Data Evaluation Report on the Acute Toxicity of Bayer AE 0172747 and Isoxadifen-ethyl SC 420+210 to *Pseudokirchneriella subcapitata*

PMRA Submission Number {.....} EPA MRID Number 466955-19

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D325337
	OECD Data Point	{.....}
	EPA MRID	466955-19
	EPA Guideline	OPPTS 850.5400

Test material: Bayer AE 0172747 and Isoxadifen-ethyl SC 420+210 **Purity:** AE 0172747: 33.9%; Isoxadifen-ethyl: 18.1%.

Common name: AE 0172747 with safener (Isoxadifen-ethyl)

Chemical name: IUPAC: 2-[2-chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoyl]cyclohexane-1,3-dione
CAS name: Not reported
CAS No.: 335-104-84-2 for AE 0172747; 163520-33-0 for Isoxadifen-ethyl SC 420+210
Synonyms: AE 0172747 02 SC52 A105

Primary Reviewer: Rebecca Bryan
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Signature: Rebecca L. Bryan
Date: 4/18/06

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Staff Scientist, Cambridge Environmental Inc.
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Date:

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{EPA/OECD/PMRA}
Date: 7/14/06

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

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Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Dorgerloh, M. 2005. *Pseudokirchneriella subcapitata* Growth Inhibition Test with AE 0172747 and Isoxadifen-ethyl SC 420+210 (code: AE 0172747 02 SC52 A105). Unpublished study performed by Bayer CropScience AG, Research/Development, Department–Ecotoxicology, Monheim, Germany. Study No. EBAEP032/E 323 2838-9. Study sponsored by Bayer CropScience AG. The final report issued May 4, 2005.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 72-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed to AE 0172747 02 SC52 A105 (formulation containing AE 0172747 and Isoxadifen-ethyl SC 420+210) at nominal concentrations of 0.868, 2.2, 5.4, 14, and 34 mg a.i./L with a negative control. The 72-hour mean measured concentrations were <0.1104 (<LOQ, negative control), 0.773, 1.92, 5.05, 12.4, and 29.1 mg a.i./L (86-93% of nominal) under static conditions. The 72-hour NOAEC was 0.773 mg a.i./L based on cell density and 1.92 mg a.i./L based on growth rate. The EC_{50} was 2.7 mg a.i./L for cell density, the most sensitive endpoint. By 72-hours, cell density percent inhibition was -6.1, 13.1, 89.1, 96.4 and 97.6% at the mean-measured 0.773, 1.92, 5.05, 12.4, and 29.1 mg a.i./L treatment levels, respectively, compared to the control. The growth rate percent inhibition was -1.6, 3.9, 55.7, 89.0 and 93.2% at the mean-measured 0.773, 1.92, 5.05, 12.4, and 29.1 mg a.i./L treatment levels, respectively, compared to the control.

In the mean-measured 12.4 and 29.1 mg a.i./L treatment groups, cells were observed to be swollen.

This toxicity study is classified as scientifically sound but does not satisfy the guideline requirement for aquatic nonvascular plant toxicity study in order to be classified as Acceptable. This study was only conducted for 72 hours and, according to EPA guidelines, should be considered for Tier I screening purposes only. The study is classified SUPPLEMENTAL.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density:

EC_{05}:	<0.773 mg a.i./L	95% C.I.: N/A
EC_{50}:	2.7 mg a.i./L	95% C.I.: 1.7-4.1 mg a.i./L
NOAEC:	0.773 mg a.i./L	
Probit Slope:	2.70±0.513	

Growth rate:

EC_{05}:	0.84 mg a.i./L	95% C.I.: 0.27-2.6 mg a.i./L
EC_{50}:	4.5 mg a.i./L	95% C.I.: 2.7-7.5 mg a.i./L
NOAEC:	1.92 mg a.i./L	
Probit Slope:	2.25±0.453	

Area under the growth curve (biomass): Not determined

Endpoint(s) Affected: Cell density and growth rate.
Most sensitive endpoint: Cell density

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test protocol was based on the U.S. EPA Pesticide Assessment Guideline 123-2, OECD Guideline #201, and EU Guideline Annex C- Part C.3. The following deviations from U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400, *Algal Toxicity, Tiers I and II* were noted:

1. The dilution water characteristics of TOC, particulate matter, metals, pesticides, and chlorine content were not reported.
2. The physiochemical properties of the test material were not reported.
3. The pre-test health of the algae cultures was not reported.
4. The size of the test vessels (300 mL) was larger than recommended (250 mL).
5. The study was conducted for only 72 hours. Three-day OECD-guideline studies will be considered as Tier I screening data.

