103938

Study Title

NTN 33893: Toxicity to Honey Bees on Alfalfa Treated Foliage

Data Requirement

FIFRA Guideline 141-2
Hazard Evaluation: Nontarget Insects

Author

[redacted]

Study Report Date

December 18, 1992

Performing Laboratory

Washington State University
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Study Number

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Miles Report Number

103938

Statement of Confidentiality

Good Laboratory Practice Certification

[redacted]

Quality Assurance Statement

[redacted]

Certificate of Authenticity

[redacted]

Inquiries

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Appendix 1

NTN 33893/Honey Bees Toxicity of Residues on Foliage

Report on the In-Life Phase by

[redacted]

Washington State University

NTN 33893/HONEY BEES TOXICITY OF RESIDUES ON FOLIAGE

FIFRA GUIDELINE 141-2

[redacted]
Washington State University
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Prosser, WA 99350
[redacted]

STUDY INITIATION: 9 September 1992

STUDY COMPLETION: 16 September l992

SUBMITTED TO

Miles Incorporated
Agriculture Division
17745 South Metcalf
Stilwell, KS 66085-9104

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SUMMARY

SPONSOR: Miles Incorporated, Agriculture Division

TEST SUBSTANCE: NTN 33893 240FS

WASHINGTON STATE UNIVERSITY PROJECT NO: 92-004

STUDY: NTN 33893/Honey Bees Toxicity of Residues on Foliage

RESULTS:

Residue bioassay of NTN 33893 240FS (0.045 lb(AI)/acre)

Bioassay on Apis mellifera L., order Hymenoptera.

The percent mortality with 2 hour old residues was 5.6, with 8 hour old residues 7.2 and with 24 hour old residues 11.9.

Residue bioassay of NTN 33893 240FS (0.167 lb(AI)/acre)

Bioassay on Apis mellifera L., order Hymenoptera.

The percent mortality with 2 hour old residues was 11.7, with 8 hour old residues 16.1 and with 24 hour old residues 15.9.

Residue bioassay of NTN 33893 240FS (0.5 lb(AI)/acre)

Bioassay on Apis mellifera L., order Hymenoptera.

The percent mortality with 2 hour old residues was 11.8, with 8 hour old residues 23.1 and with 24 hour old residues 20.8.

CONCLUSION

NTN 33893 240FS (0.045 lb(AI)/acre) was non-hazardous to honey bees if applied in early morning or late evening when bees are not foraging.

NTN 33893 240FS (0.167 lb(AI)/acre was non-hazardous to honey bees if applied in late evening when bees are not foraging.

NTN 33893 240FS (0.5 lb(AI)/acre) was moderatly hazardous to honey bees if applied in late evening.

TEST DATES: First Test:
Experimental Start - 9 September 1992
Experimental Termination - 11 September 1992
Second Test:
Experimental Start - 14 September 1992
Experimental Termination - 16 September 1992

STUDY COMPLETION: 16 September 1992

1.0 Background

This report summarizes an investigation of the hazard of NTN 33893 (also known as imidacloprid) to honey bees after application to alfalfa foliage. The study followed EPA guideline 141-2. The in-life portion was conducted by [redacted] Washington State University, Prosser, Washington. [redacted] on this phase of the study is included as Appendix 1. The residue analysis phase was conducted by Miles Residue Analysis Laboratory. Stilwell, Kansas. The Analytical report is included as Appendix 2.

2.0 Summary of Methods, Results, and Conclusions

The study was designed to determine the potential hazard of NTN 33893 to honey bees (*Apis mellifera* L.) after application to alfalfa foliage. A 240 FS end-use formulation of NTN 33893 was applied to alfalfa at approximately 0.045 lb AI/acre, 0.167 lb AI/acre, and 0.500 lb AI/acre. In addition, there was an untreated control check plot. Samples of alfalfa were collected from each test plot at 2, 8, and 24 hours post-application. The alfalfa was diced and placed in cages. Bees were exposed to the NTN 33893-treated alfalfa by placing them in the cages containing the treated foliage. At the end of a 24 hour exposure period the percent mortality in each cage was determined.

Mortality tended to increase with application rate. The 0.045 lb AI/acre rate resulted in 5.6 - 11.9% mortality. The 0.167 lb AI/acre rate resulted in 11.7 - 16.1% mortality. The 0.500 lb AI/acre rate resulted in 11.8-23.1% mortality.

Mortality did not decline greatly as the age of the residues increased from 2 to 24 hours. In fact, 2 hour residues caused less mortality than 8 or 24 hour residues. This finding, which is not explainable based on the residue data, may have been an artifact of the experiment since mortality of control bees exhibited the same pattern.

Analytical results showed an increased residue concentration with increased application rate, as would be expected (Appendix II, Table 2). Residues were reduced 11, 30 and 17% at the 0.045, 0.167, and 0.500 lbs AI/acre applications, respectively, 24 hours after application.

The RT_{25} for each application rate was calculated. RT_{25} is the residual time required to reduce the activity of the chemical and bring mortality down to 25%. RT_{25} values were used by Washington State University personnel to classify the hazard potential of the test compound.

The RT_{25} for NTN 33893 at 0.045 lb AI/acre was less than 2 hours. According to the WSU scheme, this application rate may be applied with minimal hazard during early morning or late evening when bees are not actively foraging.

The RT_{25} for NTN 33893 at 0.167 lb AI/acre was less than 8 hours. According to the WSU scheme, this application rate may be applied with minimal hazard during late evening when bees are not actively foraging.

The RT_{25} for NTN 33893 at 0.500 lb AI/acre was approximately 8 hours. According to the WSU scheme, this application may be applied with moderate hazard during late evening when bees are not actively foraging.

INTRODUCTION

The study was conducted by Washington State University for Miles Incorporated, Agriculture Division to evaluate the potential hazard of NTN 33893 240FS to honey bees exposed to treated foliage. The study was conducted at Washington State University, Irrigated Agricultural Research and Extension Center, Rt. 2, Box 2953A, Prosser, WA 99350 using treated alfalfa from a field at the station. The first test was conducted from 9 September to 11 September, 1992 and the second test was conducted from 14 September to 16 September, 1992. Raw data and a copy of the final report are filed under Project Number 92-004 in [redacted] archives located at the station.

OBJECTIVE

The purpose of this test was to evaluate the hazard of field-weathered residues of NTN 33893 240FS to honey bees (*Apis mellifera* L.). An end-use formulation of NTN 33893 was applied to alfalfa at approximately 0.045 lb(AI)/acre, 0.167 lb(AI)/acre, and 0.5 lb(AI)/acre and the residues allowed to weather under natural conditions. At specific periods of time following application, treated foliage was collected and bees confined on the foliage and foliage analyzed for residues of NTN 33893. This allowed for determination of the duration of residual toxicity of NTN 33893 to honey bees.

MATERIALS AND METHODS

The methods, species used and route of administration described in this report are based on procedures specified in section 141-2 of the Environmental Protection Agency's Registration Guidelines (Pesticide Assessment Guidelines, FIFRA Subdivision L, 141-2, Hazard evaluation: Nontarget Insects - Honey bees - toxicity of residues on foliage (Anonymous 1982).

Test Substance

The test substance received from Miles Incorporated on 3 September was a FS formulation of NTN 33893 which contains approximately 240 grams of NTN 33893 per liter of formulation. It was identified on the label as: Bay NTN 33893, volume one quart, formula 1920-A, batch 2033004, 2020075. It was assigned WSU identification number 92-004 upon receipt.

Test Bees

The studies were conducted using Carniolan honey bees belonging to [redacted] Washington State University, IAREC, Prosser, WA. All hives are maintained according to good beekeeping practices. Healthy worker honey bees 3-4 weeks old were collected from the top frames of colonies and transported to the laboratory in a holding box. For each test one frame for each of 3 different colonies was removed from the hive. The bees were anaesthetized with carbon dioxide in the holding box to facilitate handling. In order to control bias, bees were impartially distributed to test cages. About fifty bees were placed in each test cage.

Study Design

A field of 'Washo' alfalfa grown under standard agronomic practices was used. Eight plots, each approximately 0.01 acre, were established in the field and arranged in a randomized block design (Figure 1). Two tests were done. The first test series was started at 8 am on 9 September and the second test series was started at 8 am on 14 September. Different plots of alfalfa were used for each test series. Alfalfa was aged and weathered in the field under ambient outdoor conditions. At 2 hours after application foliage was picked from 12 sites within each plot and placed in separate plastic bags for transporting to the laboratory. At 8 hours after application foliage was again collected as described above. At 24 hours after application foliage was again collected as described above. Foliage was chopped, and placed in the bee test cages. About fifty bees were placed in each test cage containing the chopped foliage. The number of dead and live bees were recorded 24 hours later and percent mortality determined. Experimental design was as follows:

Test Residues and Cages

	Age of Residues	Cages *
Plot #1-1	2 hr	1, 2, 3
Plot #1-2	2 hr	1, 2, 3
Plot #2-1	2 hr	1, 2, 3
Plot #2-2	2 hr	1, 2, 3
Plot #3-1	2 hr	1, 2, 3
Plot #3-2	2 hr	1, 2, 3
Plot #4-1	2 hr	1, 2, 3
Plot #4-2	2 hr	1, 2, 3
Plot #1-1	8 hr	1, 2, 3
Plot #1-2	8 hr	1, 2, 3
Plot #2-1	8 hr	1, 2, 3
Plot #2-2	8 hr	1, 2, 3
Plot #3-1	8 hr	1, 2, 3
Plot #3-2	8 hr	1, 2, 3
Plot #4-1	8 hr	1, 2, 3
Plot #4-2	8 hr	1, 2, 3
Plot #1-1	24 hr	1, 2, 3
Plot #1-2	24 hr	1, 2, 3
Plot #2-1	24 hr	1, 2, 3
Plot #2-2	24 hr	1, 2, 3
Plot #3-1	24 hr	1, 2, 3
Plot #3-2	24 hr	1, 2, 3
Plot #4-1	24 hr	1, 2, 3
Plot #4-2	24 hr	1, 2, 3

* Each cage has about 50 bees and approximately 500 cc foliage

Pesticide Application

The formulated product of NTN 33893 was applied at 0.045 lb (AI)/acre, 0.167 lb(AI)/acre and 0.5 lb(AI)/acre using water as the carrier at 26 gallons per acre with a R&D CO_2 pressurized sprayer using a hand-held boom with 4 (TJ-60) nozzles. For the first test series the application was on 9 September and for the second test series the application was on 14 September. Each plot was approximately 0.01 acre and replicated 2 times. Spray water pH was 7.5.

Each spray solution was prepared by measuring out a calculated amount of NTN 33893 using a caliperated Eppendorf pipette. NTN 33893 was than mixed with water to prepare a 2 liter solution. Approximately 1 liter of the spray solution was applied to each replicated plot.

<u>Test Cages</u>

Cages were constructed with the tops and bottoms of 150 x 15 mm plastic petri plates. Wire screen was cut in a strip 46 x 5 cm and the ends stapled to form cylinder (Figure 2). Petri plates served as top and bottom of the cage. Bees were fed during testing by providing cotton squares (5 x 5 cm) soaked with 50 percent sugar syrup and placed under the treated foliage.

<u>Test Exposure of Bees</u>

Residues were allowed to weather in the field for a specific time prior to collection of foliage samples for testing. Foliage was collected and tested for bee toxicity 2, 8 and 24 hours after application. Foliage samples (about 0.8 kilograms) were collected from the top 15 cm portion of plants from 12 randomly chosen locations within a plot for each time interval. Samples were bulked and chopped into 2.5 cm lengths. The chopped foliage was mixed and a subsample of foliage removed (approximately 500 cc) and placed in each cage. In order to control bias, bees were impartially distributed to test cages. Bees were immobilized with carbon dioxide and about 50 bees placed in each cage. There were 3 cages for each NTN 33893 treated plot and 3 cages for each untreated check plot for each time interval.

