L. The initial-measured concentrations were <0.03 (<LLQ, negative and solvent control), 0.0586, 0.112, 0.272, 0.467, 0.952, 1.89, 7.29, and 11.5 mg ai/L; the reviewer based toxicity values on the initial-measured concentrations because the recovery of the test material after 96 hours dropped below 70% of nominal.

Cell abnormalities were not reported.

All endpoints were affected by the test material. Biomass was the most sensitive endpoint, with NOAEC and IC<sub>50</sub> values of 0.952 and 6.7 mg ai/L, respectively. Cell density had a similar IC<sub>50</sub> value, but the NOAEC was one concentration higher than that for biomass.

This toxicity study is scientifically sound and classified as Acceptable for a nonvascular aquatic plant toxicity study with the freshwater green algae, *Selenastrum capricornutum* (for the degradate TR-15)

**Results Synopsis**

Test Organism: *Selenastrum capricornutum*

Test Type (Flow-through, Static, Static Renewal): Static

Initial measured:

**Cell density**

IC<sub>05</sub>: 4.6 mg ai/L 95% C.I.: 3.9 to 5.5 mg ai/L

IC<sub>50</sub>: 6.7 mg ai/L 95% C.I.: 6.2 to 7.2 mg ai/L

Slope: 10.3 ± 1.61

NOAEC: 1.89 mg ai/L

**Biomass (Area Under the Growth Curve)**

IC<sub>05</sub>: 4.8 mg ai/L 95% C.I.: 4.0 to 5.7 mg ai/L

IC<sub>50</sub>: 6.7 mg ai/L 95% C.I.: 6.3 to 7.2 mg ai/L

Slope: 11.0 ± 1.89

NOAEC: 0.952 mg ai/L

**Growth Rate**

IC<sub>05</sub>: 6.2 mg ai/L 95% C.I.: 5.7 to 6.7 mg ai/L

IC<sub>50</sub>: 9.3 mg ai/L 95% C.I.: 9.0 to 9.6 mg ai/L

Slope: 9.18 ± 0.633

NOAEC: 1.89 mg ai/L

Endpoint(s) Effected: Cell density, biomass, and growth rate

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae  
(*Selenastrum capricornutum*)**

PMRA Submission Number {.....}

EPA MRID Number 47807005

**I. MATERIALS AND METHODS**

**GUIDELINE FOLLOWED:** This study was conducted following the OECD Guideline for Testing of Chemicals, No. 201: "Algal, Growth Inhibition Test", the EEC Commission Directive 92/69/EEC Annex, C.3 Algal Inhibition Test, U.S. EPA Pesticide Assessment Guidelines Subdivision J Hazard Evaluation: Non-target Plants, and EPA Standard Evaluation Procedure, Non-Target Plants: Growth and Reproduction of Aquatic Plants Tiers 1 and 2. The following deviations from OPPTS 850.5400 were noted:

1. The pretest health of the algae was not reported; the reporting of this parameter is suggested by OPPTS guidelines.
2. The source of the dilution water was not reported.
3. Water characterization analysis was not performed or reported, resulting in a lack of data for total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water; the reporting of these dilution water parameters is suggested by OPPTS guidelines.
4. The lighting quality was not reported.
5. The lighting intensity in the definitive test was much higher than recommended, and ranged from 6440 to 8060 lux; OPPTS guidelines suggest that the intensity for *Selenastrum capricornutum* be maintained at 4300 lux.
6. The pH in the definitive test was much higher than recommended by OPPTS guidelines, and ranged from 8.3-9.3; OPPTS guidelines suggest a pH of  $7.5 \pm 0.1$  for tests with *Selenastrum capricornutum*. The study authors did measure pH in test solutions without algae present, and obtained a range of 7.5 to 8.7.

These deviations do not impact the acceptability of the study.

**COMPLIANCE:** Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR Part 160), OECD Principles of GLP (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**                      **Trifluralin Metabolite TR-15**

**Description:**                              Solid

**Lot No./Batch No. :**                      GHD-6140-43C (Lot No.)

**Purity:**                                      99%

**Stability of compound under test conditions:**                      The time 0 measured concentrations yielded recoveries ranging from 72 to 146% of the nominal test concentrations, and the 96-hour measured concentrations yielded recoveries ranging from 55 to 75% of nominal and 45 to 97% of initial. Trifluralin Metabolite TR-15 was not stable under the

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

test conditions.  
*(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)*

**Storage conditions of test chemicals:** Not reported.

**Physicochemical properties of Trifluralin Metabolite TR-15.**

Parameter	Values	Comments
Water solubility	Not reported.	
Vapor pressure	Not reported.	
UV absorption	Not reported.	
pKa	Not reported.	
Kow	Not reported.	

