298

VOLUME ___OF___OF SUBMISSION
CGA 329351

STUDY TITLE

A 48-HOUR STATIC ACUTE TOXICITY TEST WITH THE CLADOCERAN, *Daphnia magna*

DATA REQUIREMENT

US EPA FIFRA GUIDELINE 72-2(a)

AUTHORS:

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STUDY COMPLETION DATE

AUGUST 21, 1995

PERFORMING LABORATORY

WILDLIFE INTERNATIONAL LTD.
8598 COMMERCE DRIVE
EASTON, MARYLAND 21601

LABORATORY STUDY IDENTIFICATION NUMBER

108A-166

SPONSOR

CIBA CROP PROTECTION
CIBA-GEIGY CORPORATION
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VOLUME 1 OF 1 OF STUDY
PAGE 1 OF 41

European Registration Dossier
Dossier File N°: 8.2.4/8
Ciba File N°: 329351/298

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The Good Laboratory Practice Compliance Statement found on Page 4, and signed by the Study Director, is truthful and accurate.

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Date: 17 Oct 95

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Ciba-Geigy Corporation

TITLE: CGA 329,351: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 108A-166

STUDY COMPLETION DATE: August 21, 1995

This study was conducted to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989; OECD, ISBN-92-84-12367-9, Paris 1982; and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau).

STUDY DIRECTOR:

5.1.2.e Woo DATE: 8/21/95

Senior Aquatic Biologist

QUALITY ASSURANCE STATEMENT

This study was examined for conformance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989; OECD, ISBN 92-84-12367-9, Paris 1982; and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). The dates of all inspections and audits and the dates that any findings were reported to the Study Director/Laboratory Management were as follows:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO STUDY DIRECTOR:	DATE REPORTED TO MANAGEMENT:
Test Substance Preparation, Test Initiation and Analytical Sampling	July 5, 1995	July 5, 1995	July 6, 1995
Analytical Sample Preparation	July 7, 1995	July 7, 1995	July 10, 1995
Biological Data and Draft Report	August 7 and 8, 1995	August 8, 1995	August 11, 1995
Analytical Data and Draft Report	July 26 - 31, 1995	August 2, 1995	August 2, 1995
Final Report	August 21, 1995	August 21, 1995	August 21, 1995

5.1.2.e Woo DATE 8-21-95

Quality Assurance Representative

REPORT APPROVAL

SPONSOR: Ciba-Geigy Corporation

TITLE: CGA 329,351: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 108A-166

STUDY DIRECTOR:

5.1.2.e Woo _____ 8/21/95
DATE

Senior Aquatic Biologist

MANAGEMENT:

5.1.2.e Woo _____ 8/21/95
DATE

Manager, Aquatic Toxicology

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SUMMARY

SPONSOR:	Ciba-Geigy Corporation
CONTACT:	Mr. 5.1.2.e Woo
LOCATION OF STUDY RAW DATA AND FINAL REPORT:	Wildlife International Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	108A-166
TEST SUBSTANCE:	CGA 329,351
STUDY:	CGA 329,351: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control; Solvent Control; 14, 23, 39, 65 and 113 mg a.i./L
TEST DATES:	Experimental Start - July 5, 1995 Biological Termination - July 7, 1995 Experimental Termination - July 7, 1995
LENGTH OF TEST:	48 Hours

TEST ORGANISM:	Neonate cladocerans (*Daphnia magna*)
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd. Cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	< 24 hours at test initiation

48-HOUR EC50:	> 113 mg a.i./L
95% CONFIDENCE LIMITS:	Not Calculable
NO MORTALITY/IMMOBILITY CONCENTRATION:	113 mg a.i./L
NOEC:	39 mg a.i./L

INTRODUCTION

This study was conducted by Wildlife International Ltd. for Ciba-Geigy Corporation at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The test was conducted from July 5, 1995 to July 7, 1995. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 108A-166 in archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of this study was to evaluate the acute toxicity of CGA 329,351 to the cladoceran, *Daphnia magna*, during a 48-hour exposure period under static test conditions.

EXPERIMENTAL DESIGN

Daphnids were exposed to a geometric series of five test concentrations, a solvent control and a negative (well water) control. Two replicate test chambers were maintained in each treatment and control group, with 10 daphnids in each test chamber for a total of 20 daphnids per test concentration. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the results of an exploratory range finding toxicity test. Nominal test concentrations selected were 16, 26, 43, 72 and 120 mg a.i./L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test and at test termination.

Daphnids were impartially assigned to exposure chambers at test initiation. Observations of mortality/immobility and other clinical signs of toxicity were made at approximately 2, 24 and 48 hours after test initiation. Cumulative percent mortality and immobility observed in the treatment groups were used to estimate EC50 values at 24 and 48 hours. The no mortali-

ty/immobility concentration and the no-observed-effect-concentration (NOEC) were determined by examination of the mortality, immobility and clinical observation data.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, CGA 329,351: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*). The protocol was based on procedures outlined in Series 72 of the *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms* (1); U.S. Environmental Protection Agency *Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Invertebrates* (2); and ASTM Standard E729-88, *Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians* (3).

Test Substance

The test substance was received from Ciba-Geigy Corporation on April 11, 1995 and was assigned Wildlife International Ltd. identification number WIL #3193 upon receipt. The test substance was a dark brown viscous liquid, identified on the label as: GLP Test Substance; Product: CGA-329351 Technical; ID No.: FL-950307; ARS-31012; AMT: 500 gram(s); Purity: 96.6%; Batch code: 501004; Storage Conditions: RT; Expiration: 16-Mar-97. The test substance had a reported purity of 98.2% and an expiration date of March 16, 1996. The test substance was stored at ambient room temperature.

Preparation of Test Concentrations

Test solutions were prepared individually for each of the five concentrations tested. Nominal concentrations were 16, 26, 43, 72 and 120 mg a.i./L. A primary stock was prepared by dissolving CGA 329,351 in dimethylformamide at a concentration of 0.24 g a.i./mL. The appropriate amount of primary stock was added to dilution water in a 2-L volumetric flask for each test concentration. The flasks were brought to volume and inverted to mix. Approximately 200-mL of test solution was distributed to the two replicates for each test concentration. The

remaining test solution in the volumetrics was discarded. The concentration of dimethylformamide in the solvent control and the 120 mg a.i./L test solution was 0.5 mL/L.

Test Organism

The cladoceran, *Daphnia magna*, was selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates and were selected for use in the test based upon past history of use and ease of culturing in the laboratory. Daphnid neonates used in the test were less than 24-hours old and were obtained from cultures maintained by Wildlife International Ltd., Easton, Maryland.

Adult daphnids were cultured in water from the same source and at approximately the same temperature as used during the test, except the culture water was supplemented with Selenium. Adult daphnids in the cultures were held for at least 22 days prior to collection of the juveniles for testing. The adults showed no signs of disease or stress during the holding period. During the 14-day period preceding the test, water temperatures ranged from 21.0 to 22.0°C. The pH of the water ranged from 8.2 to 8.5, and dissolved oxygen ranged from 7.4 to 8.8 mg/L. Instrumentation used for water measurements are described in the *Environmental Conditions* section of this report.

