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CGA 329351

STUDY TITLE

A DIETARY LC50 STUDY WITH THE NORTHERN BOBWHITE

DATA REQUIREMENT

US EPA FIFRA GUIDELINE 71-2(a)

AUTHORS:

5.1.2 e Woo

STUDY COMPLETION DATE

AUGUST 3, 1995

PERFORMING LABORATORY

WILDLIFE INTERNATIONAL LTD.
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LABORATORY STUDY IDENTIFICATION NUMBER

108-374

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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).

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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.

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Ciba-Geigy Corporation

TITLE: CGA 329,351: A Dietary LC50 Study with the Northern Bobwhite

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108-374

STUDY COMPLETION: August 3, 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, and OECD, ISBN 92-84-12367-9, Paris 1982, and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

STUDY DIRECTOR:

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5.1.2.e Woo DATE 8/3/95

Toxicology

QUALITY ASSURANCE STATEMENT

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108-374

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, 10 August 1984. The dates of all audits and inspections 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	MANAGEMENT
Test substance preparation	May 18, 1995	May 23, 1995	May 25, 1995
Body weights, feed consumption and observations	May 23, 1995	May 23, 1995	May 25, 1995
Sample extraction	June 12, 1995	June 12, 1995	June 13, 1995
Biological Data and Draft Report	June 1-2, 1995	June 2, 1995	June 8, 1995
Analytical Data and Draft Report	July 7-10, 1995	July 10, 1995	July 14, 1995
Final Report	August 3, 1995	August 3, 1995	August 3, 1995

5.1.2.e Woo DATE August 3, 1995

Quality Assurance Representative

REPORT APPROVAL

SPONSOR: Ciba-Geigy Corporation

TITLE: CGA 329,351: A Dietary LC50 Study with the Northern Bobwhite

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108-374

STUDY DIRECTOR:

5.1.2.e Woo _____ 8-3-95
Date

Senior Biologist

MANAGEMENT:

5.1.2.e Woo _____ 8/3/95
Date

Manager, Avian/Non-Target Insect Toxicology

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SUMMARY

SPONSOR: Ciba-Geigy Corporation

TEST SUBSTANCE: CGA 329,351

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108-374

STUDY: CGA 329,351: A Dietary LC50 Study with the Northern Bobwhite

RESULTS: The dietary LC50 value for northern bobwhite exposed to CGA 329,351 was determined to be greater than 5620 ppm a.i., the highest concentration tested. The no mortality and no observed effect concentration were both 5620 ppm a.i.

TEST DATES: Hatch - May 8, 1995
Acclimation - May 8, 1995
Experimental Start - May 18, 1995
Termination In-Life Phase - May 26, 1995
Experimental Termination - June 13, 1995

TEST LEVELS: 0, 316, 562, 1000, 1780, 3160 & 5620 ppm a.i.

TEST ANIMALS: Northern Bobwhite (*Colinus virginianus*)

AGE TEST ANIMALS: 10 days of age at test initiation.

SOURCE TEST ANIMALS: Wildlife International Ltd. Production Flock
8598 Commerce Drive
Easton, Maryland 21601

STUDY COMPLETION: August 3, 1995

INTRODUCTION

This study was conducted by Wildlife International Ltd. under contract to Ciba-Geigy Corporation. The test was conducted at the Wildlife International Ltd. avian toxicology facility in Easton, Maryland from May 18, 1995 to May 26, 1995. Raw data generated at Wildlife International Ltd. and a copy of the final report are filed under Project Number 108-374 in the archives located at Wildlife International Ltd.

OBJECTIVE

The objective of this study was to evaluate the toxicity of CGA 329,351 when administered to juvenile northern bobwhite in the diet for five days.

MATERIALS AND METHODS

The methods used in conducting this study are based upon procedures specified in Section 71-2 of the Environmental Protection Agency Registration Guidelines Pesticide Assessment Guidelines, FIFRA Subdivision E. Hazard Evaluation: Wildlife and Aquatic Organisms (1); OECD Guideline 205, Guideline for Testing of Chemicals. Avian Dietary Toxicity Test (2); and upon ASTM Standard E857-87 "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species" (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 original reported purity of the test substance was 96.6%. The test substance was later re-assayed and confirmed the original purity. The test substance was stored at ambient room temperature.

Treatment Groups

Ten northern bobwhite chicks were assigned to each of the treatment and control groups by indiscriminate draw. Birds were acclimated from the day they were hatched until test initiation. The test consisted of a geometric series of six test concentrations and three control groups. Nominal dietary concentrations used in this study were 316, 562, 1000, 1780, 3160 and 5620 parts per million (ppm) active ingredient (a.i.). The dietary concentrations were established based upon known toxicity data. Each group was fed the appropriate test or control diet for five days. During the exposure period the control group received an amount of the vehicle in their diet equivalent to the greatest amount used in the treated diets. Following the five day exposure period all groups were given untreated feed for three days.

Duration of the Test

The primary phases of this test and their durations were:

1. Acclimation - 10 days.
2. Exposure - 5 days.
3. Post-exposure observation - 3 days.

Test Birds

All northern bobwhite (*Colinus virginianus*) were 10 days of age and appeared to be in good health at initiation of the test. The birds were obtained from Wildlife International Ltd., 8598 Commerce Drive, Easton, Maryland 21601. The birds were hatched on May 8, 1995. All birds were from the same hatch, pen-reared and phenotypically indistinguishable from wild birds. Birds were assigned to six test groups and three control groups. Each treatment or control group contained ten chicks. The birds used in this study were immature and could not be differentiated by sex. All birds were acclimated to the caging and facilities from the day of hatch until initiation of the test.

Animal Diet

Throughout acclimation and testing all test birds were fed a game bird ration formulated to Wildlife International Ltd.'s specifications (Appendix I). The chicks were given a vitamin

supplement in their water from the day they were hatched until the initiation of the test. Water, from the town of Easton public water supply, and feed were provided ad libitum during acclimation and during the test. The birds received no form of antibiotic medication during acclimation or the test.

Diet Preparation

Test diets were prepared by first dissolving the test substance in acetone, and then blending the solution into the diet with corn oil in a Hobart (Model Number AS200T) mixer (Appendix II). The concentration of corn oil in the treated and control diets was 2%. Acetone was allowed to volatilize from the diets during the mixing procedure. An amount of diet sufficient to last the five day exposure period was prepared on the day of test initiation for each treatment and control group. Diets were presented to the birds at initiation of the test, and added to the feeders as needed during the five day exposure period.

All dietary test concentrations were adjusted to 100% active ingredient based upon the reassayed purity of the test substance. Therefore all dietary concentrations and the LC50 value are reported as parts per million of the active ingredient in the diet. Nominal dietary test concentrations used in this study were 316, 562, 1000, 1780, 3160 and 5620 ppm a.i.

Diet Sampling

Samples of the test diets were taken to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. Samples were frozen and transferred to Wildlife International Ltd. analytical laboratory for analysis.