These deviations did not affect the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the OECD Principles of Good Laboratory Practice and the Principles of Good Laboratory Practice according to Annex 1 of the German chemical law (ChemG). The study also met the requirements of U.S. EPA-FIFRA Good Laboratory Practice Standards (40 CFR Part 160) as well as the GLP standards of the Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF, 11 Nou(g)san No. 6283), with the exception that recognized differences exist between the GLP principles/standards of OECD and FIFRA and JMAFF.

A. MATERIALS:

1. Test material: AE0172747 02 SC52 A105 (Formulation containing Bayer AE 0172747 and Isoxadifen-ethyl SC 420+210 (safener))

Description: Light Beige milky liquid

Lot No./Batch No. : EFIM000036 (Batch Number)

Purity: AE 0172747: 33.9%; Isoxadifen-ethyl: 18.1%.

Stability of compound under test conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determinations at 0 and 72 hours. The mean recoveries at all treatment levels were 85-94% of nominal at 0 hour (except the nominal 33.9 mg a.i./L recovery of 35% due to a reported handling error) and 86-93% of nominal at 72 hours.

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

Storage conditions of test chemicals: Stored at room temperature.

Physicochemical properties of AE 0172747.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

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: SAG 61.81
Source: Collection of Algal Cultures, Institute of Plant Physiology, University of Goettingen, Goettingen, Germany.
Age of inoculum: 2-4 days old
Method of cultivation: Nutrient medium

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive test concentrations were based on a pre-experiment, but the results were not reported.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous culture; pre-culture prepared 2-4 days prior to testing. Nutrient medium; same as test. 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 N/A	*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	Growth incubator	
Duration of the test	72 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Erlenmeyer flasks 300 mL 150 mL	The size of the test vessels (300 mL) was larger than recommended (250 mL). *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*

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**):	Nutrient medium 7.8-8.1 7.8-8.3 Yes $NaHCO_3$ N/A	The nutrient medium was prepared by dissolving analytical grade salts in purified (Milli-Q-water). *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 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, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Purified (Milli-Q-water) water 7.8-8.3 (0-72-hours) N/A Sterilized and aerated Not reported Not reported Not reported Not reported Not reported	The dilution water characteristics of TOC, particulate matter, metals, pesticides, and chlorine content were 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 solutions	
Aeration or agitation	Continuously agitated at 100 rpm.	
Initial cells density	Approximately 10,000 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.*

Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Treatments:	6 N/A 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.*
Test concentrations Nominal: Measured:	0 (negative control), 0.868, 2.2, 5.4, 14, and 34 mg a.i./L <0.1104 (<LOQ, negative control), 0.773, 1.92, 5.05, 12.4, and 29.1 mg a.i./L	The nominal concentrations are equivalent to 2.56, 6.4, 16, 40, and 100 mg formulation/L. The mean measured concentrations were reviewer-calculated based on 0- and 72-hour mean concentrations. For the highest treatment group, the mean measured concentration is only based on the 72-hour samples (0-hour sample recoveries were considered outliers due to a handling error). *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.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC at 0 and 72 hours; LOQ was 0.1104 mg a.i./L.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	22.2-23.4°C Continuous 5730-7460 lux, cool-white fluorescent lighting	*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:	The algae strains were tested with 3,5-dichlorophenol or potassium dichromate (results not reported).	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density, growth rate, and doubling time.	*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	Direct cell counting with a microscope (average of two cell counts).	*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	At 24, 48, and 72 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	The pH was measured in each treatment group and control at 0, 24, 48, and 72 hours. The temperature was continuously measured in an additional incubated water replicate.	
Indicate whether there was an exponential growth in the control	Yes, the dilution water control cell density at test termination was 54.6x greater than the dilution water control cell density 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, replicate data were provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 72-hours, cell density percent inhibition was -6.1, 13.1, 89.1, 96.4 and 97.6% at the nominal 0.868, 2.2, 5.4, 14, and 34 mg a.i./L treatment levels, respectively, compared to the control. The growth rate percent inhibition was -1.6, 3.9, 55.7, 89.0 and 93.2% at the mean-measured 0.868, 2.2, 5.4, 14, and 34 mg a.i./L treatment levels, respectively, compared to the control. Both cell density and growth rate were significantly reduced compared to the control at ≥5.4 mg a.i./L treatment levels. The 72-hour NOAEC was 2.2 mg a.i./L based on cell density and growth rate.

In the nominal 14 and 34 mg a.i./L treatment groups, cells were observed to be swollen.

Table 3: Effect of AE 0172747 on algal growth (*Pseudokirchneriella subcapitata*)

Treatment measured and (nominal) concentrations (mg a.i./L)	Initial cell density	Cell density at			
		24 hours	48 hours	72 hours	
				cell count	% inhibition
Negative control	10,000	37,000	213,000	546,000	--
0.773 (0.868)	10,000	77,000	197,000	582,000	-6.1
1.92 (2.17)	10,000	58,000	153,000	477,000	13.1
5.05 (5.42)	10,000	45,000	47,000	60,000	89.1*
12.4 (13.6)	10,000	10,000	38,000	20,000	96.4*
29.1 (33.9)	10,000	10,000	10,000	13,000	97.6*

[A] Negative percent inhibition indicates promoted growth. Percent inhibition was reviewer-calculated.
* Statistically significant percent reduction compared to the control (Dunnett's multiple t-test, α=0.05).