**2. Test organism:**

**Name:** Freshwater green algae, *Selenastrum capricornutum*  
*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. capricornutum, 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:** In-house cultures originally obtained from the University of Toronto Culture Collection, Toronto, Ontario, Canada  
**Age of inoculum:** 4 weeks acclimation (3 days since previous transfer to fresh medium)  
**Method of cultivation:** Cultivated under test conditions (algal assay medium; AAM)

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding study      A range-finding study was conducted for 4 days with test concentrations of 0.016, 0.08, 0.4, 2, and 10 mg ai/L. The NOAEC was 0.4 mg ai/L, and the EC<sub>50</sub> value was between 2 and 10 mg ai/L.

b. Definitive Study

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		<i>Criteria</i>
<p>Acclimation period:</p> <p>Culturing media and conditions: (same as test or not)</p> <p>Health: (any mortality observed)</p>	<p>Continuous</p> <p>Algal assay medium Temperature, photoperiod, medium were the same; light intensity was different (4300 for culturing, 8000 for testing)</p> <p>Pretest health was not reported.</p>	<p>EPA recommends two week acclimation period.</p> <p>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.</p>
<p><u>Test system</u></p> <p>Static/static renewal</p> <p>Renewal rate for static renewal</p>	<p>Static</p> <p>N/A</p>	<p>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).</p>
<p>Incubation facility</p>	<p>Test vessels were placed in an incubator.</p>	
<p>Duration of the test</p>	<p>96 hours</p>	<p>EPA requires: 96-120 hours OECD: 72 hours</p>

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

Parameter	Details	Remarks
		-----
		<i>Criteria</i>
<u>Test vessel</u> Material: <i>(glass/stainless steel)</i> Size: Fill volume:	Glass 250 mL 100 mL	----- <i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>
<u>Details of growth medium name</u>  pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	Not reported Not reported Only for culturing NaHCO <sub>3</sub> N/A	pH range throughout entire study: 8.3-9.3 ----- <i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i>  <i>EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	A standard nutrient medium was prepared, and a detailed composition was provided.	

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

Parameter	Details	Remarks
		----- <i>Criteria</i>
<p><u>Dilution water</u>                      source/type:                      pH:                      salinity (for marine algae):                      water pretreatment (if any):                      Total Organic Carbon:                      particulate matter:                      metals:                      pesticides:                      chlorine:</p>	<p>Deionized water                      Adjusted to 7.0-7.5                      N/A                      Not reported                      Not reported                      Not reported                      Not reported                      Not reported</p>	<p>-----                      EPA pH: <i>Skeletonema costatum</i> = ~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.</p> <p>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.</p>
<p>Indicate how the test material is added to the medium (added directly or used stock solution)</p>	<p>The test substance (200 mg) was dissolved in acetone (2 mL). The stock solution was serially diluted to prepare the remaining dose stock solutions. Aliquots (50 µL) of each of the dose stock solutions were added to the algal assay medium (500 mL) to prepare bulk dose solutions (final test concentrations).</p>	<p>All test substances were adjusted to 100% active ingredient.</p>
<p>Aeration or agitation</p>	<p>Agitation (100 rpm).</p>	
<p>Initial cells density</p>	<p>Ca. <math>1 \times 10^4</math> cells/mL (average = <math>0.95 \times 10^4</math> cells/mL)</p>	<p>-----                      EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

Parameter	Details	Remarks
		<i>Criteria</i>
<u>Number of replicates</u> Control: Solvent control: Treatments:	3 3 3	<hr/> EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> 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.
<u>Test concentrations</u> Nominal:  Mean-Measured:  Initial-Measured:	0 (negative and solvent control), 0.0782, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10 mg ai/L  <0.03 (<LLQ, negative and solvent control), 0.0509, 0.101, 0.254, 0.451, 0.908, 1.86, 5.28, and 9.15 mg ai/L  <0.03 (<LLQ, negative and solvent control), 0.0586, 0.112, 0.272, 0.467, 0.952, 1.89, 7.29, and 11.5 mg ai/L	LLQ = lowest level quantified  <hr/> 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)	Acetone; 0.1 mL/L	
Method and interval of analytical verification	Samples from test solutions collected at 0 and 96 hours, method precision samples from day 0, and concurrently run analytical standards were analyzed using HPLC with UV (285 nm) detection.	



**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

Parameter	Details	Remarks
		Criteria
<u>Test conditions</u> Temperature:  Photoperiod: Light intensity and quality:	24.3°C  Continuous 6440-8060 lux Lighting not described	EPA temperature: <i>Skeletonema</i> : 20EC, Others: 24-25EC; EPA photoperiod: <i>S. costatum</i> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <i>Anabaena</i> : 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)  OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.
<u>Reference chemical (if used)</u> name: concentrations:	N/A	Results of most recent laboratory reference test resulted in a biomass EC <sub>50</sub> value of 0.73 mg/L.
Other parameters, if any	None.	

**2. Observations:**

**Table 2: Observation parameters**

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	- (algal growth) cell density -% inhibition of growth (biomass) -growth rate	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.

