VOLUME ___OF___OF SUBMISSION
CGA 329351

STUDY TITLE

A 96-HOUR STATIC ACUTE TOXICITY TEST WITH THE RAINBOW TROUT, *Oncorhynchus mykiss*

DATA REQUIREMENT

US EPA FIFRA GUIDELINE 72-1(c)

AUTHORS:

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STUDY COMPLETION DATE

SEPTEMBER 15, 1995

PERFORMING LABORATORY

WILDLIFE INTERNATIONAL LTD.
8598 COMMERCE DRIVE
EASTON, MARYLAND 21601

LABORATORY STUDY IDENTIFICATION NUMBER

108A-164

SPONSOR

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VOLUME 1 OF 1 OF STUDY
PAGE 1 OF 42

European Registration Dossier
Dossier File N°: 8.2.1/7
Ciba File N°: 329351/299

STATEMENT OF <u>NO</u> DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of FIFRA Section 10(d) (1) (A), (B), or (C).

Company: Ciba-Geigy Corporation

Company Agent: 5.1.2.e Woo Ph.D.

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Signature:

Date: 10/18/95

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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.

Submitter: 5.1.2.e Wco

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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 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 108A-164

STUDY COMPLETION DATE: September 14, 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: 9/15/95

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 and Laboratory Management were as follows:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO STUDY DIRECTOR:	DATE REPORTED TO MANAGEMENT:
Test Substance Preparation	July 20, 1995	July 20, 1995	July 25, 1995
Water Chemistry Measurements	July 21, 1995	July 25, 1995	July 26, 1995
Instrument Set-Up	July 24, 1995	July 27, 1995	July 28, 1995
Analytical Sample Collection	July 24, 1995	July 24, 1995	August 3, 1995
Biological Data and Draft Report	August 15 - 17, 1995	August 18, 1995	August 23, 1995
Analytical Data and Draft Report	August 17 - 18, 1995	August 23, 1995	August 23, 1995
Final Report	September 15, 1995	September 15, 1995	September 15, 1995

DATE 9-15-95

Senior Quality Assurance Representative

REPORT APPROVAL

SPONSOR: Ciba-Geigy Corporation

TITLE: CGA 329,351: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 108A-164

STUDY DIRECTOR:

5.1.2.e Woo 9/15/95

5.1.2.e Woo DATE
Senior Aquatic Biologist

MANAGEMENT:

5.1.2.e Woo 9/15/95

5.1.2.e Woo Ph.D. DATE
Manager, Aquatic Toxicology

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SUMMARY

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

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	108A-164
TEST SUBSTANCE:	CGA 329,351
STUDY:	CGA 329,351: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control; Solvent Control; 15, 26, 42, 72 and 121 mg a.i./L
TEST DATES:	Experimental Start - July 20, 1995 Biological Termination - July 24, 1995 Experimental Termination - July 24, 1995
LENGTH OF TEST:	96 Hours

TEST ORGANISM:	Rainbow Trout (*Oncorhynchus mykiss*)	
SOURCE OF TEST ORGANISMS:	Troutlodge, Inc. P.O. Box 11 Sumner, WA 98352	
AGE OF TEST ORGANISMS:	Juveniles	
MEASUREMENTS OF 10 NEGATIVE CONTROL FISH:		
WEIGHT (g):	Mean = 0.17;	Range = 0.15 to 0.20
STANDARD LENGTH (mm):	Mean = 24;	Range = 22 to 25

96-HOUR LC50:	> 121 mg a.i./L
95% CONFIDENCE LIMITS:	Not Calculable
NO MORTALITY CONCENTRATION:	121 mg a.i./L
NOEC:	72 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 20, 1995 to July 24, 1995. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 108A-164 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 rainbow trout, *Oncorhynchus mykiss*, during a 96-hour exposure period under static test conditions.

EXPERIMENTAL DESIGN

Rainbow trout 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 rainbow trout in each test chamber for a total of 20 rainbow trout 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 active ingredient (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.

Rainbow trout were impartially assigned to exposure chambers at test initiation. Observations of mortality and other clinical signs of toxicity were made at approximately 3, 24, 48, 72 and 96 hours after test initiation. Cumulative percent mortality observed in the treatment groups were used to estimate LC50 values at 24, 48, 72 and 96 hours. The no mortality

concentration and the no-observed-effect-concentration (NOEC) were determined by visual interpretation of the mortality and clinical observation data.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, CGA 329,351: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). 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 Fish* (2); and ASTM Standard E729-88a, *Standard Guide 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

Nominal test concentrations were 16, 26, 43, 72 and 120 mg a.i./L. A primary stock was prepared by dissolving CGA 329,351 in dimethylformamide (DMF) at a concentration of 0.240 g a.i./mL. The primary stock was inverted to mix. Four secondary stock solutions were prepared by serial dilutions of the primary stock using DMF. The concentrations of the four secondary stock solutions were 0.144, 0.0864, 0.0518 and 0.0311 g a.i./L. The test concentrations were prepared by adding 7.5 mL of the appropriate stock to 15 L of dilution water in the test chambers. The solvent control was prepared by adding 7.5 ml of DMF only to 15 L of dilution

water. The test solutions were then mixed for approximately one minute using a glass stir rod. The concentration of DMF in the solvent control and all treatment groups was 0.5 mL/L.