<u>Residue Analysis</u>

Samples of the alfalfa foliage treated with NTN 33893 at 0.045 lb(AI)/acre, NTN 33893 at 0.167 lb(AI)/acre and NTN 33893 at 0.5 lb(AI)/acre and the untreated checks were collected from the plots at 2, 8 and 24 hours after application. All samples were frozen and sent to Miles Incorporated, Stilwell, KS for analysis.

<u>Environmental Conditions</u>

The bees in the cages were held in the laboratory at 12 hours light. The temperature was 24° to 25° C. and relative humidity averaged 58%.

Weather conditions were carefully monitored using the PAWS automated weather station located about 150 yards from the plots during the testing. Temperature, solar radiation, precipitation, relative humidity and wind were monitored (Table 2).

<u>Observations</u>

At 24 hours after exposure of bees to the foliage the number of dead and live bees in each cage were recorded and the percent mortality determined.

<u>Statistical Analysis</u>

Newman-Keuls sequential studentized range was used for separation of means (Snedecor and Cochran 1980).

Mortality

The number of live bees, dead bees and percent mortality for each replication is given in Appendix III. The mean percent mortality for both tests is summarized in table 3. The mean percent mortality for NTN 33893 (0.045 lb(AI)/acre with 2 hour old residues was 5.6%, with 8 hour old residues 7.2% and with 24 hour old residues 11.9%. The mean percent mortality for NTN 33893 (0.167 lb(AI)/acre with 2 hour old residues was 11.7, with 8 hour old residues 16.1% and with 24 hour old residues 15.9%. The mean percent mortality for NTN 33893 (0.5 lb(AI)/acre with 2 hour old residues was 11.8, with 8 hour old residues 23.1% and with 24 hour old residues 20.8%. With the exception of the 2-hour residues from the 0.045 lb(AI)/acre rate there was significantly more mortality with all the rates with 2, 8 and 24 hour old residues as compared to the untreated check.

Conclusion

The residue bioassay data were used to classify the hazard of the test materials according to the scheme of Johansen and Mayer (1990). RT_{25} indicates the residual time required to reduce the activity of the chemical and bring bee mortality down to 25% in cage test exposures to field-weathered spray deposits.

RT_{25} greater than 8 hours.	Do not apply or allow to drift on blooming crops or weeds.
RT_{25} between 2 and 8 hours.	May be applied during late evening or night with minimal hazard to bees.
RT_{25} less than 2 hours.	May be applied with minimal hazard to bees when they are not actively foraging.

The RT_{25} for NTN 33893 at 0.045 lb(AI)/acre was less than 2 hours. According to this scheme NTN 33893 at 0.045 lb(AI)/acre may be applied with minimal hazard to honey bees during early morning or late evening when bees are not actively foraging.

The RT_{25} for NTN 33893 at 0.167 lb(AI)/acre was less than 8 hours. According to this scheme NTN 33893 at 0.167 lb(AI)/acre may be applied with minimal hazard to honey bees during late evening when bees are not actively foraging.

The RT_{25} for NTN 33893 at 0.5 lb(AI)/acre was about 8 hours. According to this scheme NTN 33893 at 0.5 lb(AI)/acre may be applied with moderate hazard to honey bees during late evening when bees are not actively foraging.

REFERENCES

Anonymous, 1982. Pesticide assessment guidelines, FIFRA subdivision L, hazard evaluation: non-target insects, subsection 141-2, Environmental Protection Agency, Office of Pesticide Programs.

Johansen, C.A. and D.F. Mayer. 1990. Pollinator protection: A bee and pesticide handbook. Wicwas Press, Cheshire, CT. 212 pp.

Snedecor, C.W. and W.G. Cochran. 1980. Statistical methods. 7th Edition. Ames, Iowa: Iowa State Press.

FIGURE 1

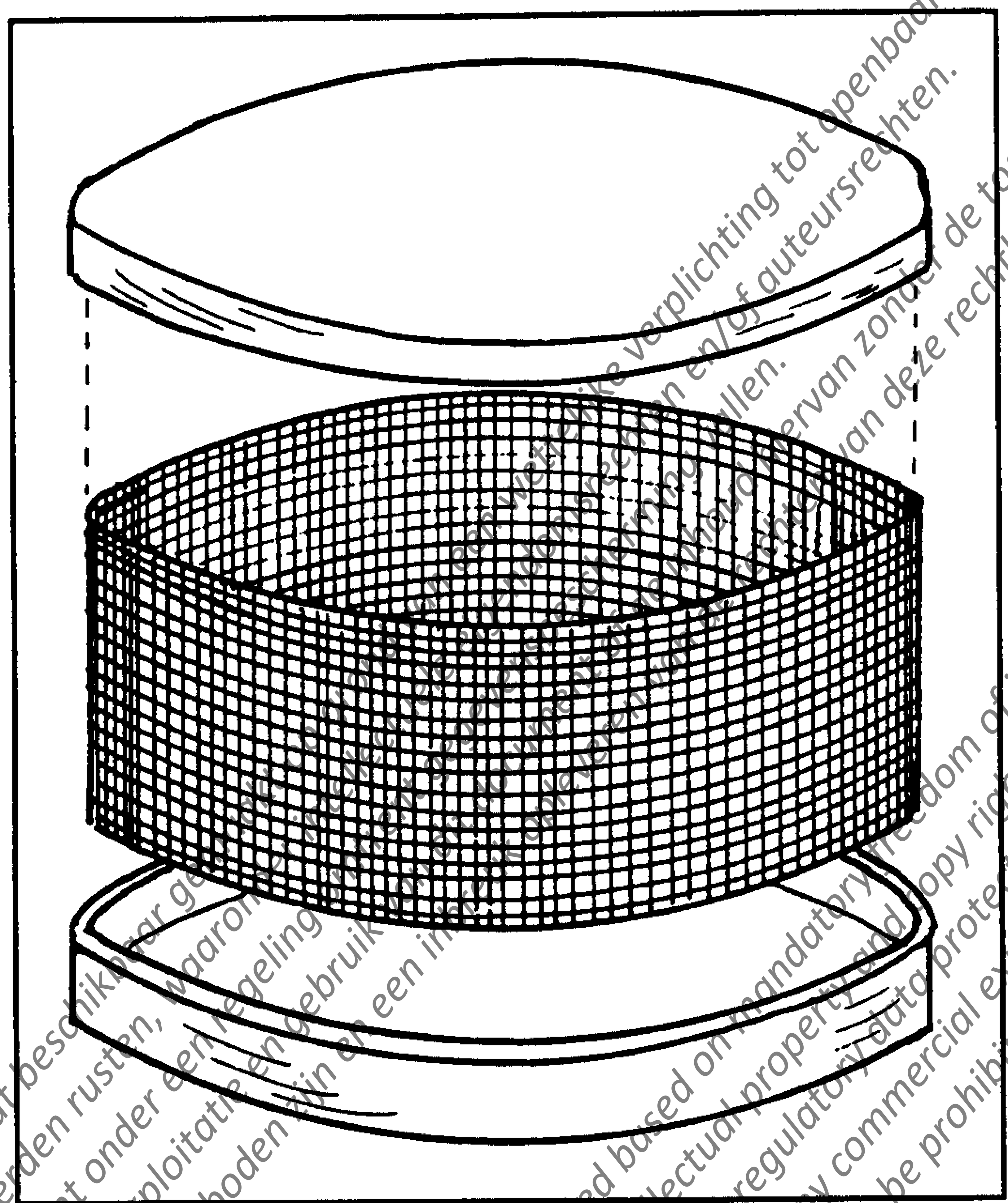
POSITION OF REPLICATED PLOTS

TEST NUMBER 1	
Plot #1-1 treated with NTN 33893 (0.045 lb(AI)/a)	Plot #4-2 untreated check
Plot #3-1 treated with NTN 33893 (0.5 lb(AI)/a)	Plot #2-2 treated with NTN 33893 (0.167 lb(AI)/a)
Plot #4-1 untreated check	Plot #3-2 treated with NTN 33893 (0.5 lb(AI)/a)
Plot #2-1 treated with NTN 33893 (0.167 lb(AI)/a)	Plot #1-2 treated with NTN 33893 (0.045 lb(AI)/a)

TEST NUMBER 2	
Plot #4-1 untreated check	Plot #1-2 treated with NTN 33893 (0.045 lb/(AI)/a)
Plot #3-1 treated with NTN 33893 (0.5 lb(AI)/a)	Plot #2-2 treated with NTN 33893 (0.167 lb(AI)/a)
Plot #1-1 treated with NTN 33893 (0.045 lb(AI)/a)	Plot #4-7 untreated check
Plot #2-1 treated with NTN 33893 (0.167 lb(AI)/a)	Plot #3-2 treated with NTN 33893 (0.05 lb(AI)/a)

FIGURE 2

DIAGRAM OF TEST CAGES



1. *Disposable cage formed from two top or bottom halves of 150 x 15 mm petri plates and 18 x 2 inch (45.7 x 5.1 cm) wire screen strip stapled to form cylinder.*

TABLE 1

APPLICATION AND COLLECTION SCHEDULE

	Date	Time
Application 9/9/92 at 8:30 am		
2-hour residues collected	9/9/92	10:30 am
8-hour residues collected	9/9/92	4:30 pm
24-hour residues collected	9/10/92	8:30 am
Application 9/14/92 at 8:30 am		
2-hour residues collected	9/14/92	10:30 am
8-hour residues collected	9/14/92	4:30 pm
24-hour residues collected	9/15/92	8:30 am

TABLE 2

WEATHER CONDITIONS DURING NTN 33893 FOLIAGE STUDY

WSU-IAREC
Public Agricultural Weather System
[redacted]