Neonate daphnids were obtained for testing from individual adult daphnids which were observed to have no neonates present less than 24 hours prior to test initiation. The progeny from seven adults were used in the test. At test initiation, the juvenile daphnids were collected from the cultures using a wide-bore, disposable pipet and indiscriminately transferred to 10-mL glass beakers. The daphnids then were transferred from the beakers to the test compartments using a wide-bore pipet and released beneath the air/water interface. Daphnids in the cultures were fed a mixture of yeast, Cerophyll®, and trout chow, as well as a suspension of the freshwater green alga, *Selenastrum capricornutum*. The adults were fed prior to test initiation, but neonates were not fed during the test.

Test Apparatus

Test chambers were 250-mL glass beakers containing approximately 200 mL of test solution. The depth of water in each test chamber was approximately 6.6 cm. Test chambers were impartially positioned in a temperature-controlled water bath designed to maintain a temperature of 20±1°C. Test chambers were labelled with the project number, test concentration and replicate.

Dilution Water

The water used for culturing and testing was freshwater obtained from a well 45 meters deep located on the Wildlife International Ltd. site. The well water is characterized as moderately hard. The specific conductance, hardness, alkalinity, and pH of the well water during the four-week period immediately preceding the test are presented in Appendix I.

The well water was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to use in the test, the water again was filtered to remove microorganisms and particles. The results of periodic analysis performed to measure the concentrations of selected contaminants in well water used by Wildlife International Ltd. are presented in Appendix II.

Environmental Conditions

Lighting used to illuminate the cultures and test systems during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity during the test was approximately 313 lux at the surface of the water.

Temperature was measured in each test chamber at the beginning and end of the test using a calibrated, hand-held thermometer. Temperature also was measured continuously in a beaker adjacent to the test using a Fulscope ER/C Recorder. The target test temperature during the study was 20±1°C. The pH and dissolved oxygen content of the water were measured in alternate replicates of each treatment and control at test initiation, at approximately 24 hours after test initiation and at the end of the test. Hardness, alkalinity and specific conductance were measured in the dilution water at test initiation.

Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Specific conductance was measured using a Yellow Springs Instruments Model 33 Salinity-Conductivity-Temperature meter. Hardness and alkalinity measurements were made by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (4).

Observations

All organisms were observed to determine the numbers of mortalities and immobile individuals. The numbers of individuals exhibiting clinical signs of toxicity or abnormal behavior also were evaluated. Observations were made approximately 2, 24, and 48 hours after test initiation.

Statistical Analyses

The 24 and 48-hour EC50 were estimated by visual inspection of the mortality and immobility data. The no mortality/immobility concentration and NOEC were determined by inspection of the mortality, immobility and clinical observation data.

Analytical Chemistry

Samples were collected from each replicate test chamber of each treatment and control group at the beginning of the test and at test termination to measure concentrations of the test substance. Samples collected from the A replicates at test initiation and the B replicates at test termination were analyzed. Samples collected from the B replicates at test initiation and A replicates at test termination were held as backups and were not analyzed. The samples were collected in glass scintillation vials and analyzed as soon as possible without storage. Analytical procedures used in the analysis of the samples are provided in Appendix III.

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Results of analyses to measure concentrations of CGA 329,351 in water samples collected during the test are presented in Table 1 and in the analytical chemistry report (Appendix III). Nominal concentrations selected for use in this study were 16, 26, 43, 72 and 120 mg a.i./L. Samples collected on Day 0 had measured values that ranged from 88 to 94% of nominal. Measured values for samples taken at 48 hours ranged from 81 to 93% of nominal. When measured concentrations of the samples analyzed at test initiation and at test termination were averaged, the mean measured concentrations for this study were 14, 23, 39, 65 and 113 mg a.i./L. Mean measured concentrations were used in the estimations of EC50 values.

Observations and Measurements

Measurements of temperature, dissolved oxygen and pH are presented in Table 2. Temperatures in all treatments and controls were within the range established for the test, or 20±1°C. Dissolved oxygen concentrations exceeded 60% of saturation throughout the test. Measurements of pH ranged from 8.1 to 8.4 during the test. Measurements of hardness, alkalinity and specific conductance are also presented in Table 2.

Daily observations of mortality, immobility and other clinical signs of toxicity observed during the test are shown in Table 3. Daphnids in the negative control and solvent control appeared normal and healthy during the test. No mortality or immobility was observed in any treatment group. Consequently, the 48-hour EC50 value was >113 mg a.i./L (Table 4) and the no mortality/immobility concentration was 113 mg a.i./L, the highest concentration tested. Several daphnids exposed to 65 and 113 mg a.i./L were observed as lethargic. Based on this lethargy, the NOEC was considered to be 39 mg a.i./L.

CONCLUSIONS

The 48-hour EC50 value for daphnids exposed to CGA 329,351 was >113 mg a.i./L. The no mortality/immobility concentration was 113 mg a.i./L, the highest concentration tested. The NOEC, determined by visual interpretation of the clinical observation data, was 39 mg a.i./L.

REFERENCES

1 U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms.*

2 U.S. Environmental Protection Agency. 1985. *Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Invertebrates.* Hazard Evaluation Division. Office of Pesticide Programs. EPA 540/9-85-005. Washington, D.C.

3 American Society for Testing and Materials. 1988. *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians,* ASTM E729-88. Philadelphia, PA.

4 APHA, AWWA, WPCF, *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, 1985. American Public Health Association, Washington, D.C.

Table 1

Summary of Analytical Chemistry Data

Sponsor: Ciba-Geigy Corporation
Test Substance: CGA 329,351
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Nominal Test Concentration (mg a.i./L)	Replicate	Sampling Time (hrs)	Measured Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)	Percent of Nominal
Negative Control	A	0	< LOQ[1]	< LOQ	--
	B	48	< LOQ		
Solvent Control	A	0	< LOQ	< LOQ	--
	B	48	< LOQ		
16	A	0	14	14	88
	B	48	13		
26	A	0	23	23	88
	B	48	23		
43	A	0	39	39	91
	B	48	39		
72	A	0	66	65	90
	B	48	64		
120	A	0	113	113	94
	B	48	112		

[1] The limit of quantitation (LOQ) was based upon the lowest matrix fortification level, 5.0 mg a.i./L, analyzed concurrently with the samples.