Test Organism

The rainbow trout, *Oncorhynchus mykiss*, was selected as the test species for this study. The rainbow trout is representative of an important group of aquatic vertebrates and was selected for use in the test based upon past history of use and ease of holding in the laboratory. Rainbow trout used in the test originated as eyed eggs from Troutlodge, Inc. Sumner, WA and were held in the culture facility of Wildlife International Ltd until they achieved the proper size for testing. Identification of the species was verified by the supplier of the original culture stock.

The rainbow trout were held at approximately the same temperature as used during the test. The fish were held for approximately 24 days prior to the testing. The fish were acclimated to test conditions for approximately 49 hours prior to test initiation. During the holding and acclimation periods, the fish showed no signs of disease or stress. During the 14-day holding period preceding the test, water temperatures ranged from 11.8 to 12.2°C. The pH of the water ranged from 8.0 to 8.4 and dissolved oxygen ranged from 8.8 to 9.8 mg/L. Instrumentation and procedures used for water measurements are described in the *Environmental Conditions* section of this report. At test initiation, the rainbow trout were collected from the acclimation tank and transferred to the test chambers.

During the holding period, the rainbow trout were fed a commercially-prepared diet supplied by Zeigler Brothers, Inc., Gardners, PA. The fish were not fed during the acclimation period (at least 48 hours prior to the test) or during the test.

All fish used in the test were from the same source and year class, and the standard length of the longest fish was no more than twice the length of the shortest. The average length of 10 negative control fish measured at the end of the test was 24 mm with a range of 22 to 25 mm. The average wet weight (blotted dry) of 10 negative control fish at the end of the test was 0.17 g

with a range of 0.15 to 0.20 g. Instantaneous loading was 0.12 g fish/L of test water present in the test chambers.

Test Apparatus

Test chambers were 38-L glass aquaria containing approximately 15 L of test solution. The depth of water in each test chamber was approximately 12 cm. Test chambers were impartially positioned in an environmental chamber set to maintain a temperature of 12±1°C. Test chambers were labelled with the project number, test concentration and replicate.

Dilution Water

The water used for holding, acclimation 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 water. 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, 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 holding, acclimation 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 measured prior to the test was approximately 845 lux at the surface of the water.

Temperature was measured in each test chamber at the beginning and end of the test using a hand-held thermometer. Temperature also was measured continuously in the negative control replicate A test chamber using a Fulscope ER/C Recorder. The target test temperature during the study was 12±1°C. The pH and dissolved oxygen content of the water were measured in alternate replicates of each treatment and control at test initiation, and daily thereafter. 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 Instrument 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. The numbers of individuals exhibiting clinical signs of toxicity or abnormal behavior also were evaluated. Observations were made approximately 3, 24, 48, 72 and 96 hours after test initiation.

Statistical Analyses

The 24, 48, 72 and 96-hour LC50 values were estimated by visual inspection of the mortality data. The no mortality concentration and NOEC were determined by visual interpretation of the mortality and clinical observation data.

Analytical Chemistry

Water 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 94 to 102% of nominal. Measured values for samples taken at test termination ranged from 94 to 100% 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 15, 26, 42, 72 and 121 mg a.i./L. Mean measured concentrations were used in the estimations of LC50 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 12±1°C. Dissolved oxygen concentrations exceeded 60% of saturation throughout the test. Measurements of pH ranged from 8.4 to 8.5 during the test. Measurements of hardness, alkalinity and specific conductance are also presented in Table 2.

Daily observations of mortality and other clinical signs of toxicity observed during the test are shown in Table 3. Rainbow trout in the negative control and solvent control appeared normal and healthy during the test; although one fish in the negative control was observed to be discolored. No mortality was observed in any treatment group. Consequently, the 96-hour LC50 was >121 mg a.i./L (Table 4) and the no mortality concentration was 121 mg a.i./L, the highest concentration tested. Several rainbow trout exposed to 121 mg a.i./L appeared darker than the control fish. Based on this discoloration, the NOEC was considered to be 72 mg a.i./L.

CONCLUSIONS

The 96-hour LC50 value for rainbow trout exposed to CGA 329,351 was >121 mg a.i./L. The no mortality concentration was 121 mg a.i./L, the highest concentration tested. The NOEC, determined by visual interpretation of the clinical observation data, was 72 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 Fish.* Hazard Evaluation Division. Office of Pesticide Programs. EPA 540/9-85-006. 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. 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition. American Public Health Association. American Water Works Association. Water Pollution Control Federation. New York.