Housing and Environmental Conditions

During acclimation and testing, all birds were housed indoors by test group in batteries of thermostatically controlled brooding pens manufactured by Beacon Steel Products Co. (Model No. B735Q). Birds were assigned to pens by indiscriminate draw. Each pen had floor space that measured approximately 72 X 90 cm. Ceiling height was approximately 23 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting. Each test or control group was assigned a pen that contained ten chicks. Each group of birds was identified by pen

number. During the test the average temperature in the brooding compartment of the pens was 38°C ± 1°C (SD). Average ambient room temperature for this study was 27.7°C ± 1.0°C (SD) with an average relative humidity of 42% ± 9% (SD). The photoperiod (maintained by a time clock) was sixteen hours of light per day during acclimation and throughout the test. The light source was fluorescent lights which closely approximate noon-day sunlight (noon-day sun - 4870° Kelvin, Chroma 50 or equivalent - 5000° Kelvin). The birds were exposed to an average of approximately 509 lux of illumination.

Housing and husbandry practices were based on guidelines established by the National Institutes of Health (4).

Observations

During acclimation all birds were observed daily. Birds exhibiting abnormal behavior or physical injury were not used. Following test initiation and continuing until termination, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity and abnormal behavior.

Animal Body Weights/Feed Consumption

Body weights by group were measured at the initiation of the test, on Day 5, and at termination of the test on Day 8. Average feed consumption values during the exposure period (Days 0-5) and the post-exposure observation period (Days 6-8) were determined for each treatment and control group. Feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time. The accuracy of feed consumption values may have been affected by the unavoidable wastage of feed by the birds.

Statistical Calculations

There were no mortalities observed in this study. Therefore, it was not possible to perform the routine calculation (6,7,8) of an LC50 value using the computer program of C.E. Stephan (5). The LC50 value was determined to be greater than the highest concentration.

RESULTS

Diet Analysis

Samples of the test diets fed to northern bobwhite quail were collected to verify test concentration, homogeneity and stability of the test substance in avian diet. These samples were analyzed at Wildlife International Ltd. analytical laboratory using gas chromatography. Analysis of homogeneity samples from the 316 ppm a.i. diet showed a range of 91 to 98% of nominal, while the 5620 ppm a.i. test diet showed a range of 85 to 88% of nominal indicating that the diets were homogeneously mixed. Analysis of the verification samples from the 562, 1000, 1780 and 3160 ppm a.i. diets showed means of 92%, 96%, 86% and 81% of nominal, respectively. Analysis of the stability samples collected from the feeders on Day 5 of the test demonstrated means of 112%, 92%, 93%, 92%, 107% and 106% of the Day 0 measured concentrations for the 316, 562, 1000, 1780, 3160 and 5620 ppm a.i. treatment groups, respectively, indicating that the test substance was stable in the diet. Details of the results of diet analyses are presented in Appendix III.

Mortalities and Clinical Observations

There were no mortalities in the control group (Table 1). With the exception of 10 birds in one unit of the control group noted as toe picked on Days 5-7, all birds in the control group were normal in appearance and behavior throughout the test. Additionally, there were no mortalities or overt signs of toxicity observed at any of the concentrations tested. All birds at all test concentrations were normal in appearance and behavior throughout the test period.

Body Weight and Feed Consumption Measurements

When compared to the control group, there did not appear to be an effect upon body weight or feed consumption at any of the concentrations tested (Tables 2 and 3).

CONCLUSION

The dietary LC50 for northern bobwhite exposed to CGA 329,351 was determined to be greater than 5620 ppm a.i., the highest concentration tested. The no mortality and no observed effect concentrations were both 5620 ppm a.i.

REFERENCES

1 Environmental Protection Agency. 1982 (October). Pesticide Assessment Guidelines, FIFRA Subdivision E. Hazard Evaluation: Wildlife and Aquatic Organisms, Subsection 71-2. Office of Pesticide Programs. Washington, D.C. 86 pp.

2 OECD Guideline 205, Guideline for Testing of Chemicals, Avian Dietary Toxicity Test, Organization for Economic Cooperation and Development, 4 April 1984.

3 ASTM Standard E857-87. 1987. "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species". American Society for Testing and Materials.

4 National Institutes of Health. 1985. Guide for the care and use of laboratory animals. NIH Pub. No. 86-23. 83 pp.

5 Stephan, C. E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.

6 Finney, D. J. 1971. Statistical Methods in Biological Assay, 2nd edition, Griffin Press, London.

7 Thompson, W. R. 1947. Bacteriological Reviews. Vol II, 2:115-145.

8 Stephan, C. E. 1977. Methods for Calculating an LC50. Aquatic Toxicology and Hazard Evaluations, Amer. Soc. Test Mat., Pub. No. STP 634:65-84.

TABLE 1

Cumulative Mortalities from a Northern Bobwhite Acute Dietary Toxicity Study with CGA-329,351

Experimental Group (ppm a.i.)	No. Dead Exposure Period						No. Dead Post Exposure Period		
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control									
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Treatment									
316	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
562	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1000	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1780	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
3160	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
5620	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

The LC50 value was determined to be greater than 5620 ppm a.i., the highest concentration tested.

TABLE 2

Mean Body Weight (g) from a Northern Bobwhite Acute Dietary Toxicity Study with CGA-329,351

Experimental Group	Test Concentration (ppm a.i.)	Exposure Period			Post Exposure Period		Total
		Day 0	Change	Day 5	Change	Day 8	Change
Control	0	18	9	27	10	37	19
	0	19	11	30	10	40	21
	0	18	13	31	10	41	23
Treatment	316	19	12	31	8	39	20
	562	19	11	30	10	40	21
	1000	19	12	31	9	40	21
	1780	19	11	30	10	40	21
	3160	19	12	31	9	40	21
	5620	18	11	29	10	39	21

TABLE 3

Feed Consumption (g/bird/day) from a Northern Bobwhite Acute Dietary Toxicity Study with CGA-329,351

Experimental Group	Test Concentration (ppm a.i.)	Exposure Period Day 0-5	Post Exposure Period Day 6-8
Control	0	7	9
	0	9	11
	0	9	10
Treatment			
	316	13	21
	562	12	12
	1000	10	8
	1780	10	11
	3160	8	9
	5620	11	12

APPENDIX I

DIET FORMULATION

WILDLIFE INTERNATIONAL LTD. GAME BIRD RATION[1]

INGREDIENTS	PERCENT (%)
Fine Corn Meal	37.45
Ground Oats	5.00
Alfalfa Meal Dehydrated, 17% Protein	3.00
CDP (Phosphate Source)	0.70
Dried Whey	2.50
Fish Meal, 60% Protein	6.00
Meat Poultry Blend, 58% Protein	4.00
Wheat Midds	5.00
Soy Bean Meal, 48% Protein	34.80
Salt Iodized	0.10
Ground Limestone	0.60
GL Ferm (Fermatco)[2]	0.25
Methionine Premix	0.20
Vitamin and Mineral Premix (see below)	0.40
Total	100.00

VITAMIN AND MINERAL PREMIX	AMOUNT ADDED PER TON
Vitamin D_3	2,000,000 I.C.U.
Vitamin A	7,000,000 I.U.
Riboflavin	6 grams
Niacin	40 grams
Pantothenic Acid	10 grams
Vitamin B_{12}	8 mgs
Folic Acid	600 mgs
Biotin	64 mgs
Pyridoxine	1.2 grams
Thiamine	1.2 grams
Vitamin E	20,000 I.U.
Vitamin K (Menadione Dimethylpyrimidinol Bisulfite)	5.8 grams
Manganese	102 grams
Zinc	47 grams
Copper	6.8 grams
Iodine	1.5 grams
Iron	51 grams
Selenium	182 mgs

[1] The guaranteed analysis is a minimum of 27% protein, a minimum of 2.5% crude fat and a maximum of 5% crude fiber.