Weather Data for: WSU-ROZA UNIT - 7.0 miles NNE of [redacted]
Hourly Values

Date mm/dd/yy	Hour of the Day PST	Total Solar Rad Langley	Avg Air Temp F 5.25ft	Avg Dewpt Temp F 5.25ft	Avg Wind Speed 6.6ft	Avg Wind Dir deg 6.6ft	Dev in Dir	Avg Rel Humid %	Total Precip inches	Leaf Wetness total minutes	Max Air Temp F 5.25ft	Min Air Temp F 5.25ft
09/09/92	0800	7255	56.97	46.33	2.37	203.7	26.44	68.01	0.00	0	58.03	56.10
09/09/92	0900	11440	59.23	47.61	3.07	187.5	19.87	65.79	0.00	0	61.09	57.33
09/09/92	1000	15086	62.62	48.61	2.79	197.2	33.37	60.62	0.00	0	64.89	60.58
09/09/92	1100	17582	65.95	50.00	2.69	155	50.21	56.85	0.00	0	67.68	64.20
09/09/92	1200	18896	68.97	49.60	2.85	162.3	49.64	50.52	0.00	0	70.74	67.05
09/09/92	1300	18872	71.33	47.88	2.68	133.9	40.67	43.64	0.00	0	72.90	69.49
09/09/92	1400	17606	73.20	47.75	2.25	152.3	62.06	40.74	0.00	0	74.53	72.03
09/09/92	1500	14754	74.52	48.60	2.40	127.2	43.25	40.27	0.00	0	75.79	73.51
09/09/92	1600	10932	74.28	49.24	4.23	80.7	29.85	41.59	0.00	0	75.27	73.71
09/09/92	1700	6634	73.40	47.46	3.89	78.1	20.04	40.03	0.00	0	74.23	72.41
09/09/92	1800	2217	69.64	45.46	3.25	63.74	20.2	42.15	0.00	0	72.45	65.71
09/09/92	1900	104.7	65.16	41.59	3.53	11.64	13.52	42.35	0.00	0	66.79	63.14
09/09/92	2000	0.00	62.82	39.11	4.85	4.161	10.04	41.62	0.00	0	64.11	61.29
09/09/92	2100	0.00	62.80	40.14	4.88	4.587	8.65	43.41	0.00	0	64.54	60.48
09/09/92	2200	0.00	63.64	40.27	4.90	6.281	8.03	43.89	0.00	0	64.09	61.47
09/09/92	2300	0.00	61.90	40.38	4.36	5.783	9.24	45.24	0.00	0	63.14	60.28
09/09/92	2400	0.00	57.49	39.51	2.27	.868	49.17	51.41	0.00	0	61.79	53.74
09/10/92	0100	0.00	55.71	40.41	3.19	353.9	8.88	56.55	0.00	0	57.94	53.96
09/10/92	0200	0.00	54.99	39.51	2.66	357.6	7.73	56.02	0.00	0	57.54	52.41
09/10/92	0300	0.00	52.97	38.93	3.91	.864	14.3	58.93	0.00	0	56.03	50.65
09/10/92	0400	0.00	52.11	38.83	2.29	4.425	20.08	60.58	0.00	0	55.44	49.95
09/10/92	0500	0.00	52.39	39.44	1.70	328.4	24.06	61.33	0.00	0	54.50	49.69
09/10/92	0600	180.4	51.87	40.27	2.56	332.6	21.84	64.7	0.00	0	53.44	49.86
09/10/92	0700	2162	53.64	41.41	0.69	18.61	58.86	63.43	0.00	0	55.20	52.66
09/10/92	0800	5253	57.90	45.25	0.82	286.1	47.19	63.14	0.00	0	62.11	54.66
09/10/92	0900	10220	61.29	47.55	1.95	181.3	34.28	61.05	0.00	0	63.81	59.77

WSU-IAREC
Public Agricultural Weather System
[redacted]

Weather Data for: WSU-ROZA UNIT - 7.0 miles NNE of [redacted]
Hourly Values

Date mm/dd/yy	Hour of the Day PST	Total Solar Rad Langley	Avg Air Temp F 5.25ft	Avg Dewpt Temp F 5.25ft	Avg Wind Speed 6.6ft	Avg Wind Dir deg 6.6ft	Dev in Dir	Avg Rel Humid %	Total Precip inches	Leaf Wetness total minutes	Max Air Temp F 5.25ft	Min Air Temp F 5.25ft
09/14/92	0800	2203	46.15	37.80	2.71	268	15.89	72.6	0.00	0	47.44	45.48
09/14/92	0900	4778	49.12	39.56	3.67	247.8	23.48	69.69	0.00	0	50.68	47.41
09/14/92	1000	6431	51.17	40.83	3.80	223.9	20.24	67.85	0.00	0	52.29	50.36
09/14/92	1100	8392	53.24	41.99	3.24	212.1	24.61	65.82	0.00	0	54.70	51.73
09/14/92	1200	7876	55.27	42.42	2.54	224.4	26.53	62.15	0.00	0	55.98	54.16
09/14/92	1300	6567	56.48	42.68	1.32	217.3	46.18	60.1	0.00	15.33	57.61	55.45
09/14/92	1400	4161	56.91	42.79	1.30	202.2	42.48	59.43	0.00	.833	57.87	56.39
09/14/92	1500	2702	56.73	43.37	1.81	195.4	23.56	61.16	0.00	2.166	57.27	56.44
09/14/92	1600	2074	57.15	43.40	0.26	265.4	60.21	60.32	0.00	0	57.40	56.77
09/14/92	1700	1089	56.53	43.32	0.19	100.3	66.56	61.49	0.00	45.32	57.15	55.53
09/14/92	1800	299.8	55.06	43.36	0.87	48.27	37.09	64.98	0.00	17.49	55.56	53.64
09/14/92	1900	6.88	52.95	43.69	1.11	39.99	16	71.1	0.00	52.31	53.91	52.50
09/14/92	2000	0.00	52.57	43.54	1.48	32.71	17.41	71.7	0.00	36.65	53.13	51.78
09/14/92	2100	0.00	52.66	43.41	2.29	9.26	10.18	71.1	0.00	12.33	53.13	51.75
09/14/92	2200	0.00	51.57	43.76	1.84	10.29	12.6	75	0.01	59.98	52.56	50.68
09/14/92	2300	0.00	50.85	44.38	1.57	8.91	15.12	79	0.00	59.98	51.71	49.59
09/14/92	2400	0.00	49.32	45.43	2.11	354.8	6.287	87	0.03	59.98	49.80	48.96
09/15/92	0100	0.00	49.10	45.54	0.81	349.9	4.827	88	0.01	59.98	49.33	48.81
09/15/92	0200	0.00	49.08	44.94	1.83	350.9	6.966	86.1	0.00	59.98	49.30	48.81
09/15/92	0300	0.00	48.87	45.39	0.10	347.4	37.41	88.3	0.03	59.98	49.28	48.61
09/15/92	0400	0.00	48.52	45.81	0.10	224.5	42.89	90.8	0.01	59.98	48.85	48.11
09/15/92	0500	0.00	48.11	45.41	0.10	249.5	28.91	90.9	0.00	59.98	48.38	47.84
09/15/92	0600	17.15	48.04	44.94	0.10	327.4	10.73	89.5	0.00	59.98	48.54	47.71
09/15/92	0700	367.9	48.54	45.50	0.10	140.6	54.88	89.7	0.01	59.98	48.85	48.18
09/15/92	0800	878.6	48.40	45.39	0.10	240.3	28.22	89.9	0.02	59.98	48.81	47.93
09/15/92	0900	2470	49.44	46.22	0.10	190.5	43.97	89.2	0.02	59.98	49.96	48.63

TABLE 3

MORTALITY (%) SUMMARY FOR HONEY BEES EXPOSED TO NTN 33893 240FS

Mortalities of honey bees (HB) exposed to different age residues of NTN 33893 applied at different rates to 0.01 acre plots of alfalfa, mean of two tests. First test started 9 September, second test started 14 September. [redacted] 1992.

Treatment	lb(AI)/a	24 hr % mortalities of HB caged with treated foliage, age of residues		
		2 hr	8 hr	24 hr
NTN 33893 240 FS	0.045	5.6a	7.2a	11.9a
NTN 33893 240 FS	0.167	11.7b	16.1b	15.9a
NTN 33893 240 FS	0.5	11.8b	23.1c	20.8b
Untreated check	--	1.7a	2.7d	4.2c

Means within a column followed by the same letter are not significantly different at the P= 0.05 level, Newman-Keuls sequential studentized range test.

APPENDIX I

TANK-MIX CALCULATIONS

Desired Rate (lb(AI)acre)
Concentration (lb(AI)/gallon of material

Plot size = 0.01 acre

Application rate = 1 liter/0.01 acre

NTN 33893 concentration = 240 grams/liter

Desired rate = 0.045 lb(AI)/acre

0.045 lb = 20.4 gms

20.4 gms divided by 240 gms/liter = 0.085 liters/acre x 1000 mls/liter = 85 mls/a

85 mls/a divided by 100 = 0.85 mls/0.01 acre

Desired rate = 0.167 lb(AI)/acre

0.0167 lb = 75.8 gms

75.8 gms divided by 240 gms/liter = 0.315 liters/acre x 1000 mls/liter = 315 mls/a

315 mls/a divided by 100 = 3.15 mls/0.01 acre

Desired rate = 0.5 lb (AI)/acre

0.5 lb = 226.8 gms

226.8 gms divided by 240 gms/liter = 0.945 liters/acre x 1000 mls/liter = 945 mls/a

945 mls/a divided by 100 = 9.45 mls/0.01 acre

APPENDIX II

TEST SPRAY MIX PREPARATION

The test mixture was prepared as follows:

0.045 lb(AI)/acre
0.85 mls NTN 33893/1 liter of water

0.167 lb(AI)/acre
3.15 mls NTN 33893/1 liter of water

0.5 lb(AI)/acre
9.45 mls NTN 33893/1 liter of water

The calculated amount of NTN 33893 was removed from the original container using a calibrated Eppendorf pipette and transferred into the 2 liter spray bottle. The mixture was shaken and turned into a milky white emulsion. The two high rates remained milky however the low rate cleared up in a few minutes. Two 10 ml samples of each solution were taken and placed in an ice chest with ice and shipped the same day to Miles Incorporated, Kansas City, MO. Approximately 1 liter was applied to each replicated plot.

APPENDIX III

HONEY BEE MORTALITY BY REPLICATE

Table 1. Mortalities of honey bees exposed to 2 hour residues of NTN 33893 applied to 0.01 acre plots of alfalfa. Applications done 9 September, Prosser, WA 1992.

Treatment	No. alive	No. dead	% Mortality
NTN 33893 (0.045 lb)			
Rep 1-1	47	1	2.1
Rep 1-2	50	2	3.8
Rep 1-3	50	2	3.8
Rep 2-1	46	2	4.2
Rep 2-2	47	1	2.1
Rep 2-3	49	1	2.0
Total	289	9	Mean 3.0
NTN 33893 (0.167 lb)			
Rep 1-1	46	6	11.5
Rep 1-2	48	2	4.0
Rep 1-3	49	1	2.0
Rep 2-1	47	2	4.1
Rep 2-2	48	1	2.0
Rep 2-3	45	5	10.0
Total	283	17	Mean 5.7
NTN 33893 (0.5 lb)			
Rep 1-1	48	2	4.0
Rep 1-2	45	5	10.0
Rep 1-3	42	7	14.3
Rep 2-1	46	3	6.1
Rep 2-2	44	6	12.0
Rep 2-3	49	3	5.8
Total	274	26	Mean 8.7
Untreated check			
Rep 1-1	49	0	0
Rep 1-2	48	0	0
Rep 1-3	50	0	0
Rep 2-1	48	0	0
Rep 2-2	50	0	0
Rep 2-3	49	1	2
Total	294	1	Mean 0.3

Table 2. Mortalities of honey bees exposed to 8 hour residues of NTN 33893 applied to 0.01 acre plots of alfalfa. Applications done 9 September. Prosser, WA 1992.

Treatment	No. alive	No. dead	% Mortality
NTN 33893 (0.045 lb)			
Rep 1-1	48	2	4.0
Rep 1-2	51	3	5.6
Rep 1-3	44	7	13.8
Rep 2-1	46	4	8.0
Rep 2-2	51	1	1.9
Rep 2-3	44	2	4.3
Total	284	19	Mean 6.3
NTN 33893 (0.167 lb)			
Rep 1-1	40	8	16.7
Rep 1-2	43	6	12.2
Rep 1-3	47	4	7.8
Rep 2-1	49	8	14.0
Rep 2-2	43	5	10.4
Rep 2-3	42	8	16.0
Total	264	39	Mean 12.9
NTN 33893 (0.5 lb)			
Rep 1-1	45	10	18.2
Rep 1-2	43	15	25.9
Rep 1-3	42	10	19.2
Rep 2-1	48	7	12.7
Rep 2-2	36	12	25.0
Rep 2-3	42	9	17.6
Total	256	63	Mean 19.7
Untreated check			
Rep 1-1	49	5	9.3
Rep 1-2	50	1	2.0
Rep 1-3	49	2	3.9
Rep 2-1	48	2	4.0
Rep 2-2	51	0	0.0
Rep 2-3	49	2	3.9
Total	296	12	Mean 4.1

Table 3. Mortalities of honey bees exposed to 24 hour residues of NTN 33893 applied to 0.01 acre plots of alfalfa. Applications done 9 September. Prosser, WA 1992.