Table 1

Summary of Analytical Chemistry Data

Sponsor: Ciba-Geigy Corporation
Test Substance: CGA 329,351
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Nominal Test Concentration (mg a.i./L)	Replicate	Sampling Time (hrs)	Measured Concentration (mg a.i./L)[1]	Mean Measured Concentration (mg a.i./L)	Percent of Nominal
Negative Control	A	0	< LOQ[2]	< LOQ	--
	B	96	< LOQ		
Solvent Control	A	0	< LOQ	< LOQ	--
	B	96	< LOQ		
16	A	0	15	15	94
	B	96	15		
26	A	0	25	26	100
	B	96	26		
43	A	0	42	42	98
	B	96	42		
72	A	0	72	72	100
	B	96	72		
120	A	0	122	121	101
	B	96	120		

[1] Measured concentrations were corrected for a mean procedural recovery of 99%.
[2] 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: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (mg a.i./L)	Replicate	0 Hour[1]			24 Hours		48 Hours		72 Hours		96 Hours		
		Temp[2] (°C)	DO[3] (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	Temp °C	DO (mg/L)	pH
Negative Control	A	11.8	10.8	8.4	--	--	10.8	8.4	--	--	11.8	10.1	8.4
	B	11.8	--	--	10.3	8.4	--	--	10.8	8.4	11.8	--	--
Solvent Control	A	12.2	10.7	8.4	--	--	10.7	8.4	--	--	12.1	10.1	8.4
	B	12.2	--	--	10.3	8.4	--	--	10.2	8.4	12.1	--	--
15	A	12.0	10.7	8.4	--	--	10.4	8.4	--	--	11.9	10.1	8.4
	B	12.0	--	--	10.3	8.4	--	--	10.6	8.4	12.0	--	--
26	A	12.0	10.7	8.4	--	--	10.4	8.4	--	--	11.9	10.1	8.4
	B	12.0	--	--	10.5	8.4	--	--	10.8	8.4	11.8	--	--
42	A	12.1	10.8	8.4	--	--	10.2	8.4	--	--	12.0	10.0	8.4
	B	12.1	--	--	10.3	8.4	--	--	10.7	8.4	12.0	--	--
72	A	12.1	10.7	8.5	--	--	10.6	8.4	--	--	12.0	10.1	8.4
	B	12.1	--	--	10.5	8.4	--	--	10.7	8.4	11.9	--	--
121	A	12.0	10.7	8.5	--	--	10.4	8.4	--	--	11.9	10.3	8.4
	B	12.0	--	--	10.5	8.4	--	--	10.7	8.4	11.9	--	--

[1] The 0-hour dilution water measurements for hardness, alkalinity and specific conductance were 120 mg/L as $CaCO_3$, 178 mg/L as $CaCO_3$ and 275 µmhos/cm, respectively

[2] Temperature measured continuously during the test ranged from approximately 11.5 to 13.0°C.

[3] A dissolved oxygen concentration of 6.5 mg/L represents 60% saturation at 12°C in freshwater.

Table 3
Cumulative Percent Mortality and Treatment-Related Effects

Sponsor: Ciba-Geigy Corporation
Test Substance: CGA 329,351
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (mg a.i./L)	Replicate	No. Exposed	3 Hours		24 Hours		48 Hours		72 Hours		96 Hours		Cumulative Percent Mortality
			No. Dead[1]	Effects[2]	No. Dead	Effects	No. Dead	Effects	No. Dead	Effects	No. Dead	Effects	
Negative Control	A	10	0	10 AN	0	9AN;1D	0	9AN;1D	0	9AN;1D	0	9AN;1D[3]	
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
Solvent Control	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
15	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
26	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
42	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
72	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
121	A	10	0	10 AN	0	1AN;7D;2D,N	0	1AN;5D;4D,C	0	1AN;8D;1D,E	0	1AN;9D[3]	
	B	10	0	10 AN	0	9D;1D,N	0	1AN;6D;3D,C	0	1AN;7D;2D,E	0	1AN;9D[3]	0%

[1] Cumulative number of dead fish.
[2] Observed Effects: AN = Appears Normal; C = Lethargic; D = Discoloration, E = Erratic swimming, N = Loss of equilibrium.
[3] Discoloration in fish does not appear as dark as it did initially.

Table 4

LC50 Values

Sponsor: Ciba-Geigy Corporation
Test Substance: CGA 329,351
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Time	LC50 (mg a.i./L)	Lower 95% Confidence Limits	Upper 95% Confidence Limits	Statistical Method
24 Hours	> 121	--[1]	--	Visual Inspection
48 Hours	> 121	--	--	Visual Inspection
72 Hours	> 121	--	--	Visual Inspection
96 Hours	> 121	--	--	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:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

	Mean	Range
Specific Conductance (µmhos/cm)	325 (N = 4)	320 - 330
Hardness (mg/L as $CaCO_3$)	133 (N = 4)	132 - 136
Alkalinity (mg/L as $CaCO_3$)	178 (N = 4)	176 - 182
pH	8.2 (N = 4)	8.2 - 8.3

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:	Rainbow Trout, *Oncorhynchus mykiss*
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:	Rainbow Trout, *Oncorhynchus mykiss*
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-164

APPENDIX III

Introduction

Freshwater samples were collected from an acute aquatic toxicity study to determine the effects of CGA-329,351 to the rainbow trout (*Oncorhynchus mykiss*). The study was conducted by Wildlife International Ltd. and identified as WIL Project No.: 108A-164. The analyses of these water samples were performed at Wildlife International Ltd. by high performance liquid chromatography (HPLC) with UV detection. The analytical method was verified on June 29, 1995. Samples were received for analysis on July 20, 1995 and July 24, 1995 and analyzed between July 20 and July 24, 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, 1996. 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.