[2] Fermentation By-Products (Source of Unidentified Growth Factors).

APPENDIX II

DIET PREPARATION

Weight and volume of constituents used to prepare test diets:

Nominal Concentrations (ppm a.i.)	Test Substance (g)	Acetone (ml)	Corn Oil (ml)	Basal Diet (g)
0	-	50	65	2940.0
316	0.9654	50	65	2939.0
562	1.7168	50	65	2938.3
1000	3.0552	50	65	2936.9
1780	5.4380	50	65	2934.6
3160	9.6537	50	65	2930.3
5620	17.1692	50	65	2922.8

Diets were prepared as follows:

- The test substance was weighed in a tared beaker.
- Acetone (40 ml) and corn oil (65 ml) were added to the beaker containing the test substance, and stirred with a glass rod, for approximately 60 seconds, until a solution was formed.
- The test substance mixture was poured over 2000 g of basal ration in a Hobart mixing bowl. The beaker was rinsed with acetone (10 ml) and the rinse poured over the ration.
- The appropriate amount of remaining ration was then was added to the mixing bowl and all constituents mixed for approximately 10 minutes.
- The control diet was prepared by weighing 2940.0 g of basal ration and placing it into a Hobart mixing bowl. Acetone (100 ml), and corn oil (130 ml) and the remaining amount of basal ration (2940.0 g) were also added to the mixing bowl. All constituents were blended for approximately 10 minutes. This procedure was performed three times in order to provide a sufficient amount of diet for the negative control birds.

APPENDIX III

THE ANALYSIS OF CGA 329,351 IN AVIAN FEED SAMPLES

IN SUPPORT OF

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 108-374

APPENDIX III

INTRODUCTION

Avian diet samples were collected from a dietary LC50 study that was conducted to determine the toxicity of CGA 329,351 to the Northern bobwhite (*Colinus virginianus*). The study was conducted by Wildlife International Ltd. and identified as Project No. 108-374. Diets were prepared in the Wildlife International Ltd. avian toxicology laboratory. The analyses of diet samples were performed at Wildlife International Ltd. using gas chromatography with flame ionization detection. The analytical methodology was verified on June 1, 1995 through a series of matrix blank and fortification samples analyzed prior to the definitive test samples. Homogeneity, verification, and stability samples were collected and submitted for analysis. Samples were received from the avian laboratory for analysis between May 18 and 23, 1995. The samples were analyzed between June 7 and 13, 1995.

Test Substance and Analytical Standards

The test substance and analytical standard were supplied by the sponsor for a dietary LC50 study. The test substance was used in the preparation of matrix fortification samples. The analytical standard was used to prepare calibration standards.

The test substance was received on April 11, 1995 and was 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 was a dark brown viscous liquid, with 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 was received on April 20, 1995 and was identified on the label as: COMPOUND CGA-329351; PURITY 99.4%; CODE S95-1785; AMOUNT 300 mg; STORAGE Freezer; DISPENSED 4/18/95 JS; REASSAY 11/96. The analytical standard was a clear viscous liquid with a reported purity of 99.4% and an expiration date of November 1996. Upon receipt,

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the analytical standard was assigned Wildlife International Ltd. Identification Number WIL 3203 AS and stored under freezer (approximately -14°C) conditions.

Analytical Method

The method used for the analysis of the avian diet was based upon methodology provided by Ciba-Geigy Corporation and entitled "Gas Chromatographic Determination of Metalaxyl (Apron®) Coatings on Seed".

A subsample of avian diet (~20 g) was weighed into an eight ounce French square bottle. One hundred milliliters of methanol was added to the bottle. The subsample was homogenized for two minutes at approximately 10,000 rpm. The extract was then vacuum filtered through Whatman #3 filter paper. An additional seventy-five milliliters of methanol was added to the subsample and it was homogenized for one minute at approximately 10,000 rpm. The resultant extract was vacuum filtered through the original filter paper. The filter paper was then rinsed with twenty-five milliliters of methanol. The extracts were combined and brought to a two hundred fifty milliliter final volume with methanol. After the extract was shaken to mix, a portion was transferred to a scintillation vial. An aliquot was diluted into the range of calibration standards using methanol. Concentrations of CGA 329,351 in extracts of the samples were determined by gas chromatography using a Hewlett-Packard Model 5890 Gas Chromatograph (GC) equipped with a flame ionization detector (FID). Gas chromatographic separations were achieved using a J & W Scientific DB-5 column (15 m x 0.53 mm ID, 1.5 µm film thickness). 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 3.00 to 10.0 µg a.i./mL, were analyzed with each sample set. Linear regression equations were generated using the peak height 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 height responses into the applicable linear regression equation. Typical chromatograms of low and high calibration standards are shown in Figures 3 and 4.

APPENDIX III

The instrument limit of detection (LOD) was set based upon the injection volume (1 µL) and the lowest standard concentration (3.00 µg a.i./mL). The LOD was set at 3 ng on column. The method limit of quantitation (LOQ) for these analyses was set at 75.0 ppm a.i. based upon the lowest matrix fortification level analyzed concurrently with the samples. The 3.00 µg a.i./mL standard was equivalent to a calculated value of 37.5 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent were reported.

Method Verification

The analytical method was evaluated for avian feed by analyzing a series of triplicate fortification samples at each of three concentrations (75.0, 750, and 7500 ppm a.i.). The recoveries, based on the measured concentrations, ranged from 77% to 87% of the nominal concentrations (Table 2). No interferences were observed at or above the ppm a.i. equivalent of the lowest standard during method verification.

Matrix Blank and Fortification Samples

Along with the sample analyses, two matrix blanks were analyzed to determine possible interferences. No interferences were observed at or above the ppm a.i. equivalent of the lowest standard during the sample analyses (Table 3). A typical chromatogram of a matrix blank is presented in Figure 5.

Avian diet samples were fortified at 75.0, 750, and 7500 ppm a.i. and analyzed concurrently with the samples to determine the mean procedural recovery (Table 3). The method yielded a mean procedural recovery of 85%. A typical chromatogram of a matrix fortification is presented in Figure 6.

Sample Analysis

Avian diet samples were analyzed to measure test substance concentrations and to evaluate homogeneity and stability. Diet samples collected to evaluate homogeneity were obtained by sampling the left and right sides of the mixing vessel at the top, middle, and bottom layers of diets prepared at the 316 and 5620 ppm a.i. concentrations (Table 4). Mean measured concentrations

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of test substance were determined by analyzing diet samples collected on Day 0 from the 562, 1000, 1780 and 3160 ppm a.i. concentrations (Table 5). Stability of CGA 329,351 in diet under actual test conditions was evaluated by comparing test substance concentrations from samples collected on Day 5 with Day 0 values (Table 6).