Treatment	No. alive	No. dead	% Mortality
NTN 33893 (0.045 lb)			
Rep 1-1	43	5	10.4
Rep 1-2	44	5	10.2
Rep 1-3	45	6	11.8
Rep 2-1	39	5	11.4
Rep 2-2	39	8	17.0
Rep 2-3	49	4	7.5
Total	259	33	Mean 11.3
NTN 33893 (0.167 lb)			
Rep 1-1	40	7	14.9
Rep 1-2	45	9	16.7
Rep 1-3	43	4	8.5
Rep 2-1	36	10	21.2
Rep 2-2	44	4	8.3
Rep 2-3	39	10	20.4
Total	247	44	Mean 15.1
NTN 33893 (0.5 lb)			
Rep 1-1	38	10	20.8
Rep 1-2	36	14	28.0
Rep 1-3	38	15	28.3
Rep 2-1	46	4	8.0
Rep 2-2	45	10	18.2
Rep 2-3	33	12	26.7
Total	236	65	Mean 21.6
Untreated check			
Rep 1-1	46	5	9.8
Rep 1-2	48	6	11.1
Rep 1-3	49	2	3.9
Rep 2-1	48	1	2.0
Rep 2-2	43	7	14.0
Rep 2-3	48	4	7.7
Total	282	25	Mean 8.1

Table 4. Mortalities of honey bees exposed to 2 hour residues of NTN 33893 applied to 0.01 acre plots of alfalfa. Applications done 14 September. Prosser, WA 1992.

Treatment	No. alive	No. dead	% Mortality
NTN 33893 (0.045 lb)			
Rep 1-1	34	13	27.7
Rep 1-2	48	3	5.9
Rep 1-3	45	1	2.2
Rep 2-1	48	0	0.0
Rep 2-2	47	3	6.0
Rep 2-3	43	4	8.5
Total	265	24	Mean 8.3
NTN 33893 (0.167 lb)			
Rep 1-1	39	10	20.4
Rep 1-2	44	4	8.3
Rep 1-3	42	12	22.2
Rep 2-1	43	8	15.7
Rep 2-2	45	9	16.7
Rep 2-3	36	11	23.4
Total	248	54	Mean 17.9
NTN 33893 (0.5 lb)			
Rep 1-1	43	5	10.4
Rep 1-2	48	2	4.0
Rep 1-3	40	8	16.7
Rep 2-1	39	11	22.0
Rep 2-2	41	9	18.0
Rep 2-3	39	9	18.8
Total	250	44	Mean 15.0
Untreated check			
Rep 1-1	51	0	0.0
Rep 1-2	44	2	4.3
Rep 1-3	50	0	0.0
Rep 2-1	43	5	10.4
Rep 2-2	50	1	2.0
Rep 2-3	48	1	2.0
Total	286	9	Mean 3.1

Table 5. Mortalities of honey bees exposed to 8 hour residues of NTN 33893 applied to 0.01 acre plots of alfalfa. Applications done 14 September. Prosser, WA 1992.

Treatment	No. alive	No. dead	% Mortality
NTN 33893 (0.045 lb)			
Rep 1-1	48	3	5.9
Rep 1-2	48	3	5.9
Rep 1-3	44	6	12.0
Rep 2-1	45	1	2.2
Rep 2-2	44	4	8.3
Rep 2-3	43	7	14.0
Total	272	24	Mean 8.1
NTN 333893 (0.167 lb)			
Rep 1-1	37	11	22.9
Rep 1-2	40	9	18.4
Rep 1-3	39	9	18.8
Rep 2-1	43	8	15.7
Rep 2-2	40	12	23.1
Rep 2-3	41	9	18.0
Total	240	58	Mean 19.5
NTN 33893 (0.5 lb)			
Rep 1-1	37	12	24.5
Rep 1-2	41	11	21.2
Rep 1-3	38	12	24.0
Rep 2-1	38	10	20.8
Rep 2-2	34	18	34.6
Rep 2-3	33	17	34.0
Total	221	80	Mean 26.6
Untreated check			
Rep 1-1	52	2	3.7
Rep 1-2	47	0	0.0
Rep 1-3	51	0	0.0
Rep 2-1	50	1	2.0
Rep 2-2	48	2	4.0
Rep 2-3	47	0	0.0
Total	295	5	Mean 1.7

Table 6. Mortalities of honey bees exposed to 24 hour residues of NTN 33893 applied to 0.01 acre plots of alfalfa. Applications done 14 September. Prosser, WA 1992.

Treatment	No. alive	No. dead	% Mortality
NTN 33893 (0.045 lb)			
Rep 1-1	44	4	8.3
Rep 1-2	50	4	7.4
Rep 1-3	54	7	11.5
Rep 2-1	44	7	13.7
Rep 2-2	40	12	23.1
Rep 2-3	56	7	11.1
Total	288	41	Mean 12.5
NTN 33893 (0.167 lb)			
Rep 1-1	38	8	17.4
Rep 1-2	46	4	8.0
Rep 1-3	40	14	25.9
Rep 2-1	49	6	11.1
Rep 2-2	44	7	13.7
Rep 2-3	38	12	24.0
Total	255	51	Mean 16.7
NTN 33893 (0.5 lb)			
Rep 1-1	40	10	20.0
Rep 1-2	37	9	19.6
Rep 1-3	41	13	24.1
Rep 2-1	49	10	16.9
Rep 2-2	36	9	20.0
Rep 2-3	38	9	19.1
Total	241	60	Mean 19.9
Untreated check			
Rep 1-1	47	0	0.0
Rep 1-2	50	0	0.0
Rep 1-3	52	0	0.0
Rep 2-1	49	0	0.0
Rep 2-2	48	0	0.0
Rep 2-3	51	1	1.9
Total	297	1	Mean 0.3

APPENDIX IV

PERSONNEL INVOLVED IN STUDY

The following people from Washington State University were involved in this study:

1. [redacted]
2.

Appendix 2

Analysis of Alfalfa for Residues of Imidacloprid

Report on the In-Life Phase by

[redacted]

Miles Inc., Agriculture Division

Study Title

Analysis of Alfalfa for Residues of Imidacloprid

Data Requirement

EPA Guideline Ref. No.: 141-2 Hazard Evaluation: Nontarget Insects

Author

[redacted]

Completion Date

October 22, 1992

Performing Laboratory

Miles Residue Analysis Laboratory
Agriculture Division
Environmental Research
Research and Development Department
17745 South Metcalf
Stilwell, Kansas 66085

Submitting Laboratory

Miles Incorporated
Agriculture Division
Research and Development Department
P. O. Box 4913
Kansas City, Missouri 64120

Performing Laboratory ID Number

PR 92321

Statement of No Data Confidentiality Claims

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

Company: Miles Incorporated
Agriculture Division
Research and Development Department
Environmental Research

[redacted]

These data are the property of the Agriculture Division of Miles Incorporated, and as such, are considered to be confidential for all purposed other than compliance with FIFRA 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

Good Laboratory Practice Certification

[redacted]

Certification of Availability of Raw Data

[redacted]

Quality Assurance Statement

[redacted]

Certification of Authenticity

[redacted]

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Stilwell, Kansas 66085

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Analysis of Alfalfa for Residues of Imidacloprid

1.0 Abstract

An imidacloprid hazard evaluation study was conducted on alfalfa by Washington State University (WSU) consisting of two tests of eight plots each. The plots were treated at rates of: control, 0.045 lbs AI/acre, 0.167 lbs AI/acre, and 0.500 lbs AI/acre. Samples were collected from each of the treatment rates of each of the tests--at predetermined intervals of 2, 8 and 24 hours--to characterize the levels of total imidacloprid residues present. Sample analyses presented in this report include measurement of alfalfa foliage only.

As stated above, samples were collected by WSU. They were then shipped to Miles Research Park. Upon receipt, samples were logged in by Miles personnel, and stored immediately in the freezer at -20°C. Samples were then analyzed for total imidacloprid residues by the Residue Analysis Laboratory (RAL) of the Environmental Research Section.

All residues in each of the control samples, not including samples suspected to be contaminated in laboratory sample preparation, were found to be below the limit of determination (0.50 ppm). At the low treatment rate of 0.045 lbs AI/acre, average residues were; 3.01 ppm at 2 hours, 4.92 ppm at 8 hours, and 2.69 ppm at 24 hours. At the medium treatment rate of 0.167 lbs AI/acre, average residues were; 18.64 ppm at 2 hours, 12.35 ppm at 8 hours, and 13.17 ppm at 24 hours. At the high treatment rate of 0.500 lbs AI/acre, average residues were; 54.72 ppm at 2 hours, 54.26 ppm at 8 hours, and 45.77 ppm at 24 hours.

2.0 Introduction

The field portion of this study was conducted by Washington State University (WSU). The analyses of alfalfa samples from the field tests were conducted by the Residue Analysis Laboratory (RAL) of Miles, Inc. (formerly Mobay Corporation), Agriculture Division, Environmental Research Section. Alfalfa residue data are presented in this report to support the registration of imidacloprid.

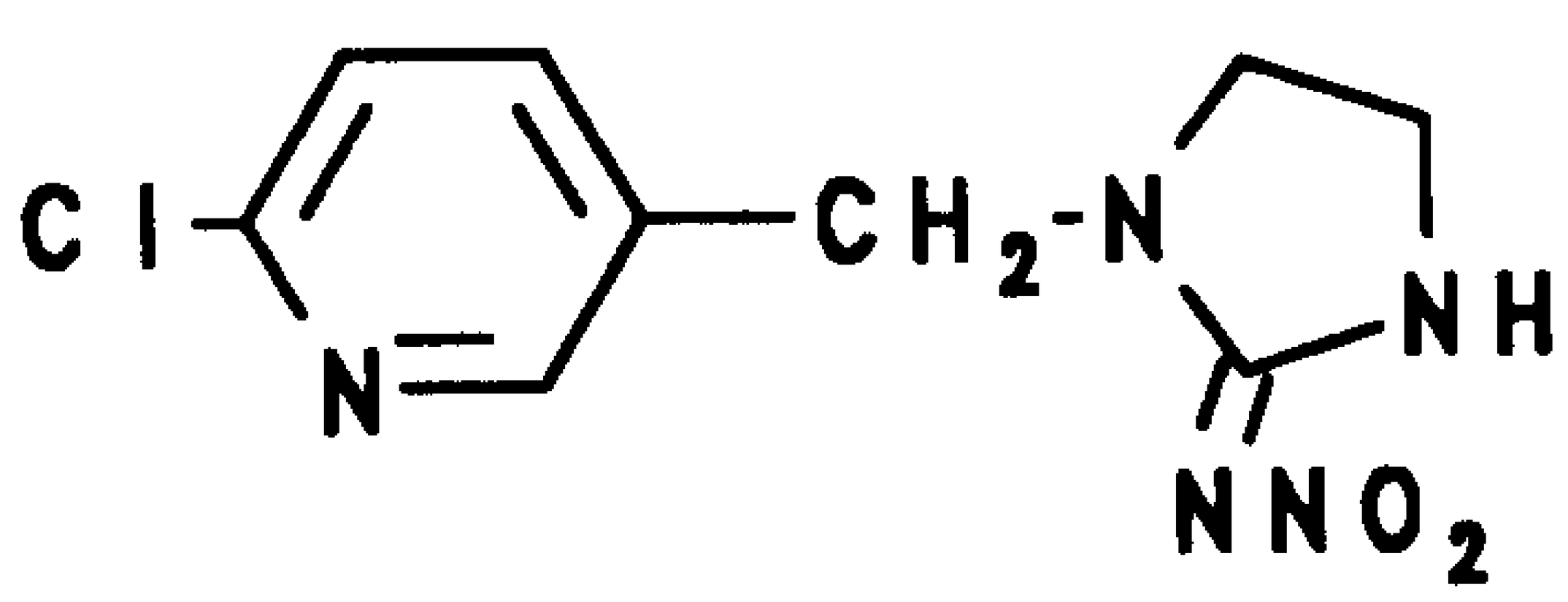
Chemical Name:

1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-*N*-nitro-1*H*-imidazol-2-amine

Common Name: Imidacloprid

Experimental Name: NTN 33893

Chemical Structure:



NTN 33893

These data are being submitted in partial fulfillment of the requirements of Section 141-2, entitled "Hazard Evaluation: Nontarget Insects", in Subdivision L of the U.S. EPA Pesticide Assessment Guidelines.