Table 2

Temperature, Dissolved Oxygen and pH of Water in the Test Chambers

Sponsor: Ciba-Geigy Corporation
Test Substance: CGA 329,351
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Concentration (mg a.i./L)	Replicate	0 Hours[1] Temp[2] (°C)	0 Hours[1] DO[3] (mg/L)	0 Hours[1] pH	24 Hours DO (mg/L)	24 Hours pH	48 Hours Temp (°C)	48 Hours DO (mg/L)	48 Hours pH
Negative Control	A	20.0	8.2	8.1	--	--	20.0	8.3	8.3
	B	19.8	--	--	8.1	8.3	20.0	--	--
Solvent Control	A	19.9	8.1	8.1	--	--	20.0	8.3	8.3
	B	20.0	--	--	8.1	8.3	20.0	--	--
14	A	19.9	8.0	8.1	--	--	20.0	8.4	8.4
	B	20.2	--	--	8.1	8.3	20.0	--	--
23	A	20.3	7.9	8.1	--	--	20.0	8.5	8.4
	B	20.2	--	--	8.1	8.3	20.0	--	--
39	A	20.3	7.7	8.1	--	--	20.1	8.3	8.4
	B	20.3	--	--	8.1	8.3	20.1	--	--
65	A	20.5	7.9	8.1	--	--	20.1	8.4	8.4
	B	20.5	--	--	8.1	8.3	20.1	--	--
113	A	20.6	7.5	8.1	--	--	20.3	8.3	8.4
	B	20.6	--	--	8.1	8.3	20.3	--	--

[1] The 0-hour dilution water measurements for hardness, alkalinity and specific conductance were 132 mg/L as $CaCO_3$, 188 mg/L as $CaCO_3$ and 330 µmhos/cm, respectively.
[2] Temperature measured continuously during the test ranged from approximately 19.5 to 20.5°C.
[3] A dissolved oxygen concentration of 5.4 mg/L represents 60% saturation at 20°C in freshwater.

Table 3
Cumulative Percent Mortality/Immobility and Treatment-Related Effects[1]

Sponsor: Ciba-Geigy Corporation
Test Substance: CGA 329,351
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Mean Measured Concentration (mg a.i./L)	Replicate	Daphnia/ Replicate	2 Hours			24 Hours			48 Hours			Percent Immobile and Dead
			Number Dead	Number Immobile	Effects	Number Dead	Number Immobile	Effects	Number Dead	Number Immobile	Effects	
Negative Control	A	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	
	B	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	0%
Solvent Control	A	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	
	B	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	0%
14	A	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	
	B	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	0%
23	A	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	
	B	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	0%
39	A	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	
	B	10	0	0	10 AN	0	0	10 AN	0	0	10 AN	0%
65	A	10	0	0	10 AN	0	0	10 AN	0	0	7AN; 3C	
	B	10	0	0	10 AN	0	0	7AN; 3C	0	0	7AN; 3C	0%
113	A	10	0	0	10 AN	0	0	5AN; 5C	0	0	4AN; 6C	0%
	B	10	0	0	10 AN	0	0	3AN; 7C	0	0	3AN; 7C	

[1] Observed Effects: AN = Appears Normal; C = Lethargic

Table 4

EC50 Values

Sponsor:	Ciba-Geigy Corporation
Test Substance:	CGA 329,351
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

Time	EC50 (mg a.i./L)	Lower 95% Confidence Limits	Upper 95% Confidence Limits	Statistical Method
24 Hours	> 113	--[1]	--[1]	Visual Inspection
48 Hours	> 113	--[1]	--[1]	Visual Inspection

[1] Confidence limits could not be calculated with the mortality data obtained.

APPENDIX I

Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured During the 4-Week Period Immediately Preceding the Test

Sponsor: Ciba-Geigy Corporation
Test Substance: CGA 329,351
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

	Mean	Range
Specific Conductance (µmhos/cm)	323 (N = 4)	320 - 330
Hardness (mg/L as $CaCO_3$)	133 (N = 4)	128 - 136
Alkalinity (mg/L as $CaCO_3$)	178 (N = 4)	172 - 182
pH	8.2 (N = 4)	8.1 - 8.2

APPENDIX II

Analyses of Pesticides, Organics, Metals and Other Inorganics Analyzed in Wildlife International Ltd. Well Water[1]

Sponsor:	Ciba-Geigy Corporation
Test Substance:	CGA 329,351
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

ANALYSIS	MEASURED CONCENTRATION	
Organophosphorus & Organonitrogen Pesticides		
Azodrin (Monochrotophos)	< 2.50	µg/L
Bolstar	< 0.266	µg/L
Chlorpyrifos	< 0.267	µg/L
Coumaphos	< 0.500	µg/L
Demeton	< 0.265	µg/L
Diazinon	< 0.265	µg/L
Dichlorvos	< 0.260	µg/L
Dimethoate	< 0.250	µg/L
Disulfoton	< 0.255	µg/L
EPN	< 0.500	µg/L
Ethoprop	< 0.275	µg/L
Fenthion	< 0.252	µg/L
Fensulfothion	< 0.512	µg/L
Guthion (Methyl Azinphos)	< 0.500	µg/L
Malathion	< 0.270	µg/L
Merphos	< 0.246	µg/L
Mevinphos	< 0.255	µg/L
Naled	< 1.34	µg/L
Methylparathion	< 0.250	µg/L
Parathion	< 0.288	µg/L
Phorate	< 0.242	µg/L
Ronnel	< 0.257	µg/L
Stirofos	< 0.500	µg/L
Sulfotepp	< 0.260	µg/L
Tepp	< 1.04	µg/L
Tokuthion	< 0.276	µg/L
Trichloronate	< 0.265	µg/L
Metals and Other Inorganics		
Aluminum	< 50.0	µg/L
Arsenic	< 2.5	µg/L
Beryllium	< 5.0	µg/L
Cadmium	< 5.0	µg/L
Calcium	32800	µg/L
Chromium	< 10.0	µg/L
Copper	< 5.0	µg/L
Iron	< 45.0	µg/L
Lead	< 2.0	µg/L
Magnesium	13.1	mg/L
Manganese	< 5.0	µg/L
Nickel	< 15.0	µg/L
Potassium	6730	µg/L
Selenium	< 2.5	µg/L
Silver	< 5.0	µg/L
Sodium	21200	µg/L
Zinc	< 30.0	µg/L
Mercury	< 0.20	µg/L
Molybdenum	< 10.0	µg/L

[1] Analyses performed by Environmental Science & Engineering, Inc., Gainesville, Florida for samples collected on May 10, 1994.