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

Parameters	Details	Remarks
		Criteria
Measurement technique for cell density and other end points	Cell counts were conducted daily on samples of each test concentration and the controls using an electronic particle counter (Coulter Multisizer). Biomass and growth rate calculations were not reported.	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i>  <i>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).</i>
Observation intervals	Every 24 hours.	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes. After 96 hours, the mean cell density was 313 x 10 <sup>4</sup> cells/mL in the negative control.	<i>EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.</i>  <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Only for cell density. Other endpoints were calculated from cell density and standard formulae.	

**II. RESULTS and DISCUSSION:**

**A. INHIBITORY EFFECTS:**

After 96 hours of exposure, cell density averaged 313 and 280 x 10<sup>4</sup> cells/mL in the negative and solvent control, respectively, yielding inhibitions of -5, 0, -4, -11, -20, 1, 63, and 99% as compared to the pooled controls in the initial-measured 0.0586, 0.112, 0.272, 0.467, 0.952, 1.89, 7.29, and 11.5 mg ai/L treatment levels, respectively. The 96-hr NOAEC and EC<sub>50</sub> values for cell density were 1.86 and 4.97 mg ai/L, respectively.

The study authors did not report averages, replicate data, or inhibitions for biomass and growth rate. The reviewer used cell density data to calculate biomass and growth rate per replicate, and then used mean values per replicate to calculate inhibitions relative to the negative control.

After 96 hours of exposure, area under the curve (biomass) averaged 853 and 850 x 10<sup>5</sup> cells/mL in the negative and solvent control, respectively, yielding inhibitions of 2, 0, 5, 4, 7, 27, 68, and 100% as compared to the negative

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
**(*Selenastrum capricornutum*)**

PMRA Submission Number {.....}

EPA MRID Number 47807005

control. The 96-hr NOAEC and EC<sub>50</sub> values for biomass were 1.86 and 2.79 mg ai/L, respectively.

After 96 hours of exposure, growth rate averaged 0.056 and 0.058 cells/mL in the negative and solvent control, respectively, yielding inhibitions of -7, -7, -7, -11, -13, -9, 9, and 79% as compared to the negative control. The 96-hr NOAEC and EC<sub>50</sub> values for growth rate were 1.86 and >9.15 mg ai/L, respectively.

Cell abnormalities were not reported. The study authors determined toxicity values using mean-measured concentrations.

**Table 3: Effect of Trifluralin metabolite TR-15 on algal growth (*Selenastrum capricornutum*)**

Initial-Measured and (Nominal) Concentrations (mg ai/L)	Initial cell Density (x10 <sup>4</sup> cells/mL)	Cell density (x10 <sup>4</sup> cells/mL) at				
		24 hours	48 hours	72 hours	96 hours	
					cell count	% inhibition*
Negative control	1.53	4.91	34.59	164.72	313.17	NA
Solvent control	1.05	5.48	37.36	175.15	279.63	11
0.0586 (0.0782)	0.94	5.02	34.8	156.11	309.75	1
0.112 (0.156)	0.92	5.73	33.10	170.65	296.81	5
0.272 (0.313)	0.93	5.29	30.16	150.54	307.6	2
0.467 (0.625)	0.88	4.88	29.25	143.6	330.24	-5
0.952 (1.25)	0.85	4.44	26.82	125.83	354.7	-13
1.89 (2.50)	0.85	3.95	22.14	90.6	294.33	6
7.29 (5.00)	0.79	2.77	15.42	44.5	109.67	65
11.5 (10)	0.72	0.7	1.23	1.15	2.32	99

\*Inhibitions calculated by the reviewer; determined by comparison to the negative control.

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

**Table 4: Effect of Trifluralin metabolite TR-15 on algal growth (*Selenastrum capricornutum*)**

Initial-Measured and (Nominal) Concentrations (mg ai/L)	Initial Cell Density (x10 <sup>4</sup> cells/mL)	Mean Growth Rate (cells/mL)		Mean Area Under the Growth Curve (Biomass) (x 10 <sup>5</sup> cells/mL)	
		0-96 Hours	Percent Inhibition*	0-96 hours	Percent Inhibition*
Negative control	1.53	0.056	N/A	853	N/A
Solvent control	1.05	0.058	-3	850	0
0.0586 (0.0782)	0.94	0.060	-7	834	2
0.112 (0.156)	0.92	0.060	-7	851	0
0.272 (0.313)	0.93	0.060	-7	808	5
0.467 (0.625)	0.88	0.062	-11	815	4
0.952 (1.25)	0.85	0.063	-13	796	7
1.89 (2.50)	0.85	0.061	-9	626	27
7.29 (5.00)	0.79	0.051	9	275	68
11.5 (10)	0.72	0.012	79	4	100

\*Inhibitions calculated by the reviewer; determined by comparison to the negative control.

