Data Evaluation Report on the acute toxicity of Metolachlor to algae *Selenastrum capricornutum*
PMRA Submission #:{.....} EPA MRID #: 44931719

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D260418
OECD Data Point	{.....}
EPA MRID	44931719
EPA Guideline	122-2 & 123-2

Test material: CGA-354743 (metabolite of CGA-24705) Purity: 99.9%
Common name: Metolachlor
Chemical name: IUPAC: Not Reported
CAS name: Not reported
CAS No.: 51218-45-2
Synonyms: Not reported

Primary Reviewer: Dana Worcester
Senior Staff Scientist, Dynamac Corporation
Siganture:
Date: 10/26/01

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation
Signature:
Date: 10/26/01

Primary Reviewer: Bill Evans
{EPA/OECD/PMRA}
Date: 2/25/02

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

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 108801

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Grade, R. 1997. Growth inhibition Test of CGA-354743 (Metabolite of CGA-24705) to Green Algae (*Selenastrum capricornutum*) Under Static Conditions. Unpublished study performed by Ciba-Geigy Limited, Basle, Switzerland. Ciba-Geigy Project ID 971645. Study submitted by Novartis Crop Protection, Inc., Greensboro, NC. Novartis Study No. 784-97. Study initiated June 16, 1997 and completed December 3, 1997.

2044748

EXECUTIVE SUMMARY:

In a 72-hour acute toxicity study, cultures of *Selenastrum capriconutum* were exposed to CGA-354743 (metabolite of CGA-24705) under static conditions. Nominal concentrations were 10, 18, 32, 58, and 100 mg a.i./L. Mean measured concentrations were 10.25, 18.20, 32.95, 60.10, and 99.45 mg a.i./L. The NOAEC based on cell density is 99.45 mg a.i./L and the EC_{50} is >99.45 mg a.i/L.

No observation of unusual cell shape, color differences, flocculation, adherence of algae to test vessels, aggregation of algal cell, precipitation in the test solution, or stimulation were reported.

This toxicity study is scientifically sound, but it only partially satisfies the guideline requirements for an acute toxicity study with algae (*Selenastrum capricornutum*), due to numerous deviations from US EPA protocol. This study is classified as Supplemental. The study should be repeated as specified in the EPA guidelines for 120 hours without the use of a chelator.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static:

EC_{05}:	N/A	95% C.I.: N/A
NOEC:	99.45 mg a.i./L	Probit Slope: N/A
EC_{50}:	>99.45 mg a.i./L	Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Guideline Subdivision J, §123-2. The following deviations are noted:

1. The chelator EDTA was used in the growth medium. EPA does not recommend the used of chelators in growth medium. This deviation may have impacted algal growth or response to Metolachlor.

2. The study period (72 hours) was shorter than recommended by US EPA (120 hours). EPA accepts three day studies for Tier I screening purposes only (US EPA memorandum, Oct 21, 1994, "Closure on Nontarget Plant Phytotoxicity Policy Issues").

3. The light intensity (8 Klux) is higher than recommended by EPA (4-5±15%. klux).

4. The initial cell density (12,000 cells/mL) was significantly higher than recommended by EPA (3,000 cells/mL).

5. The dilution water source, pH, total organic carbon, particulate matter, metals, pesticides, and chlorine were not reported.

6. The carbon source of the growth medium was not reported.

These deviations affected the acceptability, but not the validity, of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material Metolachlor Technical (CGA-354743)

Description: White powder

Lot No./Batch No. : RV-2816/3

Purity: 99.9%

Stability of Compound
Under Test Conditions: Metolachlor appeared to be stable under test conditions. On average, the 0-hour concentrations ranged from 101% to 104% and the 72-hour concentrations ranged from 98% to 103% of nominal concentrations.
(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test material was refrigerated.