RESULTS

Measured concentrations for all samples were corrected for the mean procedural recovery of 85%. Diet samples were collected from the 316 and 5620 ppm a.i. test concentrations, and analyzed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the two test concentrations were 295±10.1 ppm a.i. and 4830±76.9 ppm a.i., respectively. Coefficients of variation were 3.42% and 1.59%, respectively. Control diet samples collected during the test showed no interferences. Samples collected during the test to verify test substance concentrations for the 562, 1000, 1780 and 3160 ppm a.i. diets had means and standard deviations of 519±4.24 ppm a.i., 964±5.66 ppm a.i., 1530±49.5 ppm a.i. and 2570±113 ppm a.i., respectively. Analysis of diet samples collected from the feeders after being held at ambient temperature for 5 days averaged 112%, 92%, 93%, 92%, 107% and 106% of the Day 0 values for the 316, 562, 1000, 1780, 3160 and 5620 ppm a.i. test concentrations, respectively. A typical chromatogram of a test sample is given in Figure 7.

APPENDIX III

TABLE 1

Typical Gas Chromatographic Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 5890 Gas Chromatograph (GC) Equipped with a Model G1030A ChemStation
DETECTOR:	Hewlett-Packard Flame Ionization Detector (FID)
ANALYTICAL COLUMN:	J & W Scientific DB-5 Column (15 m x 0.53 mm, 1.5 µm film thickness)
INJECTOR TEMPERATURE:	235°C
OVEN:	Initial temperature : 150°C Initial hold time : 1.0 minute Ramp : 10°C/minute Final temperature : 250°C Final hold time : 5.0 minutes
DETECTOR TEMPERATURE:	260°C
INJECTION VOLUME:	1 µL (Splitless)
CARRIER GAS:	Helium, ~20 ml/min
AIR:	~400 ml/min
HYDROGEN:	~100 ml/min
CGA 329,351 PEAK RETENTION TIME:	Approximately 5.0 minutes

APPENDIX III

TABLE 2

Method Verification Results

Sample Number (108-374-)	Type	Concentration of CGA 329,351 (ppm a.i.) Fortified	Measured[1]	Percent Recovery
MEB-1	Method Blank	0	< 37.5	--
MAB-1	Matrix Blank	0	< 37.5	--
MAB-2	Matrix Blank	0	< 37.5	--
MAB-3	Matrix Blank	0	< 37.5	--
MAS-1	Matrix Fortification	75.0	63.2	84
MAS-2	Matrix Fortification	75.0	63.8	85
MAS-3	Matrix Fortification	75.0	63.3	84
MAS-4	Matrix Fortification	750	574	77
MAS-5	Matrix Fortification	750	640	85
MAS-6	Matrix Fortification	750	606	81
MAS-7	Matrix Fortification	7500	5920	79
MAS-8	Matrix Fortification	7500	6500	87
MAS-9	Matrix Fortification	7500	6020	80

Mean Procedural Recovery = 82%
Standard Deviation = 3.32
N = 9

[1] The lowest level analytical standard was equivalent to a calculated value of less than 37.5 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent are reported.

APPENDIX III

TABLE 3

Matrix Blanks and Fortifications Analyzed Concurrently with the Samples

Sample		Concentration of CGA 329,351 (ppm a.i.)		
Number (108-374-)	Type	Fortified	Measured[1]	Percent Recovery
MAB-4	Matrix Blank	0	< 37.5	--
MAB-5	Matrix Blank	0	< 37.5	--
MAS-10	Matrix Fortification	75.0	68.5	91
MAS-13	Matrix Fortification	75.0	65.8	88
MAS-11	Matrix Fortification	750	668	89
MAS-14	Matrix Fortification	750	610	81
MAS-12	Matrix Fortification	7500	6200	83
MAS-15	Matrix Fortification	7500	5680	76

Mean Procedural Recovery = 85%
Standard Deviation = 5.68
N = 6

[1] The lowest level analytical standard was equivalent to a calculated value of less than 37.5 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent are reported.

APPENDIX III

TABLE 4

Homogeneity of CGA 329,351 in Avian Diet

Nominal Concentration (ppm a.i.)	Sample I.D. Number (S-108-374-)	Location Sampled In Mixing Vessel	CGA 329,351 Concentration		Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Mean Percent of Nominal
			Measured (ppm a.i.)	Corrected[1] (ppm a.i.)		
316	2	Top Left	263	309		
	3	Top Right	260	306		
	4	Middle Left	244	287	$\bar{x}$ = 295	
	5	Middle Right	243	286	SD = 10.1	93
	6	Bottom Left	248	292	CV = 3.42	
	7	Bottom Right	246	289		
5620	16	Top Left	4050	4760		
	17	Top Right	4070	4790		
	18	Middle Left	4220	4960	$\bar{x}$ = 4830	
	19	Middle Right	4050	4760	SD = 76.9	86
	20	Bottom Left	4120	4850	CV = 1.59	
	21	Bottom Right	4130	4860		

[1] Values were corrected for a mean procedural recovery of 85%.

APPENDIX III

TABLE 5

Verification of CGA 329,351 Concentrations in Avian Diet

Nominal Concentration (ppm a.i.)	Sample No. (S-108-374-)	Day of Study	CGA 329,351 Concentrations		Mean ($\bar{x}$) Standard Deviation (SD) Coefficient of Variation (CV)	Mean Percent of Nominal
			Measured[1] (ppm a.i.)	Corrected[2] (ppm a.i.)		
0	1	0	< 37.5	--	--	--
562	8	0	444	522	$\bar{x}$ = 519	92
	9		439	516	SD = 4.24	
					CV = 0.817	
1000	10	0	823	968	$\bar{x}$ = 964	96
	11		816	960	SD = 5.66	
					CV = 0.587	
1780	12	0	1330	1560	$\bar{x}$ = 1530	86
	13		1270	1490	SD = 49.5	
					CV = 3.24	
3160	14	0	2250	2650	$\bar{x}$ = 2570	81
	15		2120	2490	SD = 113	
					CV = 4.40	

[1] The lowest level analytical standard was equivalent to a calculated value of less than 37.5 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent are reported.

[2] Values were corrected for a mean procedural recovery of 85%.

APPENDIX III

TABLE 6

Ambient Stability of CGA 329,351 in Avian Diet During the Northern Bobwhite LC50 Study

Nominal Concentration (ppm a.i.)	Day 0[1] Measured[2] (ppm a.i.)	Day 0[1] Corrected[3] (ppm a.i.)	Day 0[1] Mean Percent of Nominal	Nominal Concentration (ppm a.i.)	Day 5 Sample Number (S-108-374-)	Day 5 Measured[2] (ppm a.i.)	Day 5 Corrected[3] (ppm a.i.)	Day 5 Mean Percent of Day 0
0	< 37.5	--	--	0	22	< 37.5	--	--
316	251	295	93	316	23	281	331	112
					24	279	328	
562	441	519	92	562	25	401	472	92
					26	406	478	
1000	819	964	96	1000	27	756	889	93
					28	772	908	
1780	1300	1530	86	1780	29	1100	1290	92
					30	1290	1520	
3160	2180	2570	81	3160	31	2490	2930	107
					32	2170	2550	
5620	4110	4830	86	5620	33	4240	4990	106
					34	4430	5210	

[1] Day O values are means of the homogeneity samples (Table 4) and the verification samples (Table 5).
[2] The lowest level analytical standard was equivalent to a calculated value of 37.5 ppm a.i. in the matrix blank extract. Measured values greater than or equal to the ppm a.i. equivalent are reported.
[3] Values were corrected for a mean procedural recovery of 85%.