APPENDIX II (Continued)
Analyses of Pesticides, Organics, Metals and Other Inorganics
Analyzed in Wildlife International Ltd. Well Water[1]

Sponsor:	Ciba-Geigy Corporation
Test Substance:	CGA 329,351
Test Organism:	Cladoceran, *Daphnia magna*
Dilution Water:	Well Water

ANALYSIS	MEASURED CONCENTRATION
Miscellaneous Measurements	
Total Dissolved Solids	248 mg/L
Ammonia Nitrogen	< 0.050 mg/L
Total Organic Carbon[2]	< 0.5 mg/L
Total Cyanide	< 0.003 mg/L
Organochlorines and PCBs	
Aldrin	< 0.005 µg/L
Alpha BHC	< 0.005 µg/L
Beta BHC	< 0.005 µg/L
Delta BHC	< 0.005 µg/L
Gamma BHC (Lindane)	< 0.005 µg/L
Chlordane	< 0.025 µg/L
DDD, pp'	< 0.005 µg/L
DDE, pp'	< 0.005 µg/L
DDT, pp'	< 0.005 µg/L
Dieldrin	< 0.005 µg/L
Endosulfan, A	< 0.005 µg/L
Endosulfan, B	< 0.005 µg/L
Endosulfan Sulfate	< 0.005 µg/L
Endrin	< 0.005 µg/L
Endrin Aldehyde	< 0.005 µg/L
Heptachlor	< 0.005 µg/L
Methoxychlor	< 0.005 µg/L
Heptachlor Epoxide	< 0.005 µg/L
Toxaphene	< 0.500 µg/L
PCB-1016	< 0.100 µg/L
PCB-1221	< 0.100 µg/L
PCB-1232	< 0.100 µg/L
PCB-1242	< 0.100 µg/L
PCB-1248	< 0.100 µg/L
PCB-1254	< 0.100 µg/L
PCB-1260	< 0.100 µg/L
Chlorophenoxy Acid Herbicides	
2,4-D, Total	< 0.020 µg/L
2,4-DB	< 0.020 µg/L
2,4,5-T Water	< 0.020 µg/L
2,4,5-TP/Silvex	< 0.020 µg/L
Dalapon	< 0.020 µg/L
Dicamba (Banvel)	< 0.020 µg/L
Dichloroprop	< 0.020 µg/L
Dinoseb	< 0.020 µg/L
MCPA	< 0.410 µg/L
MCPP	< 0.400 µg/L

[1] Analyses performed by Environmental Science & Engineering, Inc., Gainesville, Florida for samples collected on May 10, 1994.
[2] Analyses performed by Wildlife International Ltd. for the sample collected on May 27, 1994.

APPENDIX III

THE ANALYSIS OF CGA-329,351 IN FRESHWATER

IN SUPPORT OF

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108A-166

APPENDIX III

Introduction

Water samples were collected from an acute aquatic toxicity study to determine the effects of CGA-329,351 on the cladoceran (*Daphnia magna*). The study was conducted by Wildlife International Ltd. and identified as WIL Project No.: 108A-166. The analyses of these water samples were performed at Wildlife International Ltd. by high performance liquid chromatography (HPLC) with UV detection. Samples were received for analysis on July 5, 1995 and July 7, 1995 and analyzed between July 5 and July 7, 1995.

Test Substance and Analytical Standard

The test substance, received from Ciba-Geigy Corporation on April 11, 1995, was used to prepare matrix fortification samples. The test substance was a dark brown viscous liquid, identified on the label as: GLP Test Substance; Product: CGA-329351 Technical; ID No.: FL-950307; ARS-31012; AMT: 500 gram(s); Purity: 96.6%; Batch code: 501004; Storage Conditions: RT; Expiration: 16-Mar-97. The test substance had a reported purity of 98.2% and an expiration date of March 16, 1997. Upon receipt, the test substance was assigned Wildlife International Ltd. Identification Number WIL #3193 and stored under ambient conditions.

The analytical standard, received from Ciba-Geigy Corporation on April 20, 1995, was used to prepare calibration standards. The analytical standard was a clear viscous liquid, identified on the label as: COMPOUND - CGA-329,351; PURITY 99.4%; CODE S95-1785; AMOUNT 300 mg; STORAGE: Freezer; DISPENSED 4/18/95JS; REASSAY 11/96. The analytical standard had a reported purity of 99.4% and an expiration date of November, 1996. Upon receipt, the analytical standard was assigned Wildlife International Ltd. Identification Number WIL #3203 and stored under freezer (approximately -14°C) conditions.

APPENDIX III

Analytical Method

The method used for the analysis of the water samples was based upon methodology provided by Ciba-Geigy Corporation and entitled Determination of Residues of Parent Compound and Metalaxyl Acid (CGA 62 826) by High Performance Liquid Chromatography (HPLC).

The analytical method used for the analysis of the samples consisted of diluting the samples as appropriate with 65:35:0.2 (water:acetonitrile:phosphoric acid). Concentrations of CGA-329,351 in dilutions of the samples were determined by high performance liquid chromatography using a Hewlett-Packard Model 1090 High Performance Liquid Chromatograph (HPLC) equipped with a UV detector. HPLC separations were achieved using a Supelco Hi-Sep Column (15 cm x 4.6 mm ID, 5 µm particle size). The instrument parameters are summarized in Table 1. A method flow chart is provided in Figure 1.

Calibration Curve, Limit of Detection and Limit of Quantitation

Calibration standards of CGA-329,351, ranging in concentration from 1.00 to 20.0 µg a.i./mL, were analyzed with each series of samples. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. An example of a calibration curve is presented in Figure 2. The concentration of test substance in the samples was determined by substituting the area responses into the applicable linear regression equation. Representative chromatograms of a low and high calibration standard are shown in Figures 3 and 4.

The instrument limit of detection (LOD) was set based upon the injection volume (75 µL) and the lowest standard concentration (1.00 µg a.i./mL). The LOD was set at 75 ng injected on column. The method limit of quantitation (LOQ) for these analyses was set at 5.00 mg a.i./L based upon the lowest matrix fortification level analyzed concurrently with the samples.

APPENDIX III

Matrix Blank and Fortification Samples

Along with the actual sample analyses, two matrix blanks were analyzed to determine possible interference. No interferences were observed at or above the LOQ during the sample analyses (Table 2). A representative chromatogram of a matrix blank is presented in Figure 5.

Freshwater samples were fortified at 5.00, 50.0 and 140 mg a.i./L and analyzed concurrently with the samples to determine the mean procedural recovery (Table 3). Measured concentrations for the samples were corrected for the mean procedural recovery of 101%. A representative chromatogram of a matrix fortification is presented in Figure 6.

RESULTS

Sample Analysis

Water samples were collected from the acute toxicity study with the cladoceran (*Daphnia magna*) at test initiation (Day 0), July 5, 1995, and at test termination (48 hours) on July 7, 1995. The measured concentrations of CGA-329,351 in the samples collected at initiation of exposure of the test organisms (Day 0) ranged from 88 to 94% of the nominal concentrations (Table 4). Samples collected at 48 hours (test termination) had measured concentration ranges of 81 to 93% of nominal values. A representative chromatogram of a sample is shown in Figure 7.