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Analytical Method

The method used for the analysis of the freshwater 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 Liquid Chromatograph (LC) equipped with a Diode Array Detector (DAD). HPLC separations were achieved using a Supelco Hi-Sep Column (15 cm x 4.6 mm ID, 5.0 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 low and high calibration standards are shown in Figures 3 and 4, respectively.

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.

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Method Verification

The analytical method was verified for freshwater by analyzing a series of three fortification samples at each of three concentrations (1.00, 50.0 and 140 mg a.i./L). The recoveries, based on the measured concentrations, ranged from 89% to 105% of the expected concentrations (Table 2). The overall mean recovery was 99%. No interferences at or above the LOQ were observed for the three matrix blanks analyzed during the method trial.

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 3). 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 4). Measured concentrations for the samples were corrected for the mean procedural recovery of 99%. A representative chromatogram of a matrix fortification is presented in Figure 6.

RESULTS

Sample Analysis

Freshwater samples were collected from the acute toxicity study with the rainbow trout (*Oncorhynchus mykiss*) at test initiation (Day 0), July 20, 1995, and at test termination (96 hours) on July 24, 1995. The measured concentrations of CGA-329,351 in the samples collected at initiation of exposure of the test organisms (Day 0) ranged from 94 to 102% of the nominal concentrations (Table 5). Samples collected at 96 hours (test termination) had measured concentration ranges of 94 to 100% of nominal values. A representative chromatogram of a sample is shown in Figure 7.

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Table I

Typical HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1090 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

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Table 2

Method Verification Recoveries for CGA-329,351 in Freshwater

Sample Number (108A-164-)	Type	Concentration (mg a.i./L) Fortified	Measured	Percent Recovery
VMAB-1	Matrix Blank	0.00	<1.00	--
VMAB-2	Matrix Blank	0.00	<1.00	--
VMAB-3	Matrix Blank	0.00	<1.00	--
VMAS-1	Matrix Fortification	1.00	0.990	99
VMAS-2	Matrix Fortification	1.00	0.894	89
VMAS-3	Matrix Fortification	1.00	0.903	90
VMAS-4	Matrix Fortification	50.0	48.8	98
VMAS-5	Matrix Fortification	50.0	50.9	102
VMAS-6	Matrix Fortification	50.0	49.3	99
VMAS-7	Matrix Fortification	140	147	105
VMAS-8	Matrix Fortification	140	145	103
VMAS-9	Matrix Fortification	140	147	105
			Mean Recovery =	99%
			Standard Deviation =	5.9
			N =	9

[1] The limit of quantitation (LOQ) was based upon the lowest matrix fortification level (1.00 mg a.i./L) analyzed concurrently with the samples.

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Table 3

Matrix Blanks Analyzed Concurrently During Sample Analysis

Sample: Number (108A-164-)	Sample: Type	Measured Concentration of CGA-329.351 (mg a.i./L)
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.

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Table 4

Matrix Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (108A-164-)	Concentrations of CGA-329,351 (mg a.i./L) Fortified	Measured	Percent Recovered
MAS-1	5.00	4.77	95
MAS-4	5.00	4.89	98
MAS-2	50.0	49.8	100
MAS-5	50.0	50.1	100
MAS-3	140	144	103
MAS-6	140	140	100
			Mean = 99%
			Standard Deviation = 2.66
			N = 6

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Table 5

Measured Concentrations of CGA-329,351 in Freshwater Samples from a Rainbow Trout Toxicity Test

Nominal Concentration (mg a.i./L)	Sample Number (108A-164-)	Sampling Time (Hours)	CGA-329,351 Concentration		Percent of Nominal
			Measured[1] (mg a.i./L)	Corrected[2] (mg a.i./L)	
0.0 (Negative Control)	1	0	<5.00	<5.00	--
	16	96	<5.00	<5.00	--
0.0 (Solvent Control)	3	0	<5.00	<5.00	--
	18	96	<5.00	<5.00	--
16	5	0	14.8	15	94
	20	96	15.1	15	94
26	7	0	25.1	25	96
	22	96	25.8	26	100
43	9	0	42.0	42	98
	24	96	41.8	42	98
72	11	0	71.6	72	100
	26	96	71.5	72	100
120	13	0	121	122	102
	28	96	119	120	100

[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 99%.

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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 65:35:0.2 (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.