APPENDIX III

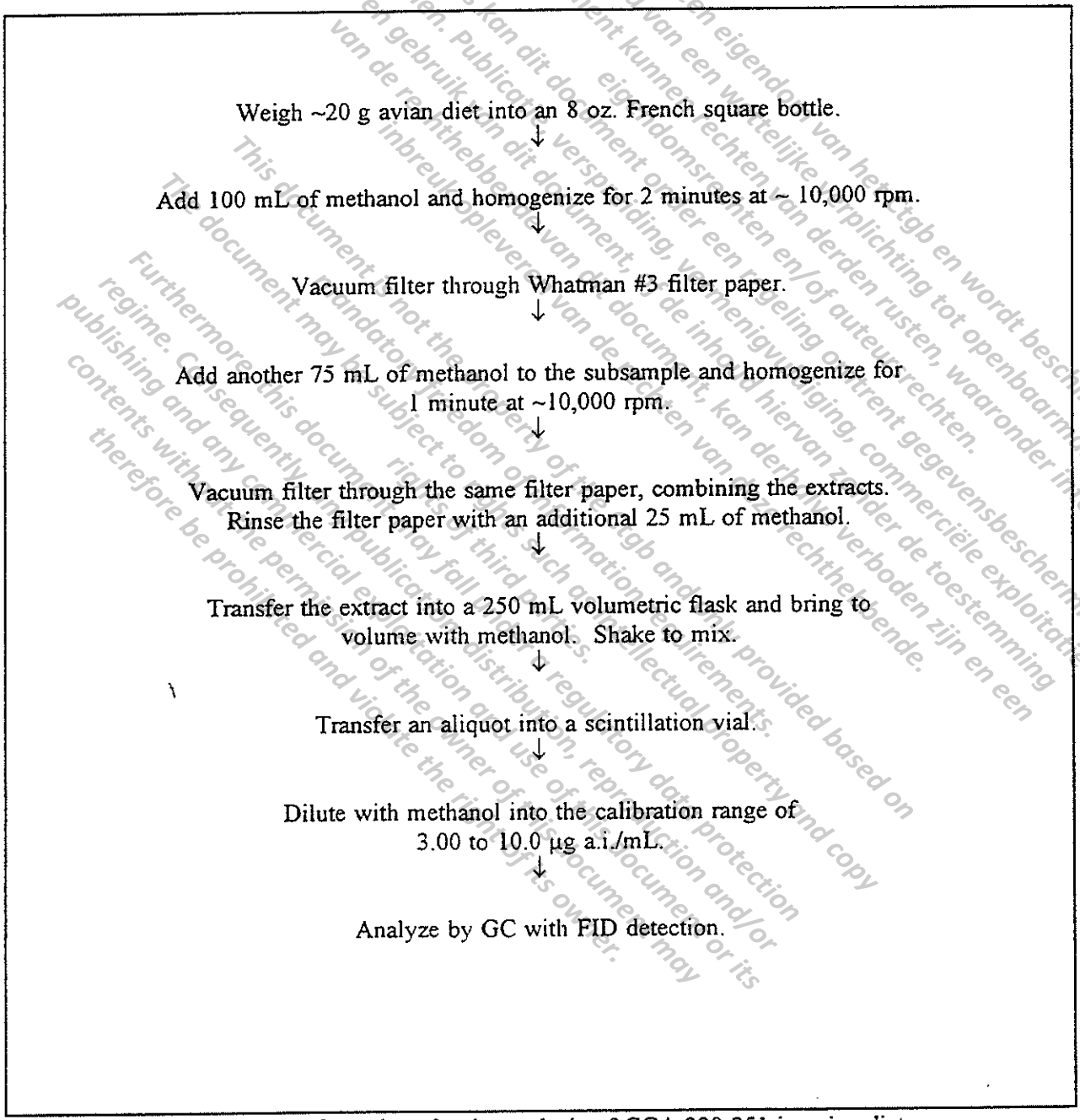

Figure 1. Analytical method flow chart for the analysis of CGA 329-351 in avian diet.

APPENDIX III

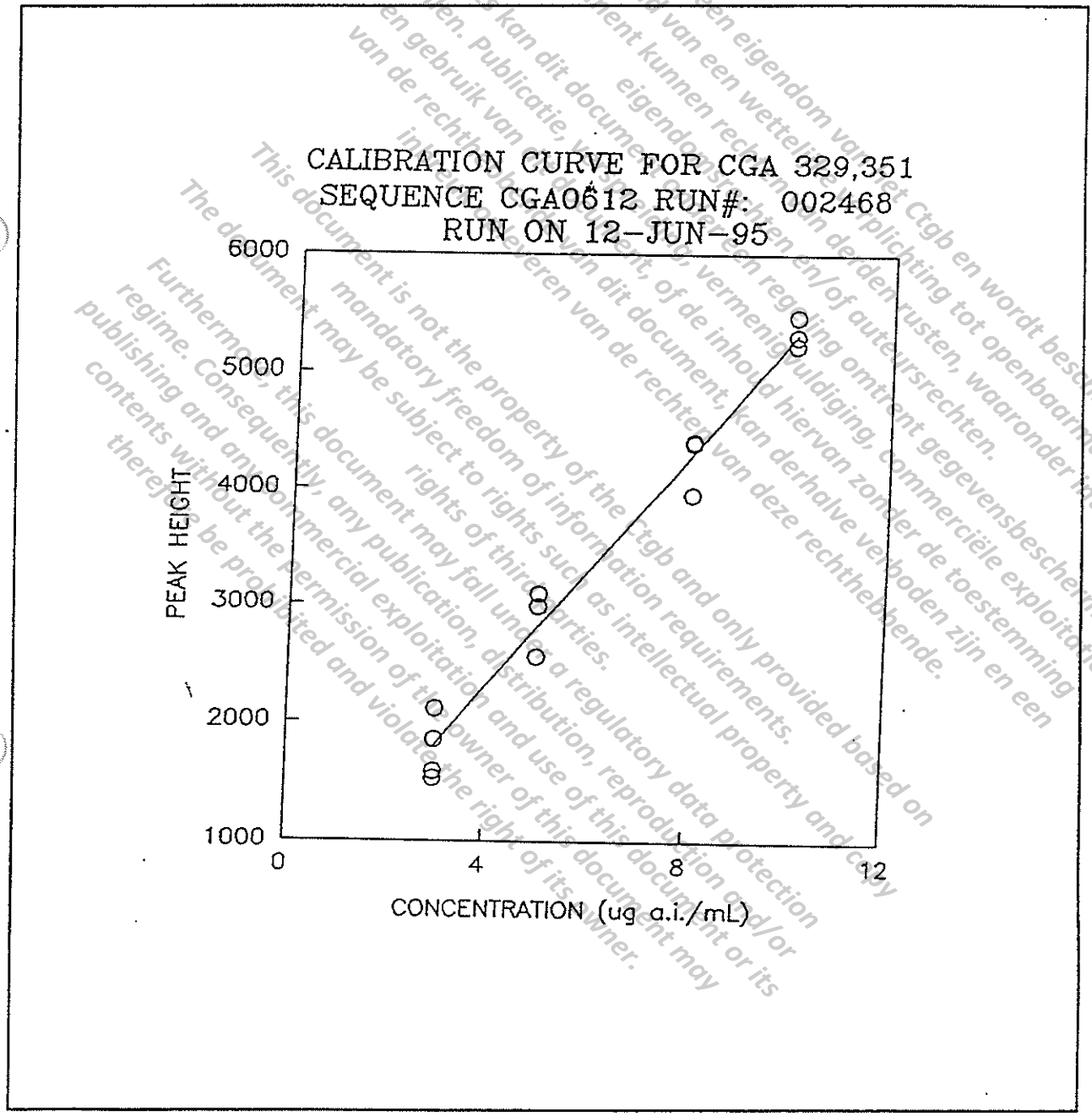

Figure 2. A typical calibration curve for CGA 329,351.