**Table 5: Statistical endpoint values – 96 hours.**

Statistical Endpoint	Cell density	Biomass (Area under the Growth Curve)	Growth Rate
NOAEC or EC <sub>05</sub> (mg ai/L)	1.86	1.86	1.86
EC <sub>50</sub> (mg ai/L)	4.97	2.79	>9.15
IC <sub>50</sub> or EC <sub>50</sub> (mg ai/L) (95% C.I.)	4.97 (2.51-7.43)	2.79 (0.11->9.15)	>9.15 (N/A)
Other (EC <sub>25</sub> )	2.87 (0.45-5.28)	ND	ND
Reference chemical, if used NOAEC IC <sub>50</sub> /EC <sub>50</sub>	N/A	N/A	N/A

ND – not determined

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Senastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

**Table 6: Statistical endpoint values – 72 hours.**

Statistical Endpoint	Cell density	Biomass (Area under the Growth Curve)	Growth Rate
NOAEC or EC <sub>05</sub> (mg ai/L)	0.451	0.451	0.451
EC <sub>50</sub> (mg ai/L)	3.82	1.67	>9.15
IC <sub>50</sub> or EC <sub>50</sub> (mg ai/L) (95% C.I.)	3.82 (1.22-6.41)	1.67 (0.22->9.15)	>9.15 (N/A)
Other (EC <sub>25</sub> )	1.53 (<0.05-4.11)	ND	ND
Reference chemical, if used NOAEC IC <sub>50</sub> /EC <sub>50</sub>	N/A	N/A	N/A

ND – not determined

**B. REPORTED STATISTICS:**

Statistical analysis was performed for the endpoints cell density, biomass, and growth rate using the pooled negative and solvent controls and the mean-measured concentrations. The EC<sub>50</sub> for biomass was calculated by regression of the differences in area under the growth curves for each dose group compared to the control group against the log of the concentrations for days 3 and 4. The EC<sub>50</sub> for growth rate was calculated by regressing the percent reduction in average growth rate for each dose group compared to the control group against the natural logarithm of the concentrations for the 0 to 72-hour and 0 to 96-hour exposure periods. The EC<sub>25</sub> and EC<sub>50</sub> values for cell density were determined by a least squares linear regression of algal cell counts against the log of the concentration on days 3 and 4. The NOAECs for algal growth were determined using ANOVA and Dunnett's test ( $\alpha = 0.05$ ).

**C. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Replicate data for all endpoints were assessed to determine toxicity values. The negative and solvent controls were compared using a t-test before further analysis. There were no statistically significant differences between the two control groups for any endpoint. Normality and homogeneity of variance were tested with Shapiro-Wilk's and Levene's tests, respectively. For all three variables, the data met the assumptions of ANOVA, and the NOAEC was determined using William's Test. Toxstat (ver3.5) was used for these analyses.

The IC<sub>x</sub> values (with 95% C.I.) and slope (based on the Bruce-Versteeg methodology) were determined using Nuthatch Statistical Software.

All toxicity values were determined using the initial-measured concentrations and 96-hr growth measurements.

Cell density and biomass values were entered into Toxstat as the value x 10<sup>4</sup> and x 10<sup>5</sup>, respectively. Growth rate data were multiplied by 1,000 before entry into Toxstat.

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
(*Selenastrum capricornutum*)

PMRA Submission Number {.....}

EPA MRID Number 47807005

Initial measured:

**Cell density**

IC<sub>05</sub>: 4.6 mg ai/L                      95% C.I.: 3.9 to 5.5 mg ai/L

IC<sub>50</sub>: 6.7 mg ai/L                      95% C.I.: 6.2 to 7.2 mg ai/L

Slope: 10.3 ± 1.61

NOAEC: 1.89 mg ai/L

**Biomass (Area Under the Growth Curve)**

IC<sub>05</sub>: 4.8 mg ai/L                      95% C.I.: 4.0 to 5.7 mg ai/L

IC<sub>50</sub>: 6.7 mg ai/L                      95% C.I.: 6.3 to 7.2 mg ai/L

Slope: 11.0 ± 1.89

NOAEC: 0.952 mg ai/L

**Growth Rate**

IC<sub>05</sub>: 6.2 mg ai/L                      95% C.I.: 5.7 to 6.7 mg ai/L

IC<sub>50</sub>: 9.3 mg ai/L                      95% C.I.: 9.0 to 9.6 mg ai/L

Slope: 9.18 ± 0.633

NOAEC: 1.89 mg ai/L

**D. STUDY DEFICIENCIES:**

There were no study deficiencies.

**E. REVIEWER'S COMMENTS:**

The reviewer's results were different from the study authors', as the reviewer used initial-measured concentrations for analysis while the study authors used mean-measured concentrations. This resulted in the study author obtaining more conservative toxicity values due to the inclusion of low recoveries at 96 hours. However, the reviewer's results did agree with the study authors' in terms of the most sensitive endpoint. Therefore, the reviewer's results are presented in the Executive Summary and Conclusions sections of this DER in terms of initial-measured concentrations.

The study authors did not provide biomass or growth rate replicate or mean value data, or % inhibitions. The reviewer independently calculated the replicate data for these endpoints using cell density data in Excel 2003. The spreadsheet containing these calculations accompanies the files associated with this DER and is titled, "036101 47807005 850.5400 calcs.xls".

The pretest health of the algae was not reported; the reporting of this parameter is suggested by OPPTS guidelines.

The source of the dilution water was not reported.