APPENDIX III

Table I

Typical HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1090 High Performance Liquid Chromatograph with Diode Array Detector (DAD)
ANALYTICAL COLUMN:	Supelco Hi-Sep Column (15 cm X 4.6 mm ID, 5 µm particle size)
STOP TIME:	10.0 minutes
POST TIME:	0.0 minutes
FLOW RATE:	1.0 mL/min.
OVEN TEMPERATURE:	40°C
SOLVENT A:	100% H_2O:CH_3CN:H_3PO_4 (65:35:0.2)
INJECTION VOLUME:	75 µL
CGA-329,351 PEAK RETENTION TIME:	Approximately 2 minutes
PRIMARY ANALYTICAL WAVELENGTH:	210 nm

APPENDIX III

Table 2

Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample		Measured Concentration of CGA-329,351 (mg a.i./L)[1]
Number (108A-166)	Type	
MAB-1	Matrix Blank	<5.00
MAB-2	Matrix Blank	<5.00

[1] The limit of quantitation (LOQ) was based upon the lowest matrix fortification level (5.00 mg a.i./L) analyzed concurrently with samples from the test.

APPENDIX III

Table 3

Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (108A-166)	Concentrations of CGA-329.351 (mg a.i./L) Fortified	Measured	Percent Recovered
MAS-1	5.00	5.05	101
MAS-4	5.00	5.06	101
MAS-2	50.0	49.8	100
MAS-5	50.0	50.4	101
MAS-3	140	141	101
MAS-6	140	143	102
		Mean =	101%
		Standard Deviation =	0.63
		N =	6

APPENDIX III

Table 4

Measured Concentrations of CGA-329,351 in Freshwater Samples from a Daphnia Acute Toxicity Test

Nominal Concentration (mg a.i./L)	Sample Number (108A-166-)	Sampling Time (Hours)	CGA-329,351 Concentration		Percent of Nominal
			Measured[1] (mg a.i./L)	Corrected[2] (mg a.i./L)	
0.0	1	0	<5.00	<5.00	--
(Negative Control)	16	48	<5.00	<5.00	--
0.0	3	0	<5.00	<5.00	--
(Solvent Control)	18	48	<5.00	<5.00	--
16	5	0	13.8	14	88
	20	48	13.3	13	81
26	7	0	23.7	23	88
	22	48	23.3	23	88
43	9	0	39.1	39	91
	24	48	39.4	39	91
72	11	0	66.7	66	92
	26	48	65.0	64	89
120	13	0	114	113	94
	28	48	113	112	93

[1] The limit of quantitation (LOQ) was based upon the lowest matrix fortification level (5.00 mg a.i./L) analyzed concurrently with the test samples.
[2] Values were corrected for a mean procedural recovery of 101%.

APPENDIX III

FLOW CHART FOR THE ANALYSIS OF CGA-329,351 IN FRESHWATER

Prepare matrix fortifications as appropriate by adding known amounts of CGA-329,351 to the matrix to achieve desired concentrations. Prepare matrix blanks as appropriate.

↓

Dilute the samples, matrix fortifications and matrix blanks as needed with 63:35:0.2 (H_2O:CH_3CN:H_3PO_4) to achieve target concentrations which fall within the linear portion of the calibration curve.

↓

Ampulate and analyze by HPLC-UV at 210 λ.

Figure 1. Analytical method flow chart for the analysis of CGA-329,351 in freshwater.

APPENDIX III

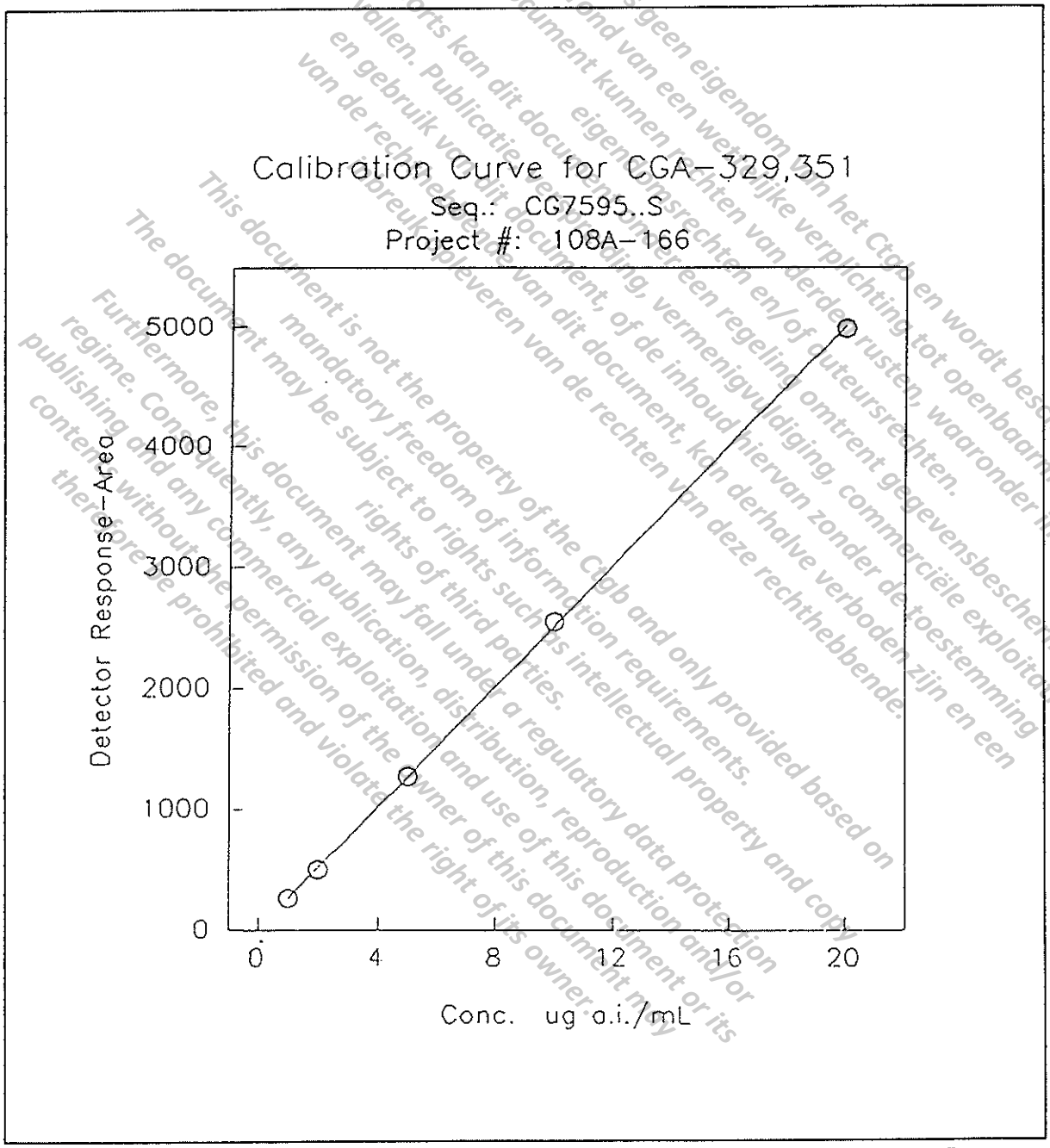

Figure 2. Representative calibration curve for CGA-329,351. Slope = 249.4220; Intercept = 17.6607.