Table 4: Effect of AE 0172747 on algal growth (*Pseudokirchneriella subcapitata*)

Treatment measured and (nominal) concentrations (mg a.i./L)	Initial cell density	Mean Growth Rate ($hours^{-1}$)		Mean Area Under the Growth Curve (cells/mL x hour)	
		0-72 hours	Percent Inhibition[a]	0-72 hours	Percent Inhibition[a]
Negative control	10,000	1.333	--	ND	ND
0.773 (0.868)	10,000	1.354	-1.6	ND	ND
1.92 (2.17)	10,000	1.282	3.9	ND	ND
5.05 (5.42)	10,000	0.590	55.7*	ND	ND
12.4 (13.6)	10,000	0.147	89.0*	ND	ND
29.1 (33.9)	10,000	0.090	93.2*	ND	ND

[a] Negative percent inhibition indicates promoted growth.
* Statistically significant percent reduction compared to the control (Dunnett's multiple t-test, α=0.05).
ND = Not determined

Table 5: Statistical endpoint values.

Statistical Endpoint	Cell density	Growth rate	Biomass
NOAEC or EC_{05}[a] (mg a.i./L)	2.2	2.2	Not determined
EC_{50} (mg a.i./L)[b]	3.35	5.21	Not determined
EC_{50} 95% C.I. (mg a.i./L)[b]	2.9-3.9	3.8-7.2	Not determined
EC_{10} (95% C.I.) (mg a.i./L)	1.92 (1.5-2.2)	2.34 (0.6-3.4)	Not determined
Reference chemical, if used NOAEC IC_{50}/EC_{50}	Not reported	Not reported	Not reported

[a] Based on 72-hour mean measured concentrations.
[b] Based on nominal (mg a.i./L) concentrations corrected for the purity of the active ingredient (33.9%).

B. REPORTED STATISTICS:

The EC_{50} values were calculated using probit analysis with the ToxRat Professional software. The NOAEC was verified using ANOVA and Dunnett's t-test. The study author's statistical calculations were performed using the nominal (mg form./L) concentrations; however, the reviewer corrected these values based on the purity of the active ingredient (33.9%) for the purposes of comparing these results to the reviewer's toxicity values.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Replicate data for cell density and growth rate were first tested for normality and homogeneity. These assumptions of ANOVA were not met; therefore, the NOAEC values were determined using the non-parametric Kruskal-Wallis test via Toxstat Statistical Software. The ECx values (with 95% C.I.) and probit slopes were determined using the probit analysis via Nuthatch Statistical Software. All toxicity values were determined using the mean-measured concentrations.

Cell density:

EC_{05}:	<0.773 mg a.i./L	95% C.I.: N/A
EC_{50}:	2.7 mg a.i./L	95% C.I.: 1.7-4.1 mg a.i./L
NOAEC:	0.773 mg a.i./L	
Probit Slope:	2.70±0.513	

Growth rate:

EC_{05}:	0.84 mg a.i./L	95% C.I.: 0.27-2.6 mg a.i./L
EC_{50}:	4.5 mg a.i./L	95% C.I.: 2.7-7.5 mg a.i./L
NOAEC:	1.92 mg a.i./L	
Probit Slope:	2.25±0.453	

Area under the growth curve (biomass): Not determined

Endpoint(s) Affected: Cell density and growth rate.
Most sensitive endpoint: Cell density

D. STUDY DEFICIENCIES:

The study was conducted for 72 hours. Three-day OECD tests will be considered as Tier I screening studies. Other than recommended guideline species was used.

E. REVIEWER'S COMMENTS:

The reviewer's results were based on mean-measured concentrations and the study author's results were based on nominal concentrations; therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The NOAEC values for cell density and growth rate (0.773 and 1.92 mg a.i./L) were determined visually based on the 13.1 and 55.7% reductions at the mean-measured 1.92 and 5.05 mg a.i./L treatment level, relative to the negative controls. The reviewer's analyses did not detect statistical differences from control and the reviewer attributes that to the use of the less sensitive, non-parametric Kruskal-Wallis test.

The percent recovery of the nominal 33.9 mg a.i./L concentration at Day 0 was 11.8 mg a.i./L (35% of nominal). The study author reported that this was most likely due to a handling error. Because concentrations remained stable throughout the definitive test (recoveries of 85-94% of nominal), the reviewer excluded the Day 0 measured value for the nominal 33.9 mg a.i./L concentration and only used the Day 3 measured value (29.1 mg a.i./L; 86% of nominal) for all analyses.