Water characterization analysis was not performed or reported, resulting in a lack of data for total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water; the reporting of these dilution water parameters is suggested by OPPTS guidelines.

The lighting quality was not reported.

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

The lighting intensity in the definitive test was much higher than recommended, and ranged from 6440 to 8060 lux; OPPTS guidelines suggest that the intensity for *Selenastrum capricornutum* be maintained at 4300 lux.

The pH in the definitive test was much higher than recommended by OPPTS guidelines, and ranged from 8.3-9.3; OPPTS guidelines suggest a pH of  $7.5 \pm 0.1$  for tests with *Selenastrum capricornutum*. The study authors did measure pH in test solutions without algae present, and obtained a range of 7.5 to 8.7.

The 72-hour toxicity values were also calculated by the study authors.

The in-life phase of the definitive algal toxicity test was conducted from September 24 to 28, 2001.

**F. CONCLUSIONS:**

This study is scientifically sound and is classified as Acceptable. Biomass was the most sensitive endpoint, with NOAEC and IC<sub>50</sub> values of 0.952 and 6.7 mg ai/L, respectively.

Test Organism: *Selenastrum capricornutum*

Test Type (Flow-through, Static, Static Renewal): Static

Initial measured:

**Cell density**

IC<sub>05</sub>: 4.6 mg ai/L                      95% C.I.: 3.9 to 5.5 mg ai/L

IC<sub>50</sub>: 6.7 mg ai/L                      95% C.I.: 6.2 to 7.2 mg ai/L

Slope:  $10.3 \pm 1.61$

NOAEC: 1.89 mg ai/L

**Biomass (Area Under the Growth Curve)**

IC<sub>05</sub>: 4.8 mg ai/L                      95% C.I.: 4.0 to 5.7 mg ai/L

IC<sub>50</sub>: 6.7 mg ai/L                      95% C.I.: 6.3 to 7.2 mg ai/L

Slope:  $11.0 \pm 1.89$

NOAEC: 0.952 mg ai/L

**Growth Rate**

IC<sub>05</sub>: 6.2 mg ai/L                      95% C.I.: 5.7 to 6.7 mg ai/L

IC<sub>50</sub>: 9.3 mg ai/L                      95% C.I.: 9.0 to 9.6 mg ai/L

Slope:  $9.18 \pm 0.633$

NOAEC: 1.89 mg ai/L

Endpoint(s) Effected: Cell density, biomass, and growth rate

**III. REFERENCES:**

Organization for Economic Co-Operation and Development (OECD) OECD Guideline for Testing of Chemicals. Algal Growth, Inhibition Test. Number 201. Adopted 7 June 1984.

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**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
**(*Selenastrum capricornutum*)**

PMRA Submission Number {.....}

EPA MRID Number 47807005

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Madsen, S. (2001). Certificate of Analysis for Test/Reference/Control/Substances, FA&PC Number 013013, Dow AgroSciences LLC, Indianapolis, Indiana, 19 March 2001.

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McClymont, E.L., Mielke, M.S. and Hales, C.A. (2001). Analytical Data for Trifluralin Metabolite TR-15: Acute Toxicity Study with the Daphnid, *Daphnia magna*, Straus, Study # 011105, 15 June 2001.

Neter, J., Wasserman, W., and Kutner, M.H. (1983). Applied Linear Regression Models. Richard D. Irwin, Inc., Homewood, Illinois.

Winer, B.J. (1971). Statistical Principles in Experimental Design. 2<sup>nd</sup> Ed., McGraw Hill, Co., New York, New York.



**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

Trifluralin metabolite & algae 96-hr cell density; mg/L  
 File: 7005c Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

---

GRP1 (SOLVENT CTRL) MEAN =	313.1667	CALCULATED t VALUE =	1.1096
GRP2 (BLANK CTRL) MEAN =	279.6667	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	33.5000		

---

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05  
 TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

Title: Trifluralin metabolite & algae 96-hr cell density; mg/L  
 File: N\_CELL~1.TXT Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 18313.7800  
 W = 0.9732

Critical W = 0.8940 (alpha = 0.01 , N = 27)  
 W = 0.9230 (alpha = 0.05 , N = 27)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Trifluralin metabolite & algae 96-hr cell density; mg/L  
 File: N\_CELL~1.TXT Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

---

SOURCE	DF	SS	MS	F
Between	8	3670.7941	458.8493	0.8402
Within (Error)	18	9829.9800	546.1100	
Total	26	13500.7741		

---

(p-value = 0.5803)

Critical F = 3.7054 (alpha = 0.01, df = 8,18)  
 = 2.5102 (alpha = 0.05, df = 8,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae  
(*Selenastrum capricornutum*)**

PMRA Submission Number {.....}

EPA MRID Number 47807005

Title: Trifluralin metabolite & algae 96-hr cell density; mg/L  
File: N\_CELL~1.TXT Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	8	338819.7941	42352.4743	41.6268
Within (Error)	18	18313.7800	1017.4322	
Total	26	357133.5741		

(p-value = 0.0000)