APPENDIX III

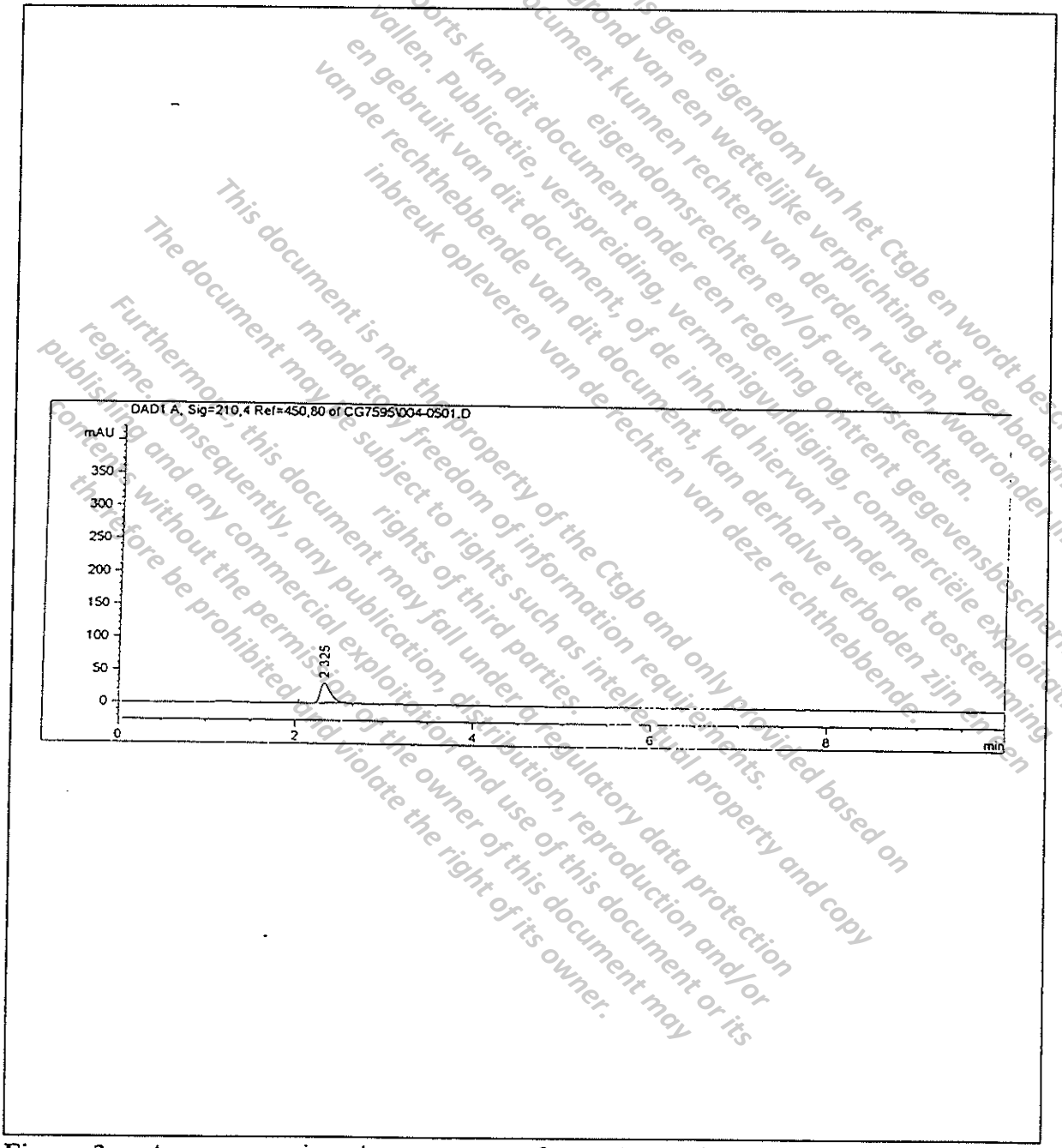

Figure 3. A representative chromatogram of a 1.00 µg a.i./mL CGA-329,351 standard (75 ng on column).