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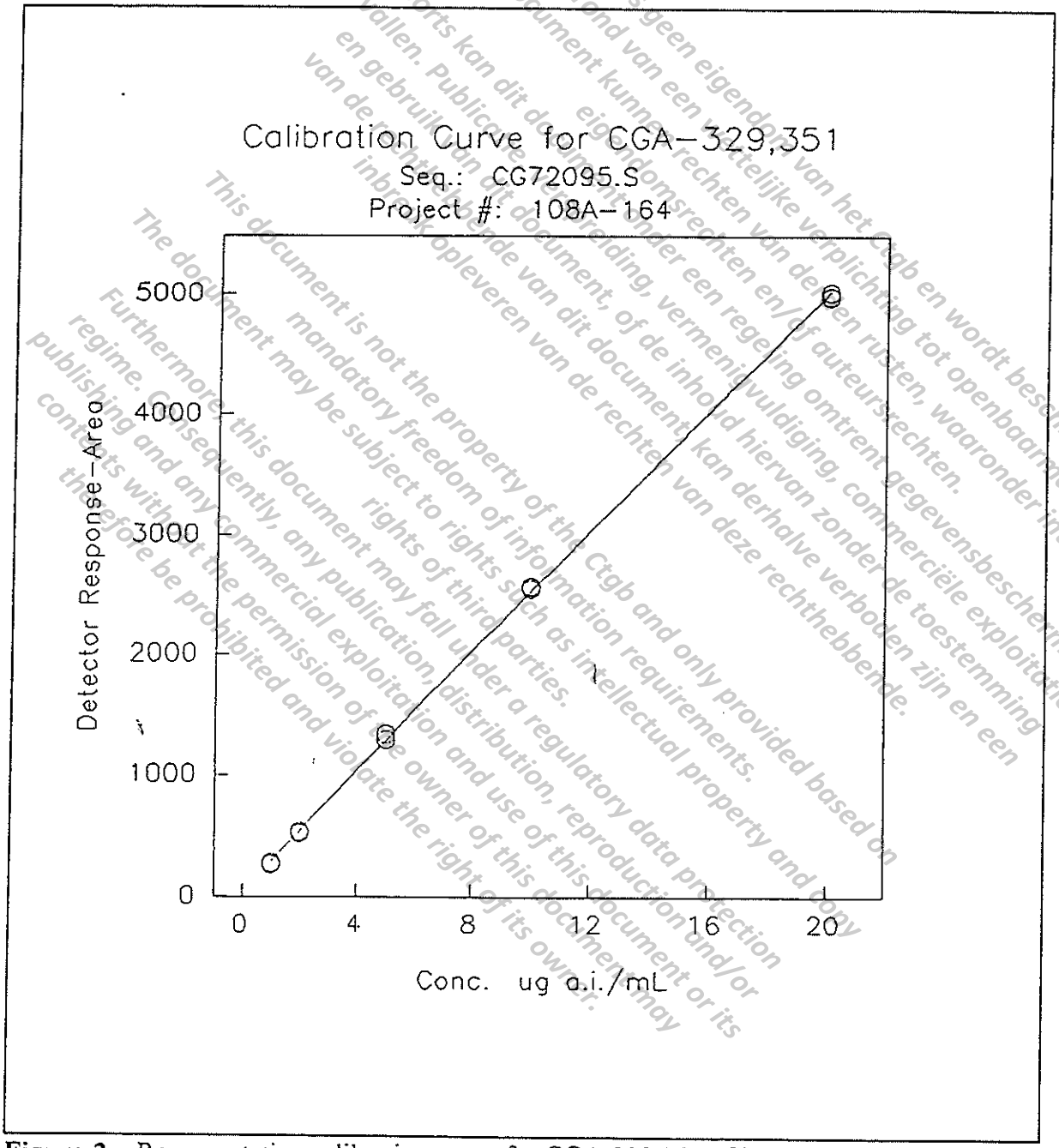

Figure 2. Representative calibration curve for CGA-329,351. Slope = 249.3553; Intercept = 39.4964.

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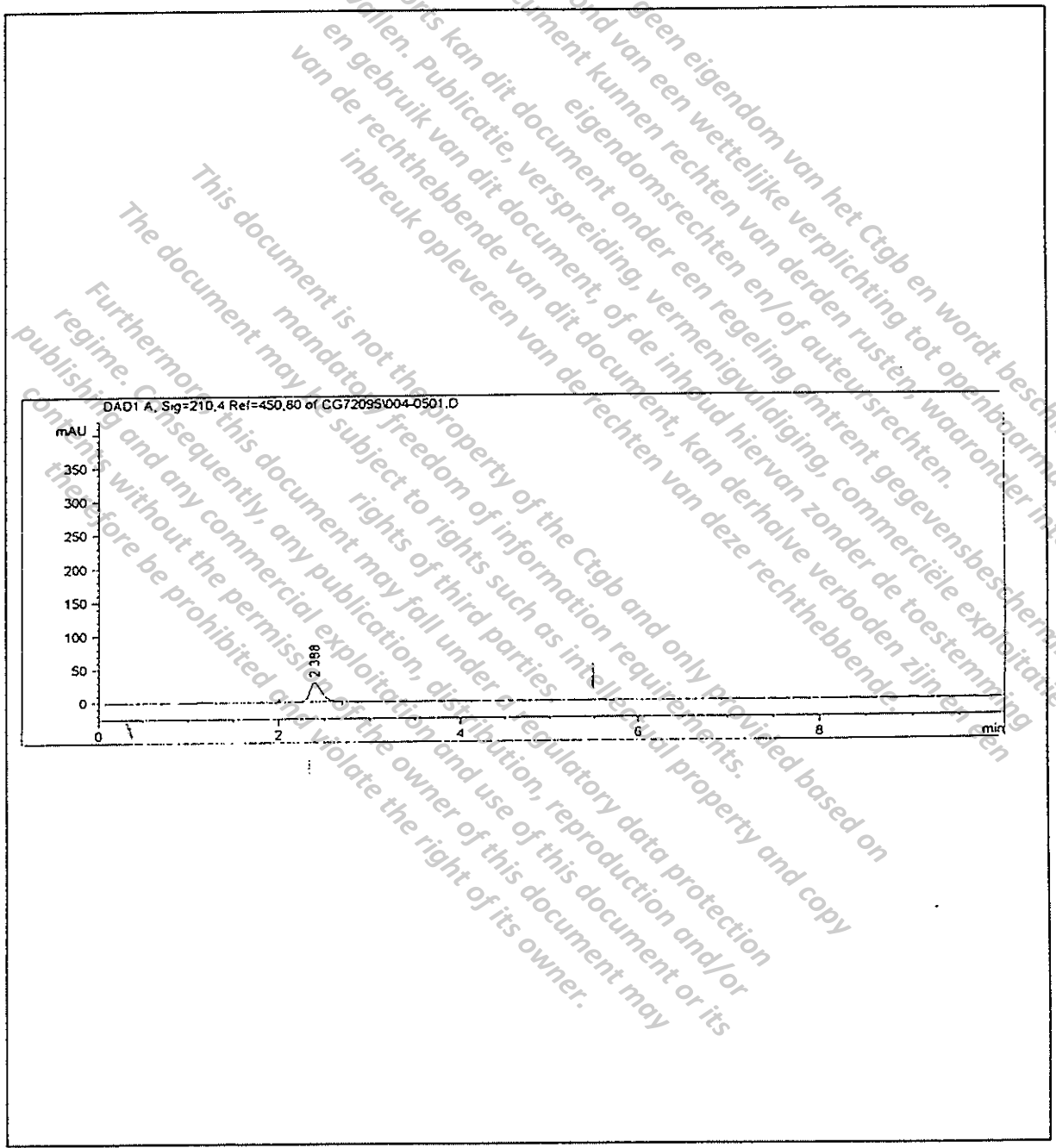