2. Test organism:

Name: *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. 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: ATCC 22662
Source: Pflanzenphysilogisches institut University, Göttingen, Germany.
Age of inoculum: 3 days
Method of cultivation: Synthetic algae culture medium

C. STUDY DESIGN:

a) Range-finding Study: A range-finding test was conducted with three replicates of 5 nominal concentrations of CGA 354743 ranging from 0.01 to 100 mg a.i./L (measured concentrations not reported), and 6 replicates of a control blank. By the conclusion of the study, "negligible effects" were observed in the 100 mg a.i./L group. Based on the results, the definitive Tier 2 test was conducted with approximate nominal concentrations ranging from 10 to 100 mg a.i./L.

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)	 3 days Sterile synthetic algae medium; same as test Not reported	*The acclimation period was shorter than recommended by US EPA. 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	Temperature controlled room	

Parameter	Details	Remarks *Criteria*
Duration of the test	72 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	 Glass 125 mL 30 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):**	 Synthetic algae culture medium 7.9 7.8-8.8 EDTA Not reported. Not applicable.	The nutrient solution contained 0.1 mg/l of EDTA. *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 and no chelators.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes, stock solution composition listed on pages 34-35 of study.	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Not reported Reconstituted bi-distilled water Not reported Not applicable Not reported Not reported Not reported Not reported Not reported 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	

Parameter	Details	Remarks *Criteria*
Aeration or agitation	Agitation, 150 rpm	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*
Initial cells density	12,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, 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:	 6 Not applicable 3	*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:	10, 18, 32, 58, and 100 mg/L. (0-hour) 10.2, 18.3, 33.2, 60.3, and 100.9 mg/L (72-hour) 10.3, 18.1, 32.7, 59.9, and 98 mg/L	*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)	Not applicable	
Method and interval of analytical verification	HPLC; 0 and 72 hours	
Test conditions temperature: photoperiod: light intensity and quality:	24±1°C Continuous approximately 8 Klux	*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:	None used	
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 count, specific growth rate, area under the growth curve (biomass)	*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	CytoFluor II	Fluorescence Multi-Well Plate Reader *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	Cell count at test termination was ≥2x initial cell count	*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	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Cell density was not reduced from exposure to any of the test concentrations of CGA 354743.

The study author did not report any unusual cell shape, color differences, flocculation, adherence of algae to test vessels, aggregation of algal cells, or precipitation in the test solution. The pH range at test initiation and termination was 7.9 and 7.8-8.8, respectively.

Table 3: Effect of Metolachlor on algal growth (*Selenastrum capricornutum*)

Treatment (record measured and nominal concentration[a] (mg a.i./L)	Initial cell density (cells/mL)	Cell density (cells/mL) at			
		24 hours[b]	72 hours[b]	72 hours	
				cell count	% inhibition[c]
Negative control	12,000	10×10^4	251×10^4	251×10^4	---
Solvent control	Not applicable	---	---	---	---
10.25 (10)	12,000	10×10^4	286×10^4	286×10^4	-13%[d]
18.2 (18)	12,000	9.7×10^4	272×10^4	272×10^4	-8%[d]
32.95 (32)	12,000	12×10^4	293×10^4	293×10^4	-14%[d]
60.1 (58)	12,000	11×10^4	316×10^4	316×10^4	-21%[d]
99.45 (100)	12,000	8.8×10^4	264×10^4	264×10^4	-5%[d]
Reference chemical (if used)	Not applicable				

[a] Mean measured concentrations are the average of concentrations measured at 0 and 72 hours. Nominal concentrations are in parentheses.
[b] Reviewer calculated from average cell density multiplied by the cell volume divided by the equivalency factor (12,000/5.84)
[c] Treatment groups were compared to the control to determine % inhibition. Reported values are reviewer-calculated.
[d] A negative number denotes an increase in cell growth compared to the control.