APPENDIX III

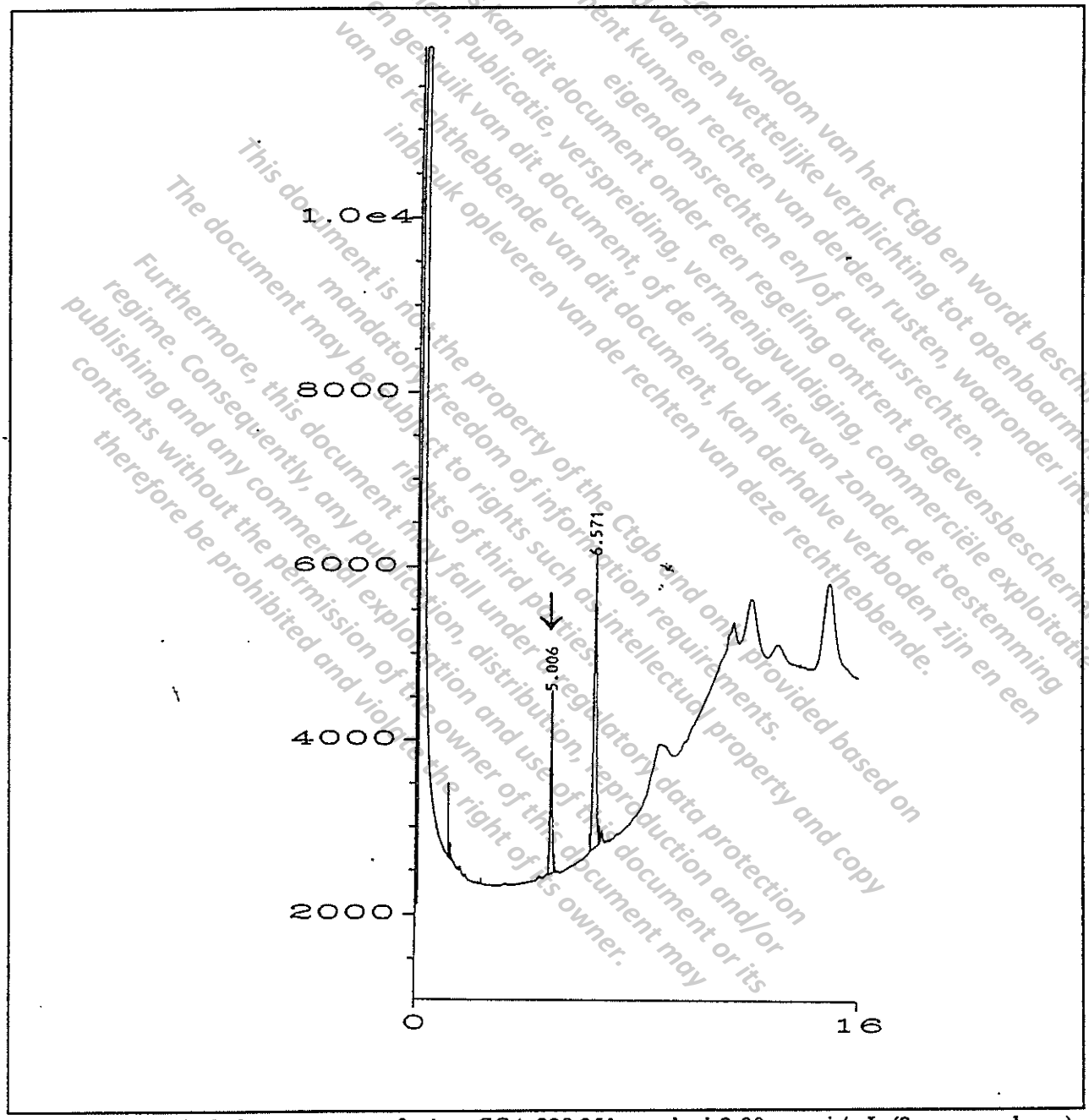

Figure 3. A typical chromatogram of a low CGA 329,351 standard 3.00 µg a.i./mL (3 ng on column).