Figure 3. A representative chromatogram of a 1.00 µg a.i./mL CGA-329,351 standard (75 ng on column).

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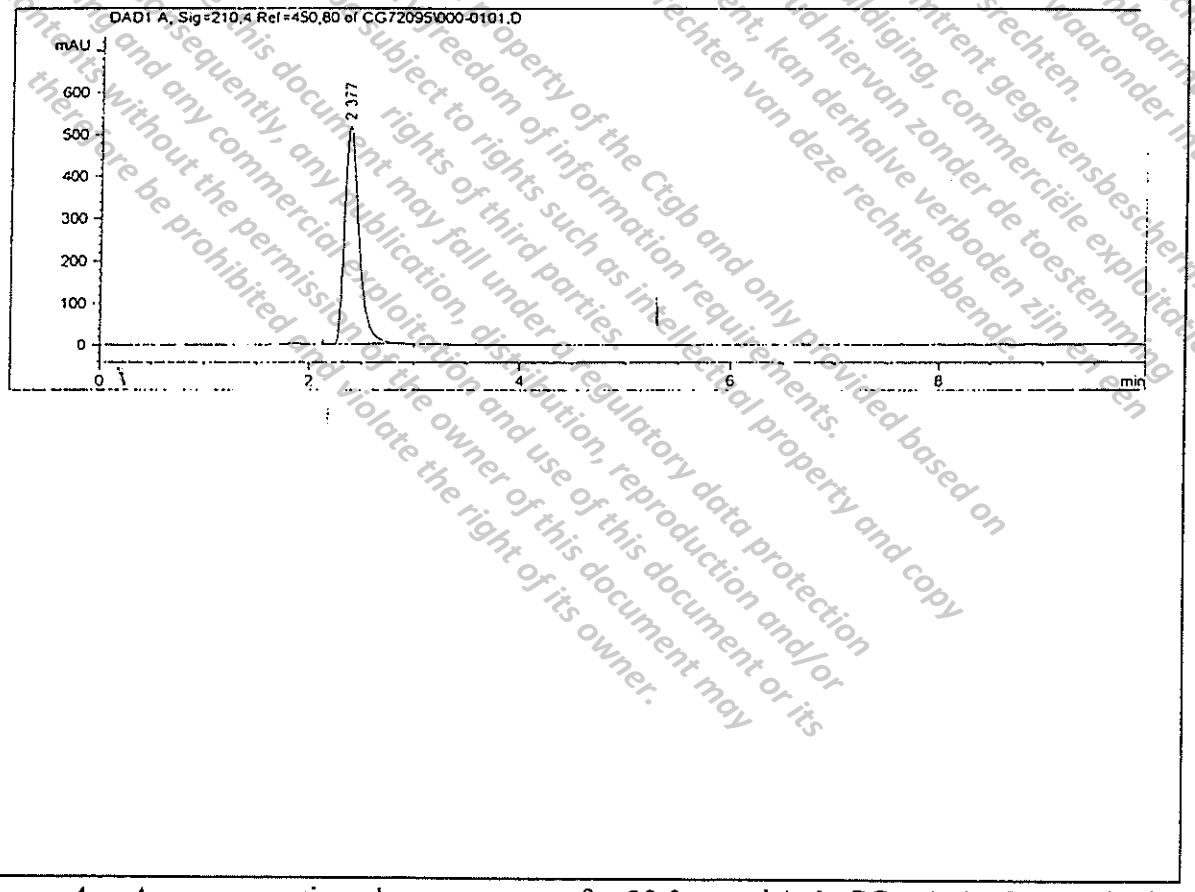

Figure 4. A representative chromatogram of a 20.0 µg a.i./mL CGA-329,351 standard.