Critical F = 3.7054 (alpha = 0.01, df = 8,18)  
= 2.5102 (alpha = 0.05, df = 8,18)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Trifluralin metabolite & algae 96-hr cell density; mg/L  
File: N\_CELL~1.TXT Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	313.1667	313.1667		
2	0.0586	309.7333	309.7333	0.1318	
3	0.112	296.8000	296.8000	0.6284	
4	0.272	307.5667	307.5667	0.2150	
5	0.467	330.2667	330.2667	-0.6566	
6	0.952	354.7000	354.7000	-1.5947	
7	1.89	294.3000	294.3000	0.7244	
8	7.29	109.6667	109.6667	7.8137	*
9	11.5	2.3333	2.3333	11.9349	*

Dunnett critical value = 2.5800 (1 Tailed, alpha = 0.05, df = 8,18)

Title: Trifluralin metabolite & algae 96-hr cell density; mg/L  
File: N\_CELL~1.TXT Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.0586	3	67.1934	21.5	3.4333
3	0.112	3	67.1934	21.5	16.3667
4	0.272	3	67.1934	21.5	5.6000
5	0.467	3	67.1934	21.5	-17.1000
6	0.952	3	67.1934	21.5	-41.5333
7	1.89	3	67.1934	21.5	18.8667
8	7.29	3	67.1934	21.5	203.5000
9	11.5	3	67.1934	21.5	310.8333

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

Title: Trifluralin metabolite & algae 96-hr cell density; mg/L  
 File: N\_CELL~1.TXT Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	313.1667	313.1667	318.7056
2	0.0586	3	309.7333	309.7333	318.7056
3	0.112	3	296.8000	296.8000	318.7056
4	0.272	3	307.5667	307.5667	318.7056
5	0.467	3	330.2667	330.2667	318.7056
6	0.952	3	354.7000	354.7000	318.7056
7	1.89	3	294.3000	294.3000	294.3000
8	7.29	3	109.6667	109.6667	109.6667
9	11.5	3	2.3333	2.3333	2.3333

Title: Trifluralin metabolite & algae 96-hr cell density; mg/L  
 File: N\_CELL~1.TXT Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	313.1667				
0.0586	318.7056	-0.2127		1.7300	k= 1, v=18
0.112	318.7056	-0.2127		1.8200	k= 2, v=18
0.272	318.7056	-0.2127		1.8500	k= 3, v=18
0.467	318.7056	-0.2127		1.8600	k= 4, v=18
0.952	318.7056	-0.2127		1.8700	k= 5, v=18
1.89	294.3000	0.7244		1.8700	k= 6, v=18
7.29	109.6667	7.8137	*	1.8800	k= 7, v=18
11.5	2.3333	11.9349	*	1.8800	k= 8, v=18

s = 31.8972

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	4.6	3.9	5.5	0.036	0.84
EC10	5.0	4.3	5.8	0.031	0.86
EC25	5.7	5.2	6.4	0.023	0.90
EC50	6.7	6.2	7.2	0.014	0.93

Slope = 10.3 Std.Err. = 1.61

Goodness of fit: p = 0.54 based on DF= 6.0 18.

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

7005C : Trifluralin metabolite & algae 96-hr cell density; mg/L

-----  
 Observed vs. Predicted Treatment Group Means  
 -----

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	313.	315.	-2.05	100.	0.00
0.0586	3.00	310.	315.	-5.49	100.	1.80e-14
0.112	3.00	297.	315.	-18.4	100.	1.80e-14
0.272	3.00	308.	315.	-7.65	100.	1.80e-14
0.467	3.00	330.	315.	15.0	100.	1.80e-14
0.952	3.00	355.	315.	39.5	100.	1.80e-14
1.89	3.00	294.	315.	-20.9	100.	7.21e-07
7.29	3.00	110.	110.	3.28e-06	34.8	65.2
11.5	3.00	2.33	2.33	-9.42e-07	0.740	99.3

Trifluralin metabolite & algae 96-hr biomass; mg ai/L

File: 7005b Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	853.0000	CALCULATED t VALUE =	0.0649
GRP2 (BLANK CTRL) MEAN =	850.0000	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	3.0000		

-----  
 TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05  
 TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01  
 -----

Title: Trifluralin metabolite & algae 96-hr biomass; mg ai/L

File: N\_BIOM-1.TXT Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

-----  
 D = 87552.6667  
 W = 0.9480

Critical W = 0.8940 (alpha = 0.01 , N = 27)  
 W = 0.9230 (alpha = 0.05 , N = 27)

-----  
 Data PASS normality test (alpha = 0.01). Continue analysis.