Table 4: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC or EC_{05} (mg a.i./L)	99.45	99.45	99.45
EC_{50} (mg a.i./L)	>99.45	>99.45	>99.45

IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	N/A	N/A	N/A
other (IC_{25}/EC_{25})	N/A	N/A	N/A
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

B. REPORTED STATISTICS: At each observation interval, a one-way analysis of variance (ECOS in SAS) was conducted with a Dunnett's comparison to the blank. A one-way Dunnett's test was performed at the 0.05 level of significance. The study authors used nominal concentrations to calculate NOEC and EC_{50} estimates.

Statistical Method: EC values and 95% confidence intervals were determined with a SAS program.

EC_{05}: Not reported 95% C.I.: Not reported

EC_{50}/IC_{50}: >100 mg/L 95% C.I.:

NOEC: >100 mg/L

Probit Slope: Not reported 95% C.I.: Not reported.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Data for cell density were analyzed using Bonferonni's t-test via TOXSTAT software. The reviewer used mean measured concentrations for the statistical analysis. There were no reductions in cell density.

EC_{05}: N/A 95% C.I.: N/A

EC_{50}/IC_{50}: >99.45 mg/L 95% C.I.: N/A

NOEC: 99.45 mg/L

Probit Slope: N/A 95% C.I.: N/A

D. STUDY DEFICIENCIES:

There were several deviations from U.S. EPA guidelines that, together, impacted the acceptability of this study. These included, use of the chelator, EDTA in the growth medium, a shorter study duration (72 hours) than recommended by US EPA (120 hours), higher light intensity (8 Klux) than recommended by EPA (4-5±15%. klux), higher initial cell density (12,000 cells/mL) than recommended by EPA (3,000 cells/mL), and failure to report the dilution water source, pH, total organic carbon, particulate matter, metals, pesticides, chlorine content, and carbon source of the growth medium.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. There were no reductions in cell density upon exposure to CGA-354743. As a result, the NOAEC and the EC_{50} were determined to be 99.45 and >99.45 mg a.i./L, respectively.

F. CONCLUSIONS: The study is scientifically sound, but it only partially fulfills the requirements for an algal toxicity study because of multiple deviations from U.S. EPA guidelines. This study is classified as Supplemental and should be repeated as specified in the EPA guidelines for 120 hours without the use of a chelator..

EC_{50}: >99.45 mg a.i./L NOAEC: 99.45 mg a.i./L

III. REFERENCES:

1. Draper, N.R. and Smith, H., 1981. Applied Regression Analysis, 2nd edition, Wiley, New York.

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

3. Dunnett, C.W., 1964. New tables for multiple comparisons with a control. Biometrics 20:482-491.

4. Finney, D.J., 1971. Probit analysis. 3rd edition, London, Cambridge University Press, Marcus, R., Peritz, E., Gabriel, K.R.: On closed testing procedures with special reference to ordered analysis of varience. Biometrika 63, 655-660.

5. Fisch, R.D. and Strehlau, G.A., 1996. Validierungsbericht ECOS, Ciba-Geigy, Basel.

6. Fisch, R.D. and Strehlau, G.A., 1994. A simplified approach to calibration confidence sets in multilinear and nonlinear regression, internal report, Mathematical Applications, Ciba-Geigy, Basel.

7. Fisch, R.D. and Strehlau, G.A., 1994. ECOS: Statistiche Analyse von Oekotoxikologie-Daten, Version 1.1. Dokumentation und Manual, Novartis Services AG, Basel.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 1719c 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	1224.333	1224.333		
2	10.25	1396.667	1396.667	-3.213	
3	18.20	1325.667	1325.667	-1.889	
4	32.95	1426.000	1426.000	-3.760	
5	60.1	1539.000	1539.000	-5.867	
6	99.45	1284.333	1284.333	-1.119	

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

cell density
File: 1719c 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	10.25	3	139.601	11.4	-172.333
3	18.20	3	139.601	11.4	-101.333
4	32.95	3	139.601	11.4	-201.667
5	60.1	3	139.601	11.4	-314.667
6	99.45	3	139.601	11.4	-60.000