3.0 Experimental

3.1 Sample Receipt and Storage

A bulk control sample of alfalfa was received by RAL personnel at Miles Research Park on July 21, 1992. This sample was shipped from Wildlife International Ltd. to use for method validation of a similar study that has since been canceled. Upon receipt, the sample was given the Miles Sample No. 93706, and stored in Freezer Storage #3 (-20°C) for later use in analyses. Miles Sample No. 93706 was utilized for method validation and some of the concurrent recoveries included in this report.

Field samples of alfalfa included in this report were collected in the field by personnel from WSU and shipped to Miles Research Park. Two shipments of samples from the field were made, these being received at Miles Research Park on September 16, and September 17, 1992. Upon receipt, samples were logged in and immediately transferred to Freezer Storage #3 (-20°C) by RAL personnel. Samples were removed from the freezer, as needed for homogenizing, aliquoting and analysis. Following subsampling, the original sample remainder was returned to the freezer for storage.

3.2 Materials

3.2.1 Apparatus

Beaker, Berzelius tall-form, 600-mL
Beakers, Griffin, 100-mL, 200-mL, 500-mL
Blender, e.g. Polytron (Brinkmann Instruments, Westbury, NY)
Chromatography column, 25cm X 10 mm ID, with glass frit, or equivalent
Condenser, reflux, with standard taper 24/40 inner joint
Cylinders, graduated, 100-mL, 250-mL, 500-mL, 1000-mL
Hobart Food Processor, VCM 25 or 40 (or equivalent)
Flasks, flat-bottom, with standard taper 24/40 joint, 250-mL, 500-mL
Flask, vacuum filter, 1000-mL, 6-liter
Funnel, Buchner, 110-mm with fast filter paper, e.g. Whatman No. 541
Funnel, glass, 80-mm
Funnel, separatory, 500-mL
Funnel, sintered glass filter, 4-liter
Gas chromatography - mass spectrometry (GC-MS) system comprised of:
  Hewlett Packard HP 5890-Series II gas chromatograph
  Hewlett Packard HP 7673 autosampler
  Hewlett Packard HP 5971 mass specific detector
  Hewlett Packard HP DOS Workstation data system
  GC capillary column, 0.20 mm ID, with dimethylsilicone stationary phase, 0.33 μm film, e.g. Hewlett Packard ULTRA-1
Ice bath
Laboratory hotplate/stirrer, e.g. Corning PC-320 (Corning, NY)
Magnetic stirring bar

Microliter syringe, 250-μL
Pipettes, bulb, 2-mL, 5-mL, 25-mL, 50-mL
Pipettes, calibrated, 5-mL, 10-mL, 50-mL
Pipettes, graduated, 2-mL, 5-mL, 10-mL
Rotary evaporator, with controlled temperature water bath
Teflon collars for standard taper 24/40 joints
Thermometer

3.2.2 <u>Reagents/Supplies</u>

Acetonitrile, HPLC grade
Amberlite XAD-4 resin, 20-60 mesh, SIGMA 37380-42-0
tert-butyl methyl ether (MTBE), pesticide grade
6-Chloronicotinic acid, e.g. Aldrich 15,635-3 (Milwaukee, WI)
Dry Ice Pellets (to be mixed with sample during initial homogenization)
Filter aid, e.g. Celite 545
Glass wool
Methanol, pesticide grade
Methanol/1% aqueous sulfuric acid mixture, 3:1 (v:v)
Methanol/water mixture, 3:1 (v:v)
N-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), e.g. Aldrich 24,210-1 (Milwaukee, WI)
pH paper
Potassium permanganate, 50 g/liter aqueous solution
Sodium bisulfite, anhydrous
Sodium hydroxide, 32% aqueous solution
Sodium sulfate, anhydrous granular
Sulfuric acid, 10% aqueous solution
Water, HPLC grade

3.2.3 <u>Analytical Standards</u>

Three analytical standards were utilized in the analyses of samples included in this report. These being the following:

1) Imidacloprid
[RAL Lot No. K-335 (PT1701/88)]
Received from Miles Inc., Agriculture Division

Formula Wt (255.6) Percent Purity (95.9)

Chemical Name: Presented in Section 2.0

Experimental Name: NTN 33893

Chemical Structure: Presented in Section 2.0

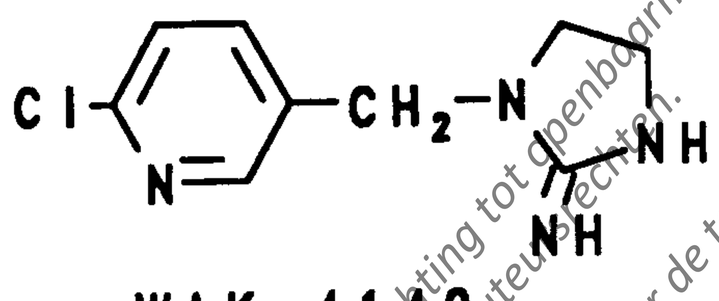
2) Desnitro-metabolite of Imidacloprid
[RAL Lot No. K-337 (Reference Substance No. 890913ELB01)]
Received from Bayer Pflanzenschutz Zentrum, Germany

Formula Wt (247.1) Percent Purity (89.0)

Chemical Name: 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dyhydro-1*H*-imidazol-2-amine HCl hydrate

Experimental Name: WAK 4140

Chemical Structure:



WAK 4140

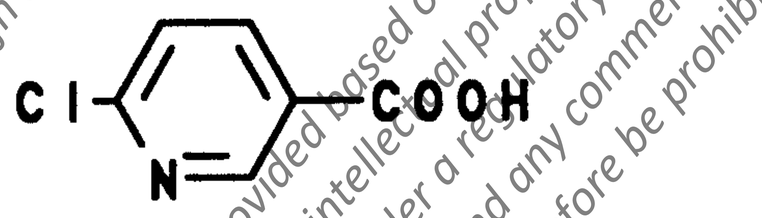
3) 6-Chloronicotinic acid
[RAL Lot No. R-200 (01513KT)]
Received from Aldrich Chemical Co.

Formula Wt (157.6) Percent Purity (99.0)

Chemical Name: 4-chloro-3-pyridine carboxylic acid

Experimental Name: None

Chemical Structure:



6-Chloronicotinic acid

All analytical standards listed above were received and stored in the freezer of RAL refrigerator R-1. Listed below are all standard dilution levels utilized and their dilution solvents.

Compound	µg/mL Parent Equivalents	Diluting Solvent
Parent Imidacloprid	250	Acetonitrile
Desnitro-metabolite	250	10% Water in Acetonitrile

Compound	µg/mL Parent Equivalents	Diluting Solvent
Analytical Reference Standards		
6-Chloronicotinic Acid	500	Acetonitrile
	250	Acetonitrile
	5.00	Acetonitrile
	1.25	Acetonitrile
Fortification Spiking Solutions		
Parent + Desnitro Mixed	50.0	Acetonitrile
	5.00	Acetonitrile

In order to alleviate needed calculations required to correct instrumentally measured residues of derivatized 6-chloronicotinic acid back to parent imidacloprid equivalents, corrections to parent equivalents were made at the time of first dilution of each neat standard in solvent to be used. Corrections were made to all standards utilized, including parent imidacloprid, for formula weight and percent purity.

Standard solutions prepared were stored in the R-1 freezer until needed. Calculations for equivalent ppm values of standards relative to matrix, following solution concentration correction to parent equivalents, were based on a nominal sample weight of 25.0 g, followed by an extract aliquot of either 10% or 20% of total volume, and a final volume of 2.00 mL.

3.3 Preparation for Extraction

Prior to extraction, whole samples of alfalfa foliage received were removed from the freezer, chopped to homogenize each individual sample, and immediately returned to freezer storage. This is performed in order to reduce particle size of the plant tissue and increase efficiency of extraction at the later needed date. All samples were processed prior to extraction by RAL personnel, following SOP No. S-00011. This procedure involves chopping of the plant tissue sample in a HOBART VCM 25 or 40 in the presence of dry ice, then allowing the dry ice to sublime while in the freezer before repackaging.

Due to the field labeling of samples #8-21, #7-31 and #8-31, these samples were processed as treated samples without knowledge of them being field controls. Therefore, it is suspected that these samples were contaminated during homogenization at RAL prior to their being extracted.

3.4 Analytical Method

Field alfalfa samples were analyzed for residues of imidacloprid by a modification of the method described in Miles Report No. 102624-R[1]. The modified method, as utilized, is presented below.

3.4.1 Extraction

Place 25.0 g of the plant material in a 600-mL beaker. Add 300 mL of methanol/1% aqueous sulfuric acid (3:1 v/v), and allow the sample to soak for 30 min.

Blend the sample using a Polytron blender (or equivalent) for approximately 3 min.

Vacuum filter the suspension through 10 g of Celite filter aid, using Whatman 541 filter paper supported on a Buchner funnel, into a 1000-mL vacuum filter flask. Wash the filtered solids 2X with 100 mL each of acidified methanol/water (3:1).

Transfer the filtrate and wash to a 500-mL graduated cylinder. Add sufficient methanol to bring the total volume of the extracts to 500 mL. Mix the solution well, and transfer 100 mL (20% sample equivalent) to a 500-mL flat-bottom boiling flask.

NOTE: During the initial method validation work, this procedure was proven to be effective with the bulk control sample that was received. However, when the field samples were commenced, the permanganate solution utilized for oxidation was immediately exhausted, inferring that the matrix had consumed it and complete oxidation of the analytes could not be trusted. Therefore, the amount of extract aliquoted was reduced to 50 mL (10% sample equivalent) and the permanganate appeared to function as normal. The final volume listed later in this report was kept at 2.00 mL for all samples resulting in a matrix concentration in the final extracts of 2.5 g/mL for the method validation analyses, and 1.25 g/mL for the analyses of field samples.

Concentrate the aliquot to an aqueous remainder of 10 mL using a rotary vacuum evaporator with a bath temperature of 60°C.

3.4.2 XAD-4 Cleanup

Bulk Resin Preparation

For multiple analyses, 1 kg of XAD-4 resin was cleaned for analyses in bulk as follows:

1) Place 500 g of XAD-4 resin in a 2-liter Erlenmeyer flask, and add 500 mL of methanol. Swirl the flask and allow the resin to settle. Decant and discard the supernatant.

2) Repeat Step 1 twice for a total of three methanol rinses.

3) Repeat Steps 1 and 2 with a second 500 g portion of resin.

4) Combine the two methanol washed portions of resin and place them in a 4-liter sintered glass filter funnel mounted on a 6-liter vacuum filter flask.

5) Rinse the resin by allowing 1500 mL of fresh methanol to slowly percolate through the resin bed without applied vacuum.

6) Repeat Step 5 three times for a total of four methanol rinses.