APPENDIX III

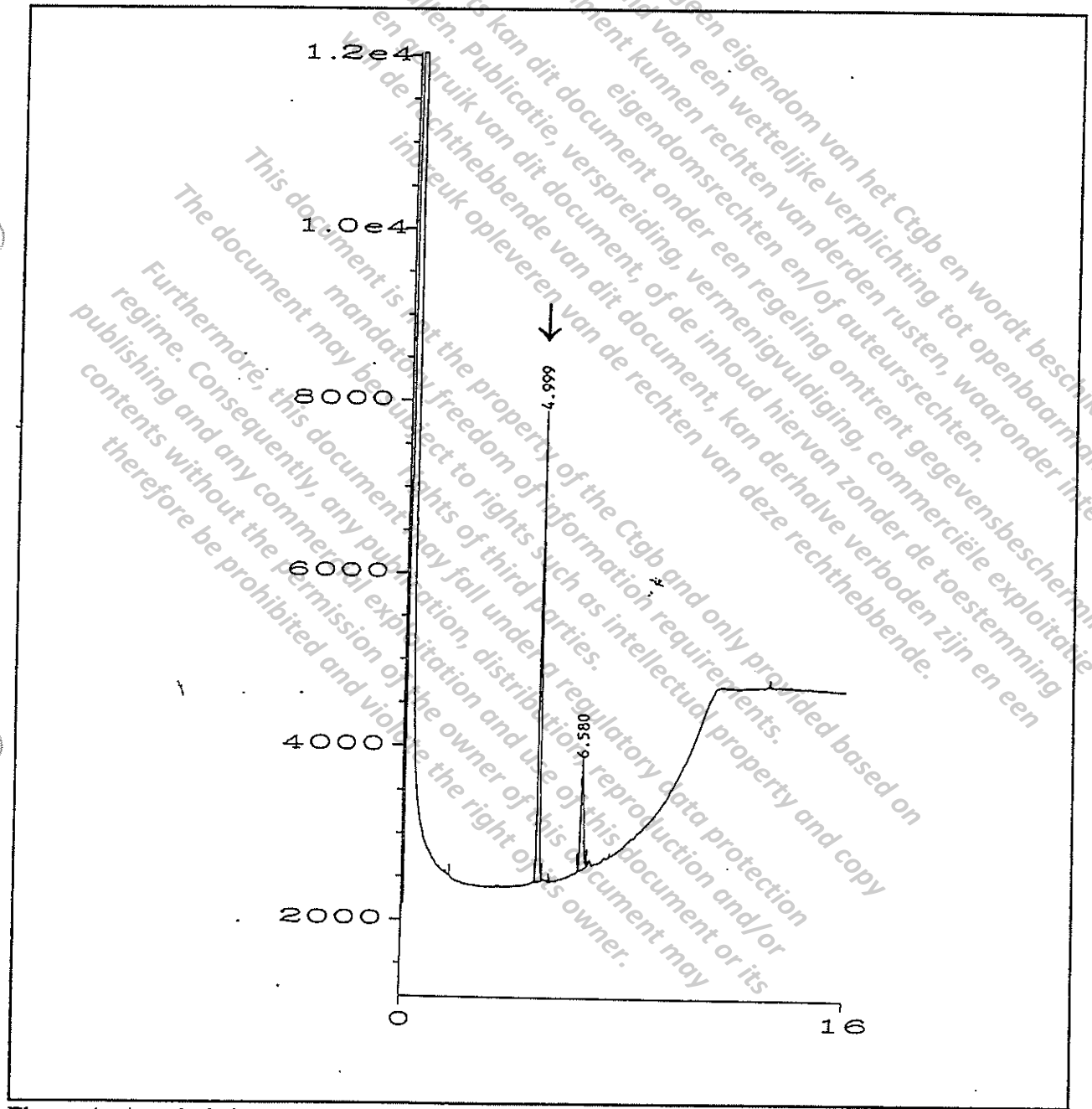

Figure 4. A typical chromatogram of a high CGA 329,351 standard 10.0 µg a.i./mL (10 ng on column).

APPENDIX III

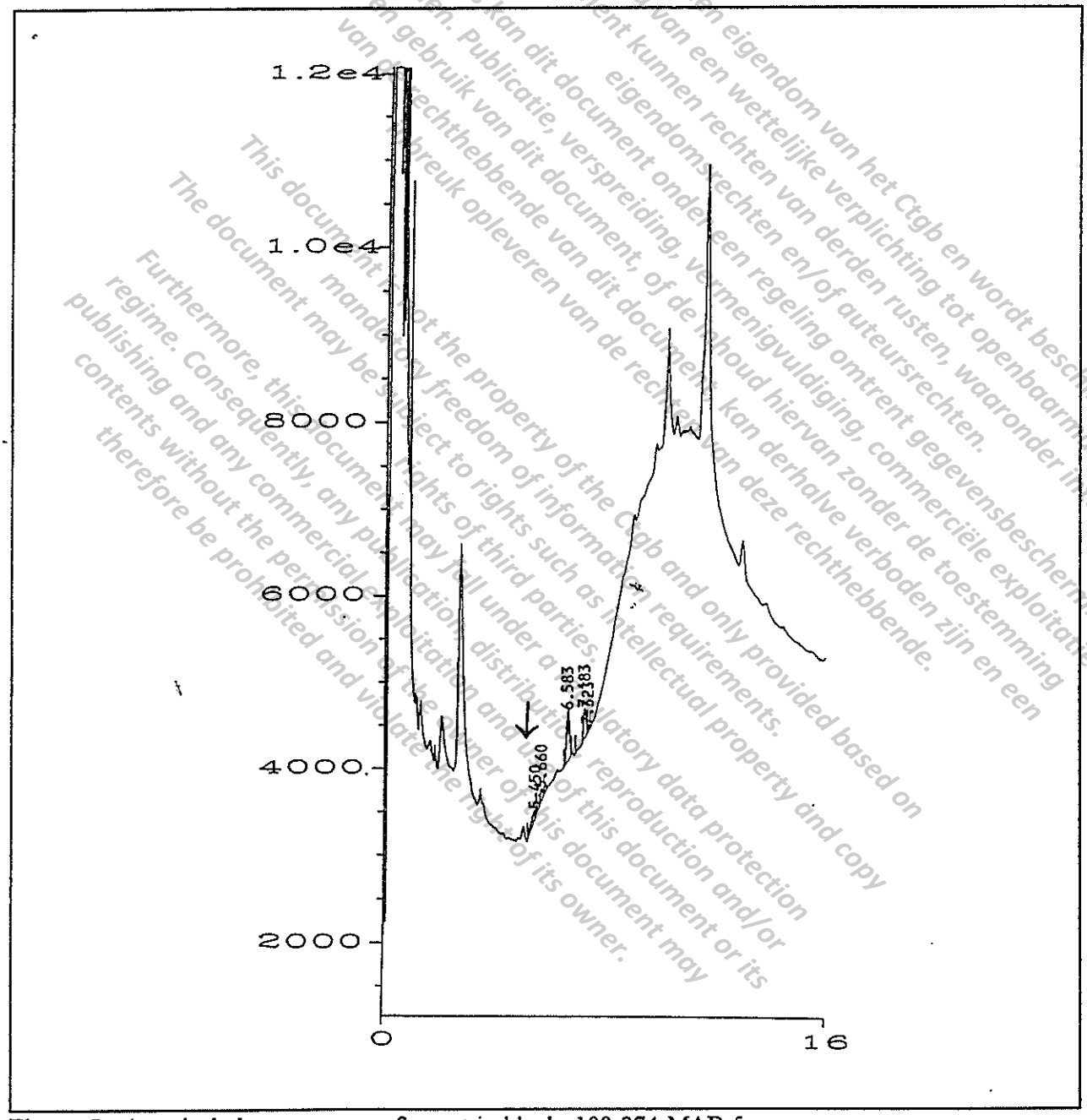

Figure 5. A typical chromatogram of a matrix blank, 108-374-MAB-5.