APPENDIX III

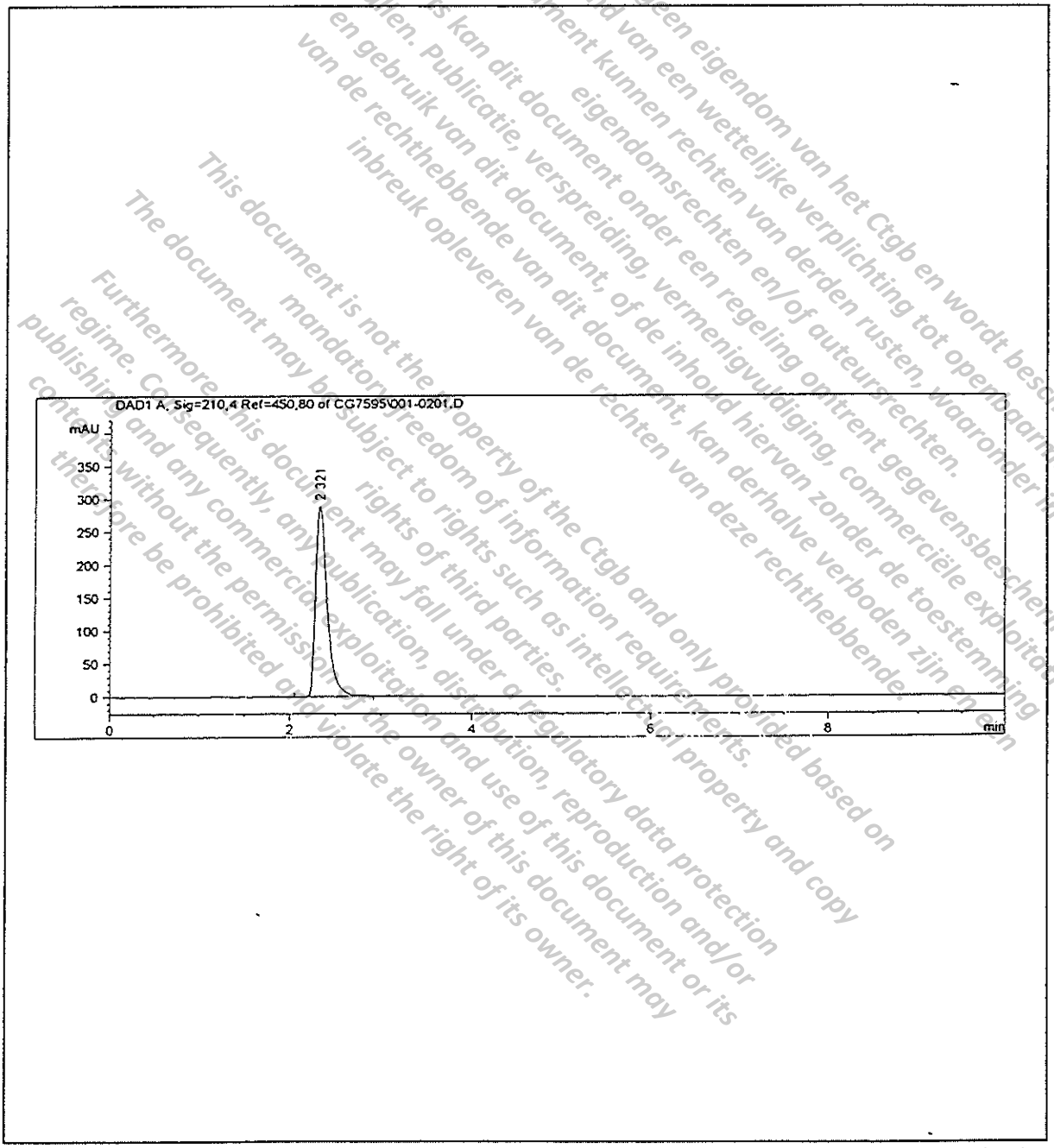

Figure 4. A representative chromatogram of a 10.0 µg a.i./mL CGA-329,351 standard.

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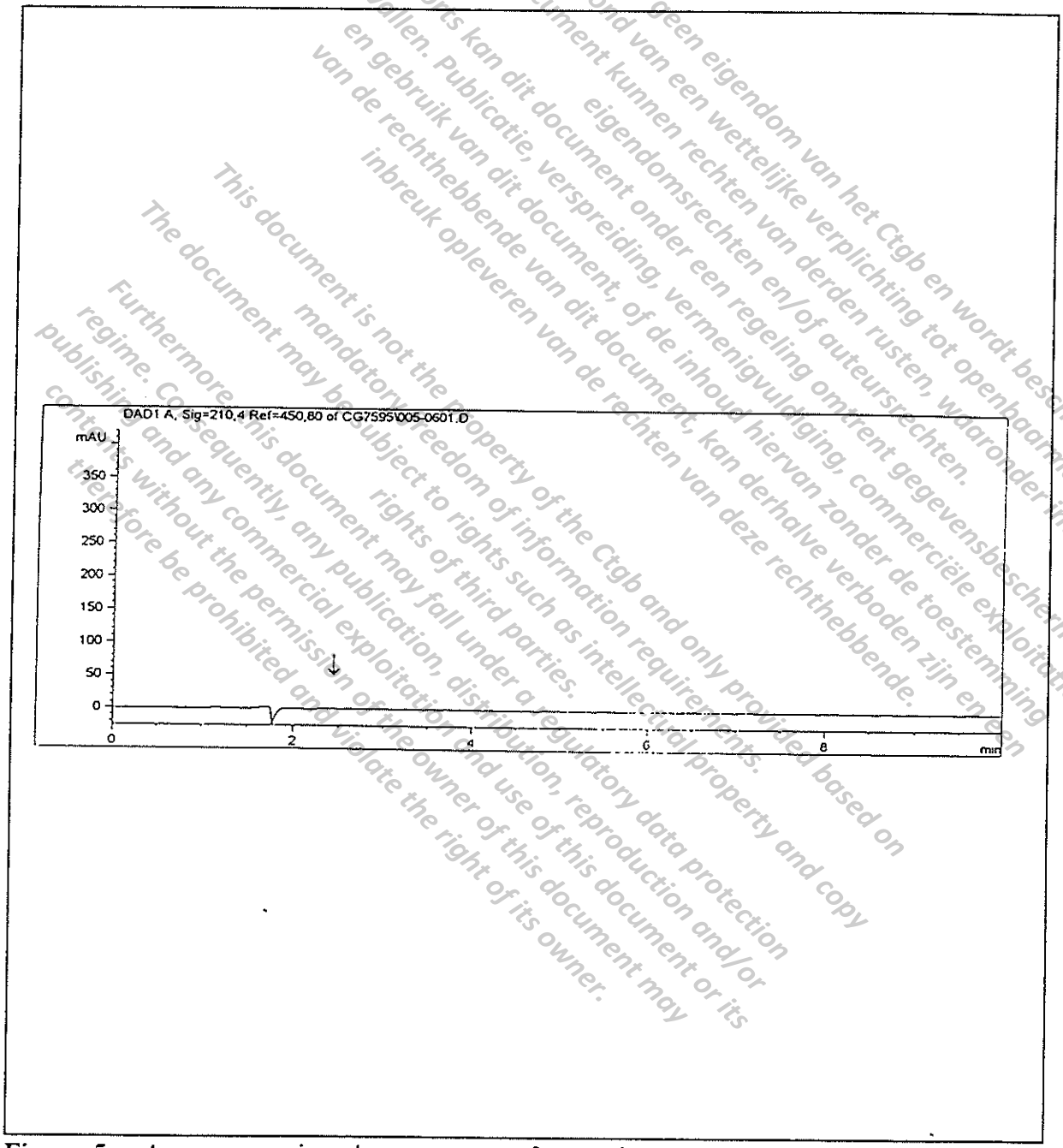

Figure 5. A representative chromatogram of a matrix blank, 108A-166-MAB-1.