7) Following the fourth rinse, remove the remaining methanol from the resin bed by applying a gentle vacuum to the flask until no more methanol is eluted. Discard the solvent.

8) Repeat Steps 5 through 7, substituting toluene for methanol.

9) Repeat Steps 5 through 7, once again with methanol

10) Repeat Steps 5 through 7, substituting water for methanol.

11) Store the prepared XAD-4 resin in a tightly sealed glass jar.

Suspend 10.0 g of the prepared XAD-4 resin in 30 mL of water, and slurry-pack a 25cm X 10 mm ID chromatography column with the suspension. Allow the water to drain just to the top of the resin bed and place a plug of glass wool on top of the resin.

Place the aqueous solution from the extraction procedure on the top of the XAD-4 column. Allow the solution to pass through the column at a rate of 2 mL/min. Discard the aqueous eluate.

Rinse the flask that held the aqueous solution with 20 mL of fresh water and place the rinse water on the column. Allow the rinse to pass through the column at a rate of 2 mL/min. Discard the aqueous eluate.

Repeat above step.

Elute the imidacloprid residues from the column with 100 mL of fresh methanol at a rate of 5 mL/min. Collect the eluate in a 500-mL flat-bottom boiling flask.

Concentrate the column eluate to 1 mL (or less) total volume using a rotary vacuum evaporator with a bath temperature of 60°C.

Dissolve the residue in 100 mL of fresh water.

3.4.3 Oxidation to 6-Chloronicotinic Acid

Add 5 mL of 32% (12N) aqueous sodium hydroxide solution to the 500-mL flat-bottom boiling flask containing the final aqueous solution from the XAD-4 column.

Using pH paper determine the pH of the aqueous solution. If the pH of the solution is ≥14 then proceed past next step.

If the pH of the solution is <14, add 32% aqueous sodium hydroxide solution, in 1.0 mL portions, to the flask until the pH of the sample solution is ≥14.

Add 50 mL of the 50 g/liter aqueous potassium permanganate solution to the flask containing the sample solution.

Add a magnetic stirring bar to the flask, attach a reflux condenser equipped with a teflon joint sleeve, and place the flask on a magnetic stirring hot plate.

Rapidly heat the sample, with vigorous stirring, so that the sample begins to reflux within 10 min. Continue to heat the sample at reflux, with stirring, for 6 min.

Immediately remove the flask from the hot plate and add 50 mL of fresh water to the reaction through the condenser to rinse any residues back into and cool the reaction flask.

Remove the reflux condenser, and quickly place the flask in an ice-water bath. Cool the solution, with agitation, until the internal temperature is $\leq$15°C.

Slowly add 50 mL of 10% (1.8<u>M</u>) sulfuric acid with continued cooling and stirring, making sure that the internal temperature of the solution does not exceed 15°C.

Add solid sodium bisulfite in 1.0 g portions, with continued cooling and stirring, at approximately 1 min intervals. Continue adding the sodium bisulfite until the solution becomes clear and colorless. Generally, about 5 g total of sodium bisulfite are required.

Determine the pH of the solution using pH paper. If the pH is $\leq$1, then proceed past the next step.

If the pH of the solution is >1, add 10% aqueous sulfuric acid to the solution, in 1.0 mL portions, to make the pH $\leq$1.

Transfer the aqueous solution to a 500-mL separatory funnel. Add 50 mL of t-butyl methyl ether (MTBE). Stopper and shake the funnel for 30 sec. Allow the phases to separate.

Drain the lower aqueous layer back into the 500-mL flat-bottom boiling flask.

Drain the MTBE layer into a 250 mL flat-bottom flask, passing the solution through a bed of 30 g of anhydrous sodium sulfate supported in a glass funnel equipped with a glass wool plug, the sodium sulfate being pre-rinsed with 30 mL of MTBE.

Repeat the above given extraction with MTBE two more times for a total of three extractions. Combine all of the dried MTBE extracts. Discard the aqueous layer following the third extraction.

Rinse the sodium sulfate with a 30 mL portion of fresh MTBE. Combine the rinse with the MTBE extracts. Discard the sodium sulfate.

Evaporate the combined MTBE solution <u>almost to dryness</u> using a rotary vacuum evaporator with a bath temperature of 40°C.

Complete evaporation of the solution under a gentle stream of dry nitrogen at ambient temperature.

Dissolve the residue in 2.00 mL of derivatization grade acetonitrile.

3.4.4 Derivatization

Place 250 µL of the acetonitrile solution from the last step of the oxidation in a GC autosampler vial.

Add 250 µL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA). Seal the vial, and mix the contents thoroughly. At the same time as the addition of derivatizing agent to the sample extracts, instrumental reference standards of 6-chloronicotinic acid are derivatized in like fashion to be utilized for instrumental calibration. As solutions of sample extract and standard are treated in a like manner, reference to concentration for all calculations did not include the two fold dilution factor resulting from the addition of MSTFA.

Allow the reaction to stand at ambient temperature for 1 hour prior to instrumental analysis, bracketing each injection of derivatized sample extract with that of derivatized 6-chloronicotinic acid.

3.4.5 Gas Chromatographic Analysis

Instrument:	HP 5890-Series II with HP 7673 Autosampler
Column:	12m HP ULTRA-1 (dimethyl silicone) quartz capillary 0.2mm ID with 0.33µ film thickness
Sample Injection:	Capillary injection in splitless mode Split flow rate 30 mL/min, valve on 1.0 min. Injector temperature 250°C Injection volume 1.0 µL
Temperature Program:	80°C for 1 min. isothermal 7.5°C/min linear ramp to 160°C 30°C/min linear ramp to 300°C 300°C for 4 min. isothermal
Detector:	HP 5971 mass selective detector in SIM mode Detection of ions at m/z 214 (primary method) 170 (confirmatory) The detector is turned on <u>after</u> the initial 3 min. of the GC run to allow solvents and excess MSTFA to elute prior to data acquisition.

3.5 Calculations

Presented below are examples for various calculations performed to arrive at residues and recoveries presented in this report.

3.5.1 Standard Solutions Preparation

The following calculation was utilized to prepare primary dilutions of neat standard, correcting to parent equivalents, so that any further calculations would remain straightforward when the needed nominal concentration is known:

$$G = A \times B \times \frac{C}{255.6\ g/mol} \times \frac{100\%}{D} \times \frac{1\ g}{1000000\ \mu g}$$

OR

$$G = \frac{A \times B \times C}{D \times 2556000\ \mu g/mol\%}$$

where G = amount of neat standard to be weighed out (g)
A = final needed solution concentration in parent equivalents (µg/mL)
B = final volume of solution (mL)
C = formula weight of neat standard (g/mol)
D = percent purity of neat standard (%)

Example: Desnitro- standard needed to be 250 µg/mL parent equivalents with a final volume of 100 mL

$$G = \frac{250\ \mu g/mL \times 100\ mL \times 247.1\ g/mol}{89.0\% \times 2556000\ \mu g/mol\%} = 0.02716\ g$$

3.5.2 Bulk Control and Field Samples

Total residues of imidacloprid and its metabolites in bulk control and field alfalfa samples were calculated by the following equation:

$$RES = \frac{SPL \times STDCONC \times DF}{STD}$$

where RES = apparent residue of sample rounded to three digits to the right of the decimal point (ppm)

SPL = instrumental response for sample (area)

STDCONC = equivalent standard conc. (ppm) relative to matrix

$$DF = \frac{25.0g\ Init.\ Sample\ Wt}{Actual\ Init.\ Sample\ Wt} \times \frac{Actual\ Final\ Volume}{2.00\ mL\ Final\ Volume}$$

STD = average of instrumental response (area) for preceding and trailing standards

Example: RAL Sample No. 93706A-1 (Unfortified Bulk Control Sample)

$$RES = \frac{(3516) \times (0.50) \times 1}{(505514 + 613274)/2} = 0.003 \text{ ppm} \quad (<1.00 \text{ ppm})$$

3.5.3 Recoveries

Total residues of imidacloprid in laboratory fortified alfalfa controls were calculated from raw data by the following equation:

$$REC = \frac{(RESR - RESC) \times 100}{FORT}$$

where REC = percent recovery for fortification performed

RESR = residue determined for recovery sample using equation given above (ppm)

RESC = residue for corresponding control sample using equation given above (ppm)

FORT = fortification level performed relative to matrix (ppm)

Example: RAL Sample No. 93706A-2 (Bulk Control Spiked at 1.00 ppm)

$$REC = \frac{(0.885 - 0.003) \times 100}{1.00} = 88\% \text{ Recovery}$$

4.0 Results and Discussion

4.1 Method Validation

4.1.1 Instrumental Response Linearity

The instrumental response of imidacloprid to GC/MS analysis in the presence of field control alfalfa matrix was characterized. It was found to be linear over the range of sample extract residue solution concentrations analyzed. These data are presented in Figures 1 and 2.

Figure 1 presents linearity of the derivatized 6-chloronicotinic acid in solvent. The levels of analyte relative to matrix presented are 0.025-1.00 ppm when observed as the 2.5 g matrix/mL equivalence, and 0.050-2.00 ppm when observed as the 1.25 g matrix/mL equivalence.

Figure 2 presents linearity of the derivatized 6-chloronicotinic acid in the presence of alfalfa extract. The levels of analyte present relative to matrix are 0.025-1.00 ppm. This curve was injected with 2.5 g matrix/mL equivalence present. Since the field samples were analyzed resulting in an extract for injection of 1.25 g matrix/mL this same curve with said matrix present would be presented as 0.050-2.00 ppm. Actual injection of this linearity was not required as the presence of less matrix in solution would have even less effect on the instrumental linearity of response.

4.1.2 Recovery Studies Performed

Recoveries of mixed fortifications of parent imidacloprid and its desnitro-metabolite from alfalfa were conducted. Apparent residues following analyses were reported to three digits to the right of the decimal point in ppm prior to calculating net recovery percentages. All recovery data are presented in Table 1.

The method was validated with mixed fortifications of parent imidacloprid and its desnitro-metabolite, in the bulk control alfalfa sample received. The levels of fortification were 0.50, 5.00, and 15.00 ppm of each analyte with recoveries of 77-106%, a mean of 90%, and a standard deviation of 14.6.

In addition to this, recoveries of mixed fortifications of parent imidacloprid and its desnitro-metabolite were conducted with each analytical run of the field treated samples. These were performed to verify the integrity of analyzed residues found in sets of samples analyzed on respective days. The concurrent recoveries were routinely run at the 1.00 or 10.00 ppm total fortification level, relative to matrix, with additional recoveries run at higher levels to encompass the highest measured field residue. Recoveries ranged 71-131%, with a mean of 100%, and a standard deviation of 17.2.

Example chromatography for injections of the instrumental reference standard, control extract, and a recovery extract are presented as Figures 3 through 5.

4.1.3 Resultant Limit of Determination

The limit of determination is defined as being the lowest detectable residue level at which reliable measurement may be performed, this being supported by acceptable recoveries from the matrix at that level.

During the method validation, the lowest fortification level performed relative to matrix was 0.50 ppm of each of the analytes in mixture. As this resulted in an acceptable subtracted recovery with residues in the corresponding control less than 0.25 ppm, the nominal limit of determination of 0.50 ppm was validated. Therefore, in subsequent analyses of field samples, apparent residues calculated to be less than 0.50 ppm were identified as <0.50 ppm.

4.2 Residues of Imidacloprid Found

The total residues of imidacloprid in each of the field control and treated samples are presented in Table 2. This table also presents averaged residues for given intervals grouped by treatment levels.