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

Title: Trifluralin metabolite & algae 96-hr biomass; mg ai/L  
 File: N\_BIOM-1.TXT Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	8	19243.8519	2405.4815	1.1269
Within (Error)	18	38422.0000	2134.5556	
Total	26	57665.8519		

(p-value = 0.3920)

Critical F = 3.7054 (alpha = 0.01, df = 8,18)  
 = 2.5102 (alpha = 0.05, df = 8,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Trifluralin metabolite & algae 96-hr biomass; mg ai/L  
 File: N\_BIOM-1.TXT Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	8	2241228.0741	280153.5093	57.5969
Within (Error)	18	87552.6667	4864.0370	
Total	26	2328780.7407		

(p-value = 0.0000)

Critical F = 3.7054 (alpha = 0.01, df = 8,18)  
 = 2.5102 (alpha = 0.05, df = 8,18)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Trifluralin metabolite & algae 96-hr biomass; mg ai/L  
 File: N\_BIOM-1.TXT Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	853.0000	853.0000		
2	0.0586	834.0000	834.0000	0.3337	
3	0.112	851.0000	851.0000	0.0351	
4	0.272	808.0000	808.0000	0.7902	
5	0.467	815.6667	815.6667	0.6556	
6	0.952	795.6667	795.6667	1.0068	
7	1.89	626.3333	626.3333	3.9805	*
8	7.29	275.6667	275.6667	10.1385	*
9	11.5	4.0000	4.0000	14.9092	*

Dunnett critical value = 2.5800 (1 Tailed, alpha = 0.05, df = 8,18)

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae  
(*Selenastrum capricornutum*)**

PMRA Submission Number {.....}

EPA MRID Number 47807005

Title: Trifluralin metabolite & algae 96-hr biomass; mg ai/L  
File: N\_BIOM~1.TXT Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho: Control<Treatment

---

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.0586	3	146.9172	17.2	19.0000
3	0.112	3	146.9172	17.2	2.0000
4	0.272	3	146.9172	17.2	45.0000
5	0.467	3	146.9172	17.2	37.3333
6	0.952	3	146.9172	17.2	57.3333
7	1.89	3	146.9172	17.2	226.6667
8	7.29	3	146.9172	17.2	577.3333
9	11.5	3	146.9172	17.2	849.0000

---

Title: Trifluralin metabolite & algae 96-hr biomass; mg ai/L  
File: N\_BIOM~1.TXT Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

---

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	853.0000	853.0000	853.0000
2	0.0586	3	834.0000	834.0000	842.5000
3	0.112	3	851.0000	851.0000	842.5000
4	0.272	3	808.0000	808.0000	811.8333
5	0.467	3	815.6667	815.6667	811.8333
6	0.952	3	795.6667	795.6667	795.6667
7	1.89	3	626.3333	626.3333	626.3333
8	7.29	3	275.6667	275.6667	275.6667
9	11.5	3	4.0000	4.0000	4.0000

---

Title: Trifluralin metabolite & algae 96-hr biomass; mg ai/L  
File: N\_BIOM~1.TXT Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

---

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	853.0000				
0.0586	842.5000	0.1844		1.7300	k= 1, v=18
0.112	842.5000	0.1844		1.8200	k= 2, v=18
0.272	811.8333	0.7229		1.8500	k= 3, v=18
0.467	811.8333	0.7229		1.8600	k= 4, v=18
0.952	795.6667	1.0068		1.8700	k= 5, v=18
1.89	626.3333	3.9805	*	1.8700	k= 6, v=18
7.29	275.6667	10.1385	*	1.8800	k= 7, v=18
11.5	4.0000	14.9092	*	1.8800	k= 8, v=18

---

s = 69.7426

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae  
(*Selenastrum capricornutum*)**

PMRA Submission Number {.....}

EPA MRID Number 47807005

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	4.8	4.0	5.7	0.037	0.84
EC10	5.1	4.4	6.0	0.031	0.86
EC25	5.8	5.2	6.5	0.023	0.90
EC50	6.7	6.3	7.2	0.014	0.94

Slope = 11.0 Std.Err. = 1.89

Goodness of fit: p = 0.058 based on DF= 6.0 18.

7005B : Trifluralin metabolite & algae 96-hr biomass; mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	853.	798.	55.3	100.	0.00
0.0586	3.00	834.	798.	36.3	100.	2.85e-14
0.112	3.00	851.	798.	53.3	100.	2.85e-14
0.272	3.00	808.	798.	10.3	100.	2.85e-14
0.467	3.00	816.	798.	18.0	100.	2.85e-14
0.952	3.00	796.	798.	-2.00	100.	2.85e-14
1.89	3.00	626.	798.	-171.	100.	7.18e-08
7.29	3.00	276.	276.	-1.75e-05	34.6	65.4
11.5	3.00	4.00	4.00	-2.35e-05	0.501	99.5

Trifluralin metabolite & 96-hr growth rate; mg ai/L  
File: 7005g Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	55.6667	CALCULATED t VALUE =	-1.1314
GRP2 (BLANK CRTL) MEAN =	58.3333	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	-2.6667		

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05  
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae  
(*Selenastrum capricornutum*)**

PMRA Submission Number {.....}

EPA MRID Number 47807005

Title: Trifluralin metabolite & 96-hr growth rate; mg ai/L  
File: N\_GROW~1.TXT Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 128.0000  
W = 0.9320