The experiments started on January 13, 2005 and were completed on January 20, 2005.

F. CONCLUSIONS:

This study is scientifically sound but is classified as SUPPLEMENTAL because of shorter test duration and use of different algal species. Cell density was the most sensitive endpoint, with a 72-hour EC_{50} of 2.7 mg a.i./L; the 72-hour NOAEC for cell density was 0.773 mg a.i./L.

Cell density:

EC_{05}:	<0.773 mg a.i./L	95% C.I.: N/A
EC_{50}:	2.7 mg a.i./L	95% C.I.: 1.7-4.1 mg a.i./L
NOAEC:	0.773 mg a.i./L	
Probit Slope:	2.70±0.513	

Growth rate:

EC_{05}:	0.84 mg a.i./L	95% C.I.: 0.27-2.6 mg a.i./L
EC_{50}:	4.5 mg a.i./L	95% C.I.: 2.7-7.5 mg a.i./L
NOAEC:	1.92 mg a.i./L	
Probit Slope:	2.25±0.453	

Area under the growth curve (biomass): Not determined

Endpoint(s) Affected: Cell density and growth rate.
Most sensitive endpoint: Cell density

III. REFERENCES:

Draft Proposal for Updating OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (Feb. 18, 2004)

Statistical Software "ToxRat Professional", version 2.09, produced by ToxRat Solutions GmbH, 52477 Alsdorf, Germany (Feb 6, 2004)

ToxRat Validation Document from ToxRat Solutions GmbH, valid for ToxRat Version 2.09 (released January 25, 2004)

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell density (x 10E+04/mL)
File: 5519cd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	54.833	54.833	91.000
2	0.773	58.167	58.167	54.000
3	1.92	47.667	47.667	41.000
4	5.05	6.000	6.000	24.000
5	12.4	2.000	2.000	12.000
6	29.1	1.333	1.333	9.000

Calculated H Value = 16.617 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Cell density (x 10E+04/mL)
File: 5519cd 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 4 3 1 2
6	29.1	1.333	1.333	\
5	12.4	2.000	2.000	. \
4	5.05	6.000	6.000	. . \
3	1.92	47.667	47.667	. . . \
1	neg control	54.833	54.833	. . . . \
2	0.773	58.167	58.167	* . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.66	0.26	1.6	0.19	0.40
EC10	0.89	0.40	2.0	0.17	0.45
EC25	1.5	0.80	2.8	0.13	0.54
EC50	2.7	1.7	4.1	0.091	0.64

Slope = 2.70 Std.Err. = 0.513

!!!Poor fit: p < 0.001 based on DF= 3.00 15.0

5519CD : Cell density (x 10E+04/mL)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	54.8	58.5	-3.70	100.	0.00
0.773	3.00	58.2	54.2	3.92	92.7	7.32
1.92	3.00	47.7	38.0	9.64	65.0	35.0
5.05	3.00	6.00	13.2	-7.24	22.6	77.4
12.4	3.00	2.00	2.08	-0.0761	3.55	96.5
29.1	3.00	1.33	0.146	1.19	0.250	99.8

!!!Warning: EC5 not bracketed by doses evaluated.

Cell growth rate (0-72hours)
File: 5519gr Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	1.334	1.334	91.000
2	0.773	1.354	1.354	54.000
3	1.92	1.282	1.282	41.000
4	5.05	0.590	0.590	24.000
5	12.4	0.147	0.147	12.000
6	29.1	0.090	0.090	9.000

Calculated H Value = 16.617 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Cell growth rate (0-72hours)
File: 5519gr 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 4 3 1 2
6	29.1	0.090	0.090	\
5	12.4	0.147	0.147	. \
4	5.05	0.590	0.590	. . \
3	1.92	1.282	1.282	. . . \
1	neg control	1.334	1.334	. . . . \
2	0.773	1.354	1.354	* . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.84	0.27	2.6	0.23	0.32

EC10	1.2	0.45	3.3	0.20	0.37
EC25	2.3	1.1	4.8	0.15	0.47
EC50	4.5	2.7	7.5	0.11	0.60

Slope = 2.25 Std.Err. = 0.453

Goodness of fit: p = 0.35 based on DF= 3.0 15.

5519GR : Cell growth rate (0-72hours)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	1.33	1.39	-0.0572	100.	0.00
0.773	3.00	1.35	1.33	0.0223	95.8	4.21
1.92	3.00	1.28	1.11	0.172	79.8	20.2
5.05	3.00	0.590	0.634	-0.0443	45.6	54.4
12.4	3.00	0.147	0.224	-0.0776	16.1	83.9
29.1	3.00	0.0900	0.0474	0.0426	3.41	96.6