5.0 Bibliography

1. [redacted] "Method for the Determination of Total Residues of Imidacloprid in Plant Materials and Drinking Water", Miles Ag. Div. Report No. 102624-R (U.S. EPA MRID No. Currently Not Available).

Table 1. Laboratory Fortification (Parent + Desnitro-) Recovery Summary

Miles Sample Number	Total[1] Conc Added (ppm)	Extract	Analysis	Gross Residue (ppm)	Percent Recovery
Controls Utilized for Fortifications					
93706[2]	NA	08/03/92	08/06/92	0.003	-
#7-11	NA	10/01/92	10/08/92	0.009	-
#7-22	NA	10/14/92	10/16/92	0.099	-
#8-11	NA	10/01/92	10/08/92	0.006	-
#8-31	NA	09/30/92	10/06/92	0.291	-
Method Validation					
93706	1.00	08/03/92	08/06/92	0.885	88
93706	10.00	08/03/92	08/07/92	10.557	106
93706	30.00	08/03/92	08/07/92	23.018	77
				Mean =	90
				Std Deviation =	14.6
Concurrent Recoveries					
93706	1.00	09/30/92	10/06/92	1.031	103
93706	10.00	09/30/92	10/06/92	10.080	101
93706	10.00	10/01/92	10/08/92	9.021	90
93706	30.00	10/07/92	10/15/92	31.273	104
Reinjection of above extract ->			10/18/92	30.849	103
#8-31	1.00	10/07/92	10/15/92	0.996	71
Reinjection of above extract ->			10/18/92	1.020	73
#7-11	10.00	10/12/92	10/15/92	9.911	99
#8-11	10.00	10/14/92	10/16/92	10.620	106
#8-11	121.00	10/14/92	10/16/92	158.178	131
#7-22	10.00	10/19/92	10/22/92	11.644	116
				Mean =	100
				Std Deviation =	17.2

[1]Total concentration of equal parent equivalent additions of Imidacloprid and its Desnitro-metabolite.
[2]93706 = Bulk alfalfa sample.

Table 2. Apparent Total Imidacloprid Residues in Field Samples

Treatment Level (lbs AI/acre)	Interval (hours)		Test #1		Test #2		Average Residue Ppm
Control	2	WSU ID No.	#7-11	#8-11	#7-12	#8-12	
		Residue ppm	<0.50	<0.50	<0.50	<0.50	0.50
Control	8	WSU ID No.	#7-21	#8-21	#7-22	#8-22	
		Residue ppm	<0.50	3.63[1]	<0.50	<0.50	0.50
Control	24	WSU ID No.	#7-31	#8-31	#7-32	#8-32	
		Residue ppm	1.11[1]	<0.50[1]	<0.50	<0.50	0.50
0.045	2	WSU ID No.	#1-11	#2-11	#1-12	#2-12	
		Residue ppm	4.81	4.38	0.97	1.86	3.01
0.045	8	WSU ID No.	#1-21	#2-21	#1-22	#2-22	
		Residue ppm	4.50	5.77	4.43	4.97	4.92
0.045	24	WSU ID No.	#1-31	#2-31	#1-32	#2-32	
		Residue ppm	4.36	4.16	0.99	1.24	2.69
0.167	2	WSU ID No.	#3-11	#4-11	#3-12	#4-12	
		Residue ppm	16.61	19.51	18.62	19.82	18.64
0.167	8	WSU ID No.	#3-21	#4-21	#3-22	#4-22	
		Residue ppm	21.45	4.27	19.92	3.76	12.35
0.167	24	WSU ID No.	#3-31	#4-31	#3-32	#4-32	
		Residue ppm	22.94	18.15	7.37	4.23	13.17
0.500	2	WSU ID No.	#5-11	#6-11	#5-12	#6-12	
		Residue ppm	51.77	55.20	53.85	58.07	54.72
0.500	8	WSU ID No.	#5-21	#6-21	#5-22	#6-22	
		Residue ppm	56.46	50.82	54.33	55.42	54.26
0.500	24	WSU ID No.	#5-31	#6-31	#5-32	#6-32	
		Residue ppm	68.25	77.26	16.64	20.91	45.77

[1]Residue value not included in average as sample suspected to be contaminated during homogenization.

MILES INCORPORATED
AGRICULTURE DIVISION
RESIDUE ANALYSIS LABORATORY
Linear Response Data Report Sheet

103938

Compound: NTN 33893
Crop: APPLICABLE TO ALL
Matrix: SOLVENT ONLY
Analysis Method: 102624
Analysis Type: GC/MSD

(RAL) Sample No.	(MILES) Sample No.	Date ANAL	STD Conc (ppm) [1]	AVG STD Response (Ht/Area)	Matrix Present (Yes/No)
0.025 SOLVENT ①	NA	05/27/92	0.025	28118	NO
0.050 SOLVENT ②	NA	05/27/92	0.050	51035	NO
0.10 SOLVENT ③	NA	05/27/92	0.100	104462	NO
0.50 SOLVENT ④	NA	05/27/92	0.500	516453	NO
1.00 SOLVENT ⑤	NA	05/27/92	1.000	1085680	NO

↑ For 2.5 grams/mL matrix equiv. TG 10/19/92

ND = NON DETECT
(1) ppm relative to matrix

Regression Output:	
Constant	−5233.462
Std Err of Y Est	12897.345
R Squared	0.99939
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	1081740.484
Std Err of Coef.	15393.301

Entered by: [redacted]
Date: [redacted]
Reviewed by: [redacted]
Date: [redacted]

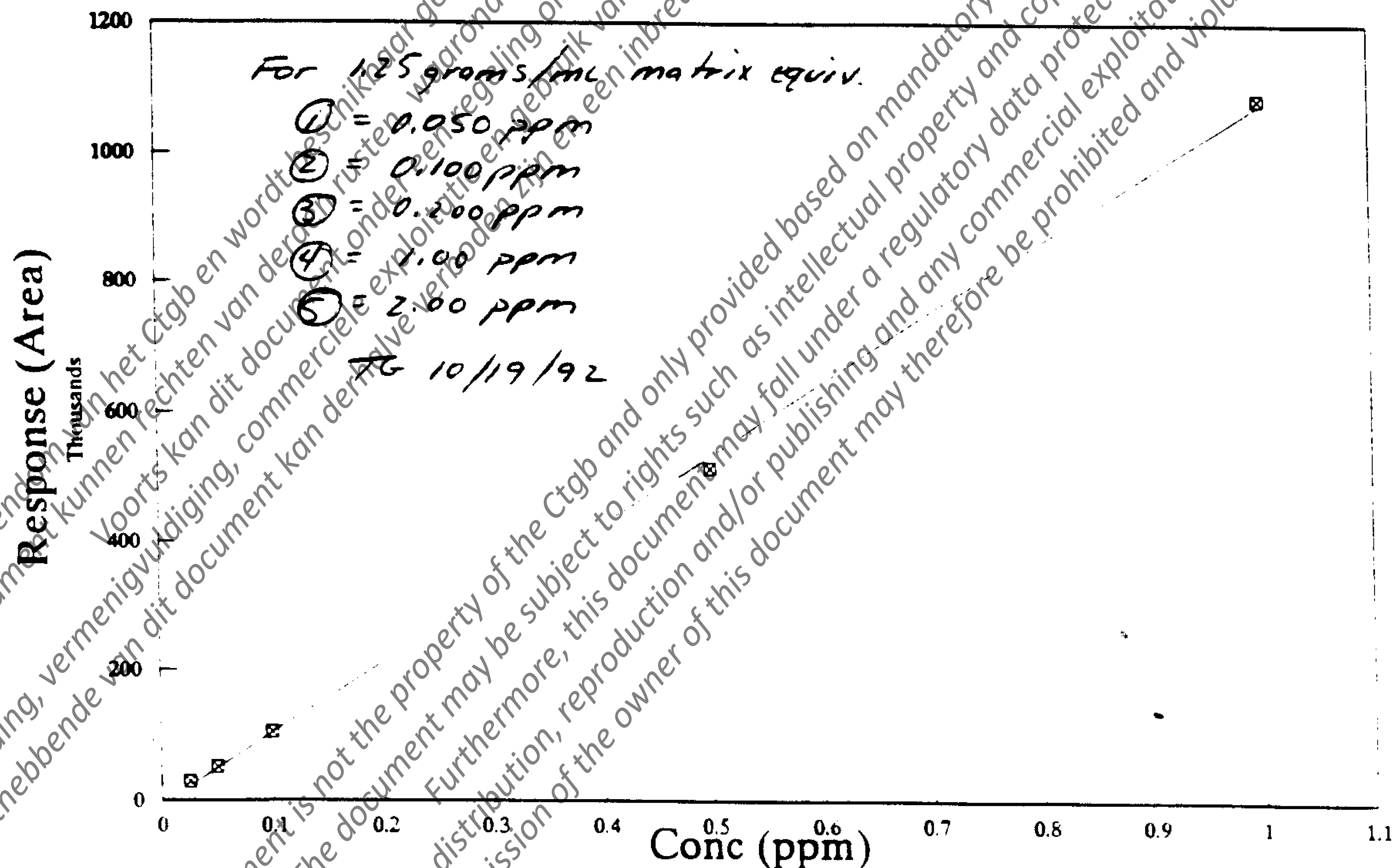


Figure 1. Linear Response Data Report Sheet - (Derivatized 6-Chloronicotinic Acid Linearity in Solvent)

MILES INCORPORATED
AGRICULTURE DIVISION
RESIDUE ANALYSIS LABORATORY
Linear Response Data Report Sheet 103938

Compound: NTN 33893
Crop: ALFALFA
Matrix: FORAGE (2.5 GRAMS MATRIX/ML)
Analysis Method: 102624
Analysis Type: GC/MSD

(RAL) Sample No.	(Miles) Sample No.	Date ANAL	STD Conc (ppm) [1]	STD Response (Ht/Area)	Matrix Present (Yes/No)
93706A-1(0.025)	93706	09/24/92	0.025	25529	YES
93706A-1(0.05)	93706	09/24/92	0.05	51729	YES
93706A-1(0.10)	93706	09/24/92	0.10	107732	YES
93706A-1(0.50)	93706	09/24/92	0.50	477962	YES
93706A-1(1.00)	93706	09/24/92	1.00	966655	YES

Regression Output:

Constant	4418.975
Std Err of Y Est	6024.769
R Squared	0.99983
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	959708.732
Std Err of Coef.	7190.711