Critical W = 0.8940 (alpha = 0.01 , N = 27)  
W = 0.9230 (alpha = 0.05 , N = 27)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Trifluralin metabolite & 96-hr growth rate; mg ai/L  
File: N\_GROW~1.TXT Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	8	24.2963	3.0370	0.6508
Within (Error)	18	84.0000	4.6667	
Total	26	108.2963		

(p-value = 0.7263)

Critical F = 3.7054 (alpha = 0.01, df = 8,18)  
= 2.5102 (alpha = 0.05, df = 8,18)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All equal (alpha = 0.01)

Title: Trifluralin metabolite & 96-hr growth rate; mg ai/L  
File: N\_GROW~1.TXT Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	8	6267.4074	783.4259	110.1693
Within (Error)	18	128.0000	7.1111	
Total	26	6395.4074		

(p-value = 0.0000)

Critical F = 3.7054 (alpha = 0.01, df = 8,18)  
= 2.5102 (alpha = 0.05, df = 8,18)

Since  $F > \text{Critical } F$  REJECT  $H_0$ : All equal (alpha = 0.05)



**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae**  
*(Selenastrum capricornutum)*

PMRA Submission Number {.....}

EPA MRID Number 47807005

Title: Trifluralin metabolite & 96-hr growth rate; mg ai/L  
 File: N\_GROW~1.TXT Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	55.6667	55.6667		
2	0.0586	60.3333	60.3333	-2.1433	
3	0.112	60.3333	60.3333	-2.1433	
4	0.272	60.3333	60.3333	-2.1433	
5	0.467	61.6667	61.6667	-2.7557	
6	0.952	63.0000	63.0000	-3.3680	
7	1.89	61.0000	61.0000	-2.4495	
8	7.29	50.3333	50.3333	2.4495	
9	11.5	12.0000	12.0000	20.0552	*

Dunnett critical value = 2.5800 (1 Tailed, alpha = 0.05, df = 8,18)

Title: Trifluralin metabolite & 96-hr growth rate; mg ai/L  
 File: N\_GROW~1.TXT Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.0586	3	5.6175	10.1	-4.6667
3	0.112	3	5.6175	10.1	-4.6667
4	0.272	3	5.6175	10.1	-4.6667
5	0.467	3	5.6175	10.1	-6.0000
6	0.952	3	5.6175	10.1	-7.3333
7	1.89	3	5.6175	10.1	-5.3333
8	7.29	3	5.6175	10.1	5.3333
9	11.5	3	5.6175	10.1	43.6667

Title: Trifluralin metabolite & 96-hr growth rate; mg ai/L  
 File: N\_GROW~1.TXT Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	55.6667	55.6667	60.3333
2	0.0586	3	60.3333	60.3333	60.3333
3	0.112	3	60.3333	60.3333	60.3333
4	0.272	3	60.3333	60.3333	60.3333
5	0.467	3	61.6667	61.6667	60.3333
6	0.952	3	63.0000	63.0000	60.3333
7	1.89	3	61.0000	61.0000	60.3333
8	7.29	3	50.3333	50.3333	50.3333
9	11.5	3	12.0000	12.0000	12.0000

**Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Algae  
(*Selenastrum capricornutum*)**

PMRA Submission Number {.....}

EPA MRID Number 47807005

Title: Trifluralin metabolite & 96-hr growth rate; mg ai/L  
File: N\_GROW~1.TXT Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	55.6667				
0.0586	60.3333	-2.1433		1.7300	k= 1, v=18
0.112	60.3333	-2.1433		1.8200	k= 2, v=18
0.272	60.3333	-2.1433		1.8500	k= 3, v=18
0.467	60.3333	-2.1433		1.8600	k= 4, v=18
0.952	60.3333	-2.1433		1.8700	k= 5, v=18
1.89	60.3333	-2.1433		1.8700	k= 6, v=18
7.29	50.3333	2.4495	*	1.8800	k= 7, v=18
11.5	12.0000	20.0552	*	1.8800	k= 8, v=18

s = 2.6667

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	6.2	5.7	6.7	0.018	0.92
EC10	6.7	6.3	7.2	0.015	0.93
EC25	7.9	7.5	8.3	0.011	0.95
EC50	9.3	9.0	9.6	0.0068	0.97

Slope = 9.18 Std.Err. = 0.633

Goodness of fit: p = 0.14 based on DF= 6.0 18.

7005G : Trifluralin metabolite & 96-hr growth rate; mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	55.7	60.3	-4.67	100.	0.00
0.0586	3.00	60.3	60.3	-1.84e-10	100.	2.36e-14
0.112	3.00	60.3	60.3	-1.84e-10	100.	2.36e-14
0.272	3.00	60.3	60.3	-1.84e-10	100.	2.36e-14
0.467	3.00	61.7	60.3	1.33	100.	2.36e-14
0.952	3.00	63.0	60.3	2.67	100.	2.36e-14
1.89	3.00	61.0	60.3	0.667	100.	1.07e-08
7.29	3.00	50.3	50.3	-6.48e-08	83.4	16.6
11.5	3.00	12.0	12.0	7.17e-08	19.9	80.1