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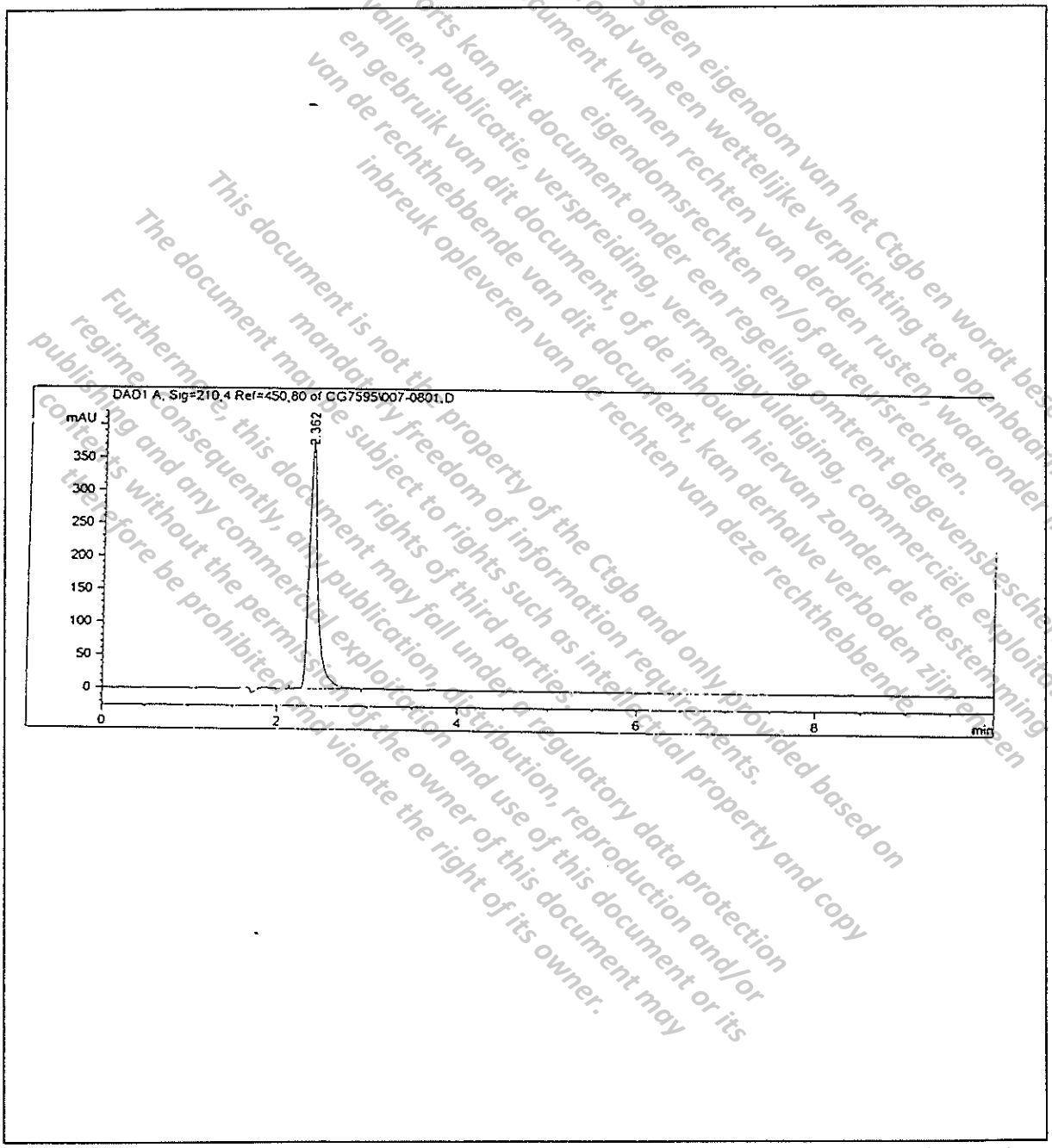

Figure 6. A representative chromatogram of a matrix fortification, 108A-166-MAS-2, 50.0 mg a.i./L.

APPENDIX III

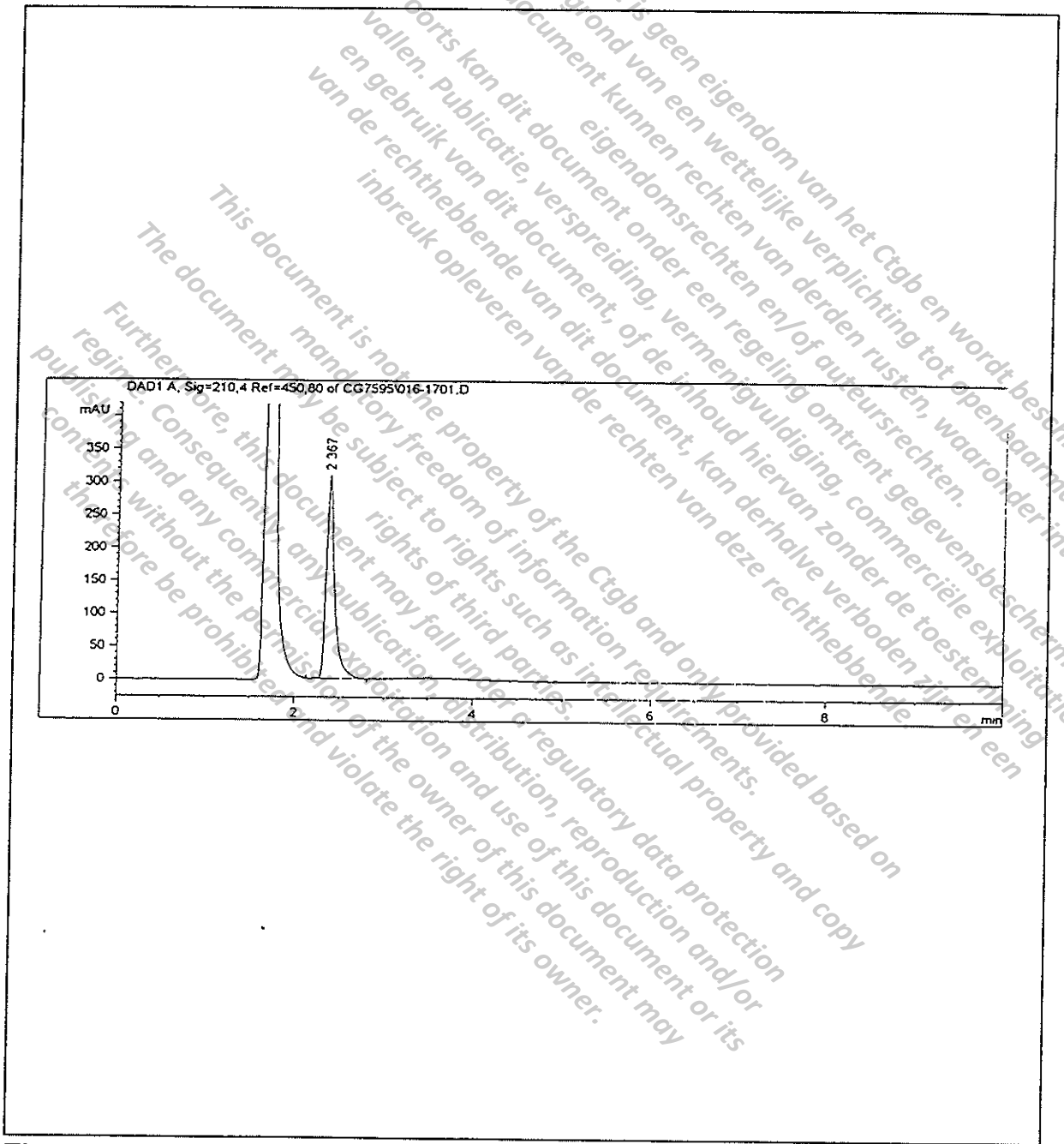

Figure 7. A representative chromatogram of a sample on Day 0, 108A-166-9, 43 mg a.i./L nominal concentration.

APPENDIX IV

Changes to Protocol

This study was conducted in accordance with the approved Protocol with the following changes:

1. The protocol was amended to add the proposed experimental start and termination dates, study room and test concentrations.

2. The protocol was amended to change the frequency of water and feed analyses.

In the opinion of the Study Director, the above changes in the approved Protocol did not adversely affect the results of this study.

APPENDIX V

Personnel Involved in the Study

The following key personnel were involved in the conduct or management of this study:

1. [redacted] Ph.D., Manager, Aquatic Toxicology
2. [redacted], Senior Aquatic Biologist
3. [redacted], Senior Aquatic Biologist
4. [redacted] Aquatic Biologist
5. [redacted], Aquatic Biologist
6. [redacted] Scientist