APPENDIX III

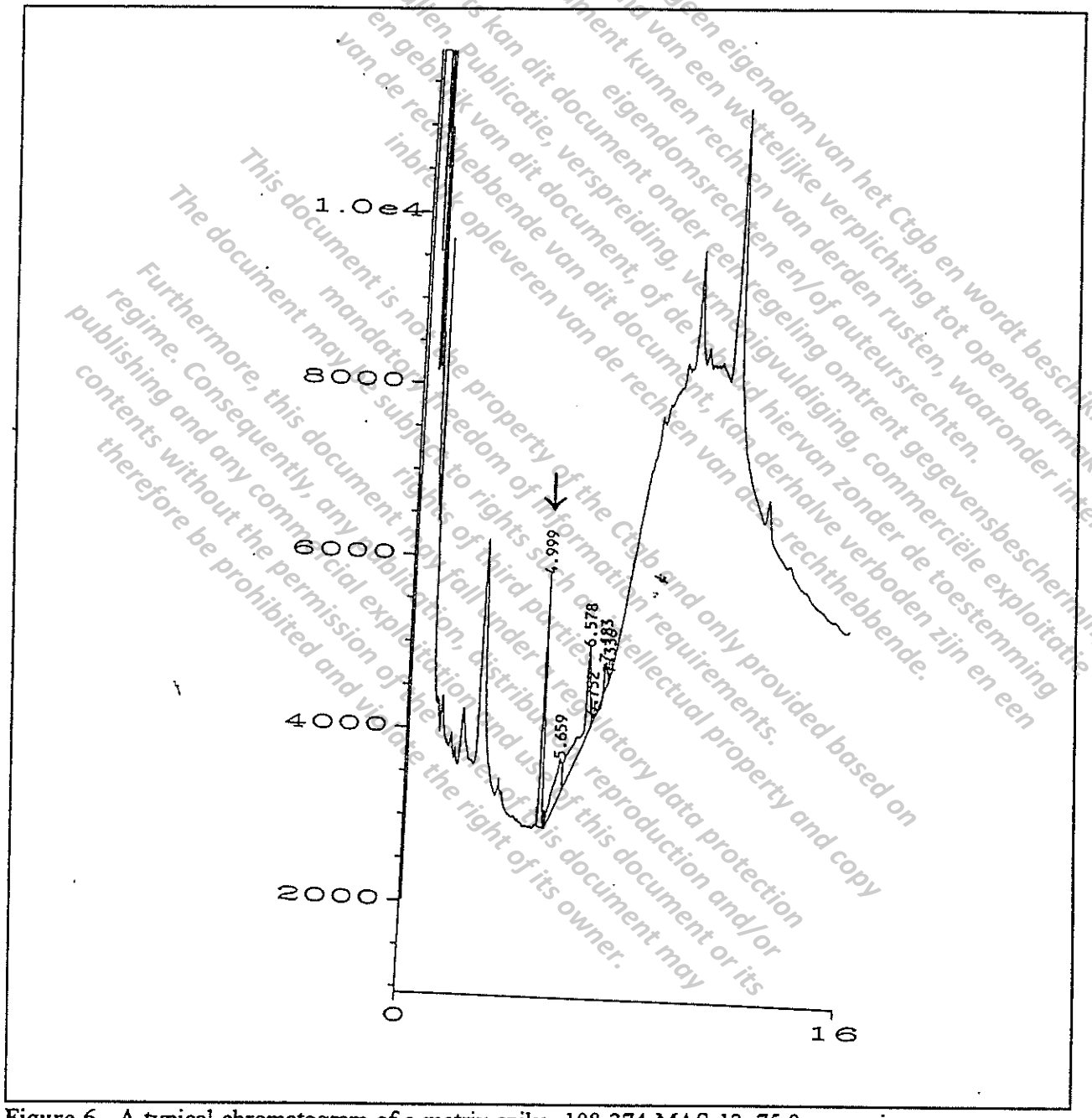

Figure 6. A typical chromatogram of a matrix spike, 108-374-MAS-13, 75.0 ppm a.i.

APPENDIX III

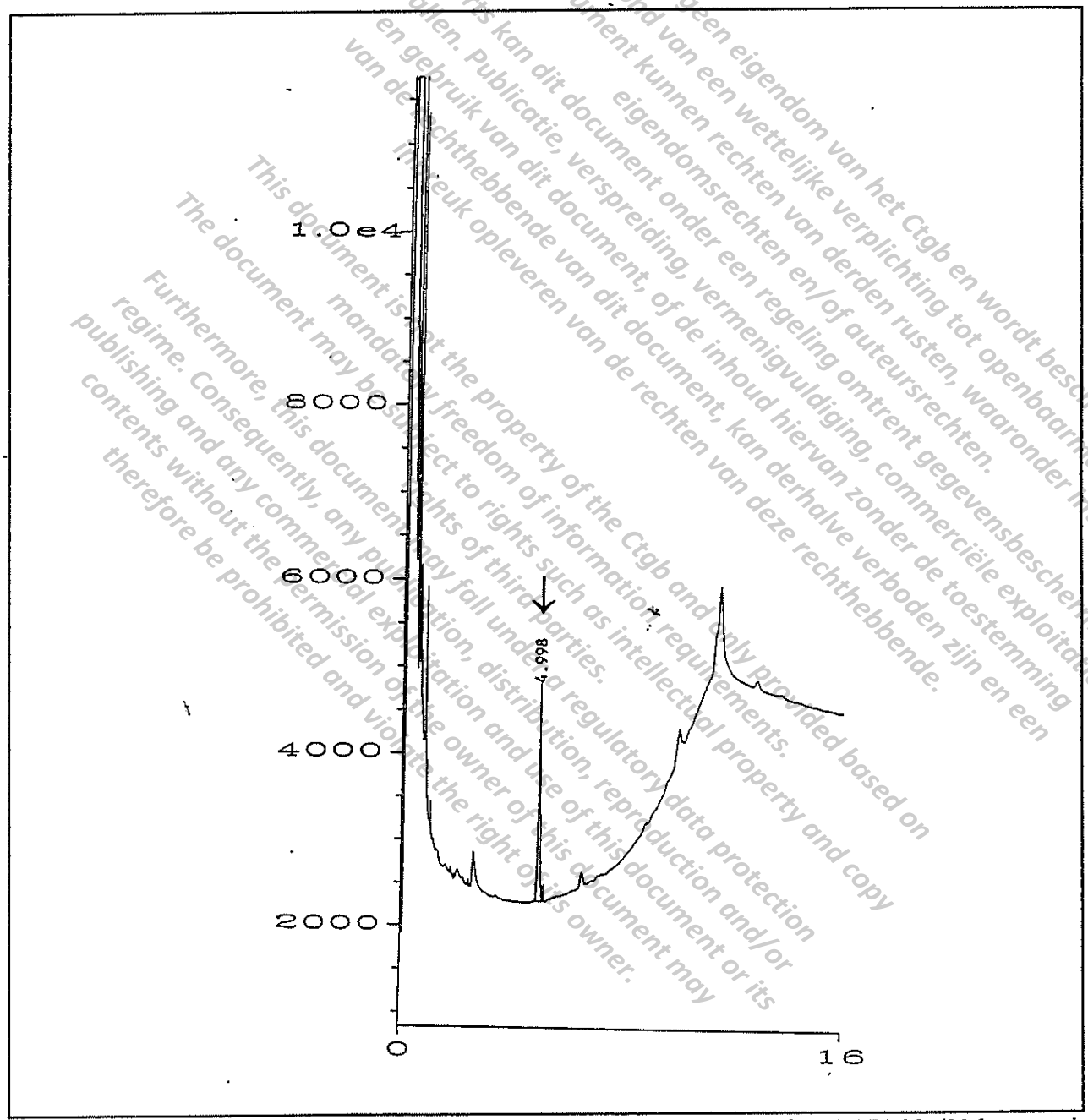

Figure 7. A typical chromatogram of an avian diet sample on Day 5, S-108-374-23 (316 ppm a.i. nominal).

APPENDIX IV

CHANGES TO PROTOCOL

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

- The experimental start date, experimental termination date, study room, test substance number and receipt date information were amended to the protocol.
- Study director responsibilities were reassigned.

The above changes to the approved Protocol did not adversely affect the results of this study.

APPENDIX V

PERSONNEL INVOLVED IN STUDY

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

(1) 5.1.2.e Woo Wildlife Toxicologist
(2) 5.1.2.e Woo Toxicology
(3) 5.1.2.e Woo Biologist
(4) 5.1.2.e Woo Senior Biologist
(5) 5.1.2.e Woo Research Biologist
(6) 5.1.2.e Woo , Research Biologist
(7) 5.1.2.e Woo Senior Chemist