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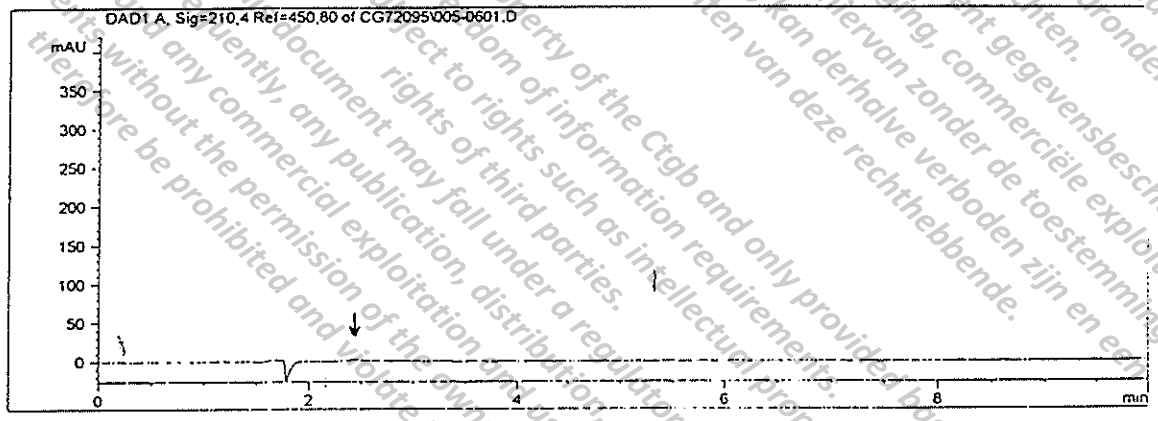

Figure 5. A representative chromatogram of a matrix blank, 108A-164-MAB-1.

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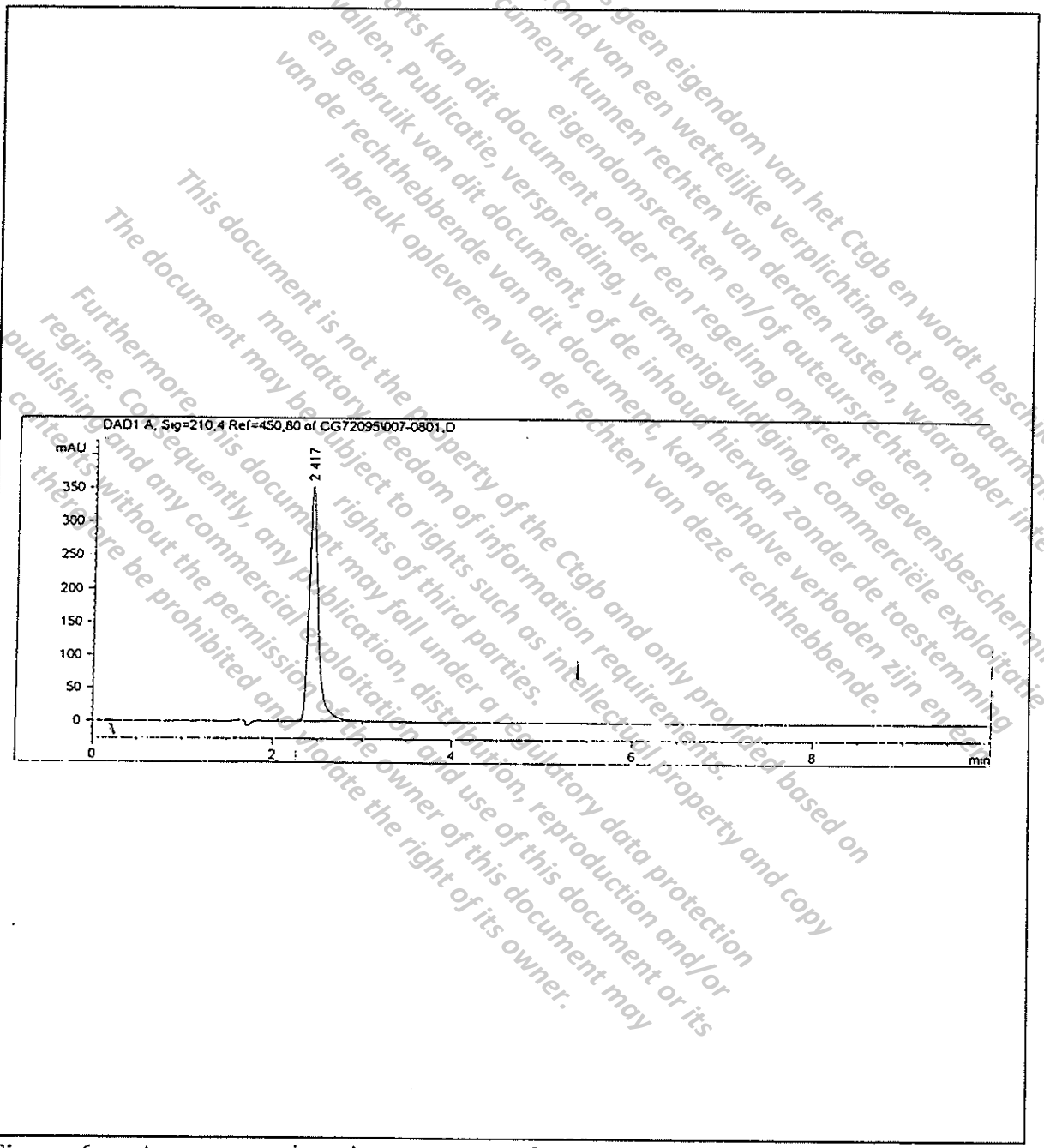

Figure 6. A representative chromatogram of a matrix fortification, 108A-164-MAS-2, 50.0 mg a.i./L.

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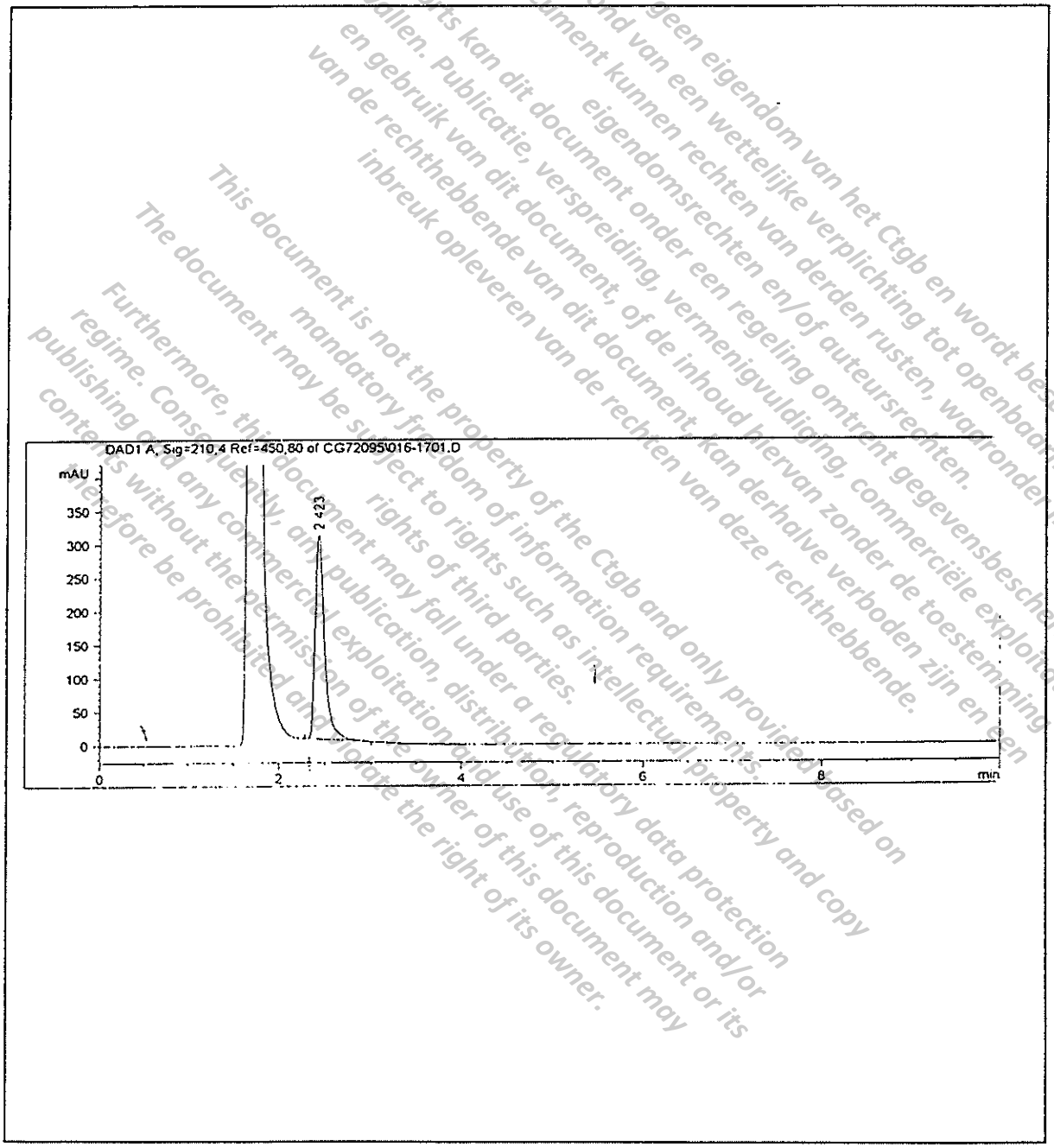

Figure 7. A representative chromatogram of a sample on Day 0, 108A-164-9, 43 mg a.i./L nominal concentration.

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.

3. The protocol was amended to add the analytical method verification.

4. Dissolved oxygen and pH were not measured at approximately 24-hour intervals on Day 3 of the test.

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. 5.1.2.e Woo, Ph.D., Manager, Aquatic Toxicology
2. [redacted] Senior Aquatic Biologist
3. 5.1.2.e Woo, Senior Aquatic Biologist
4. 5.1.2.e Woo, Aquatic Biologist
5. 5.1.2.e Woo Aquatic Biologist
6. 5.1.2.e Woo, Scientist