ND = NON DETECT
(1) ppm relative to matrix

Entered by: [redacted]
Date:
Reviewed by:
Date: 10/26/92

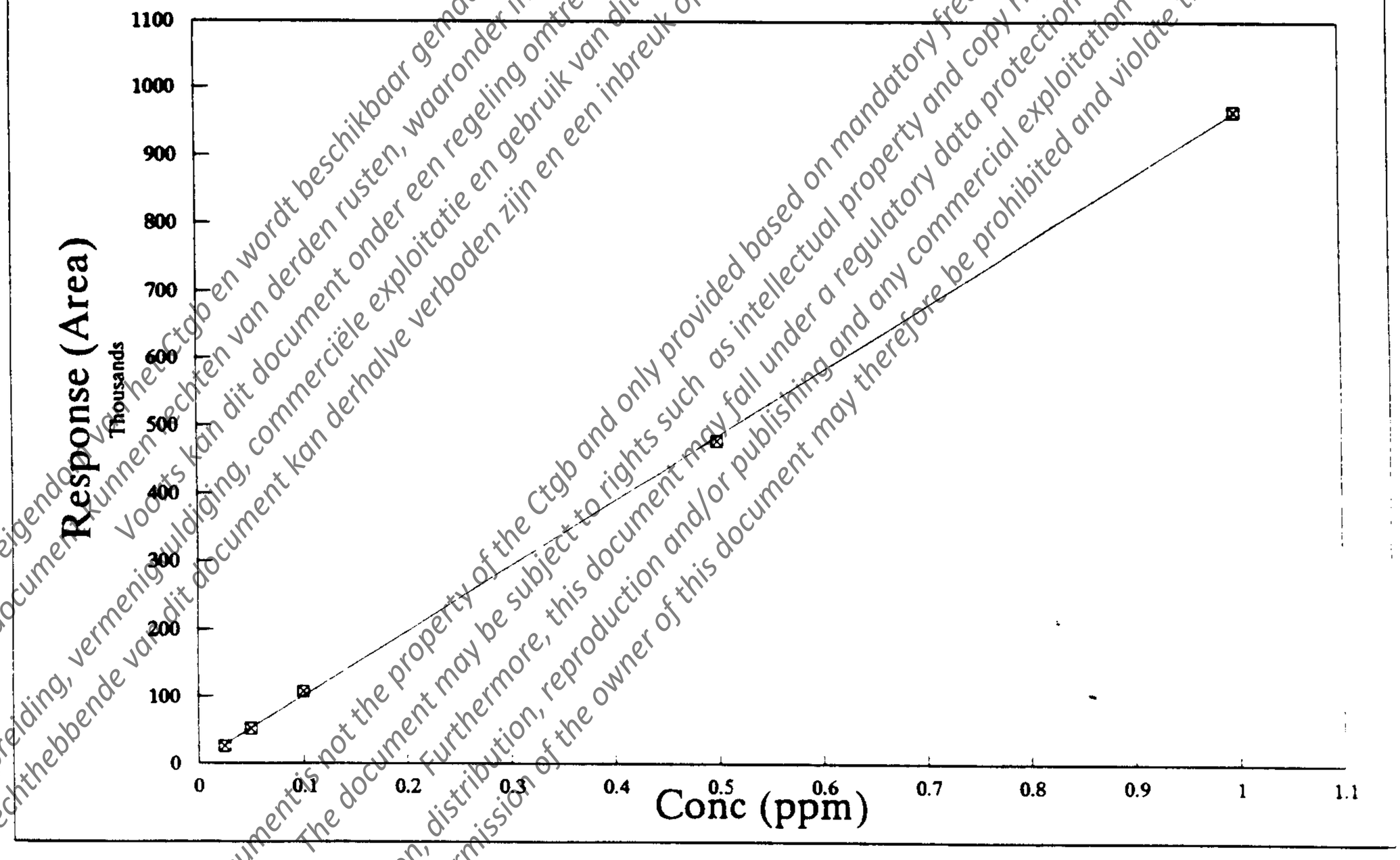
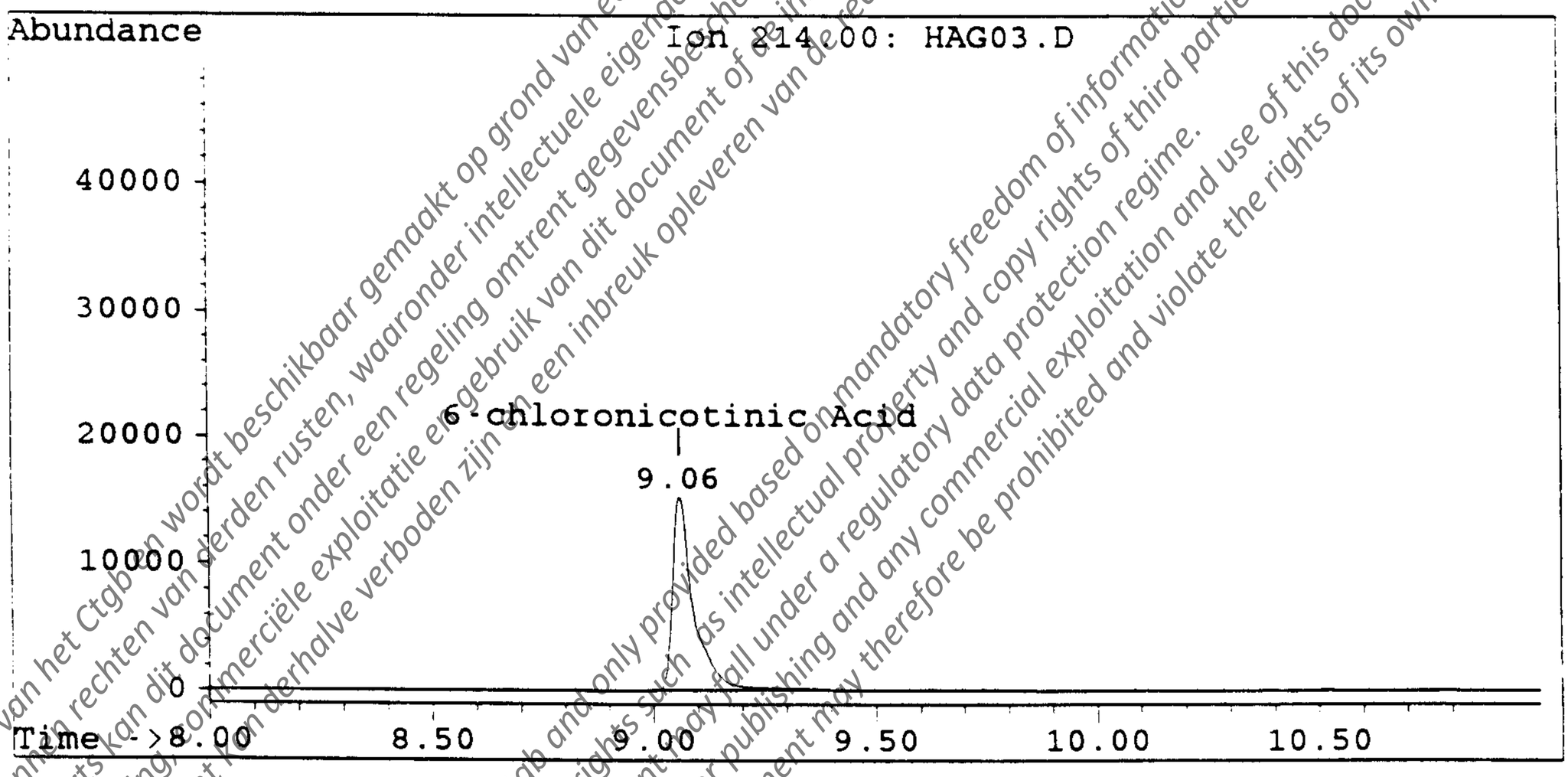


Figure 2. Linear Response Data Report Sheet - (Derivatized 6-Chloronicotinic Acid Linearity in Alfalfa Extract @ 2.5 g matrix/mL)

MILES INCORPORATED
AGRICULTURE DIVISION
Residue Analysis Laboratory
Hewlett Packard GC/MSD

File: C:\CHEMPC\DATA\HAG03.D
Operator: CAL
Date Acquired: 6 Aug 92 11:10 am
Method File: TOTAL2.M
Sample Name: STD 1.25 UG/ML 072392-3
Date Analyzed Thu Aug 06 11:30:56 1992
ALS vial: 3



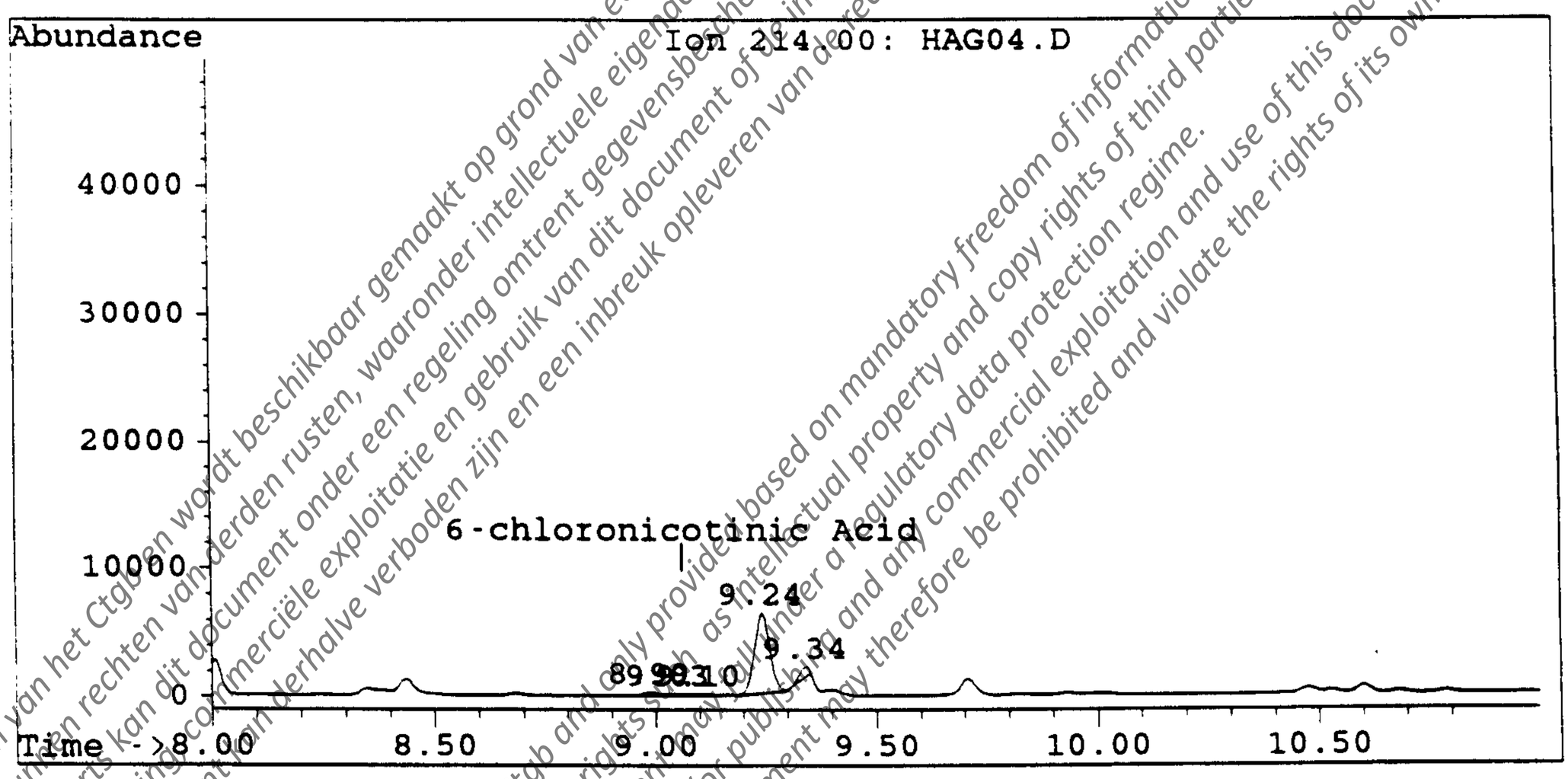
Ion 214.00: HAG03.D
STD 1.25 UG/ML 072392-3

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.063	BBA	0.048	505514	8.874	9.351

Figure 3. Example Chromatogram - Derivatized 6-Chloronicotinic Acid (0.50 ppm Relative to Matrix @ 2.5 g matrix/mL)

MILES INCORPORATED
AGRICULTURE DIVISION
Residue Analysis Laboratory
Hewlett Packard GC/MSD

File: C:\CHEMPC\DATA\HAG04.D
Operator: CAL
Date Acquired: 6 Aug 92 11:35 am
Method File: TOTAL2.M
Sample Name: 93706A-1
Date Analyzed Thu Aug 06 11:55:50 1992
ALS vial: 4



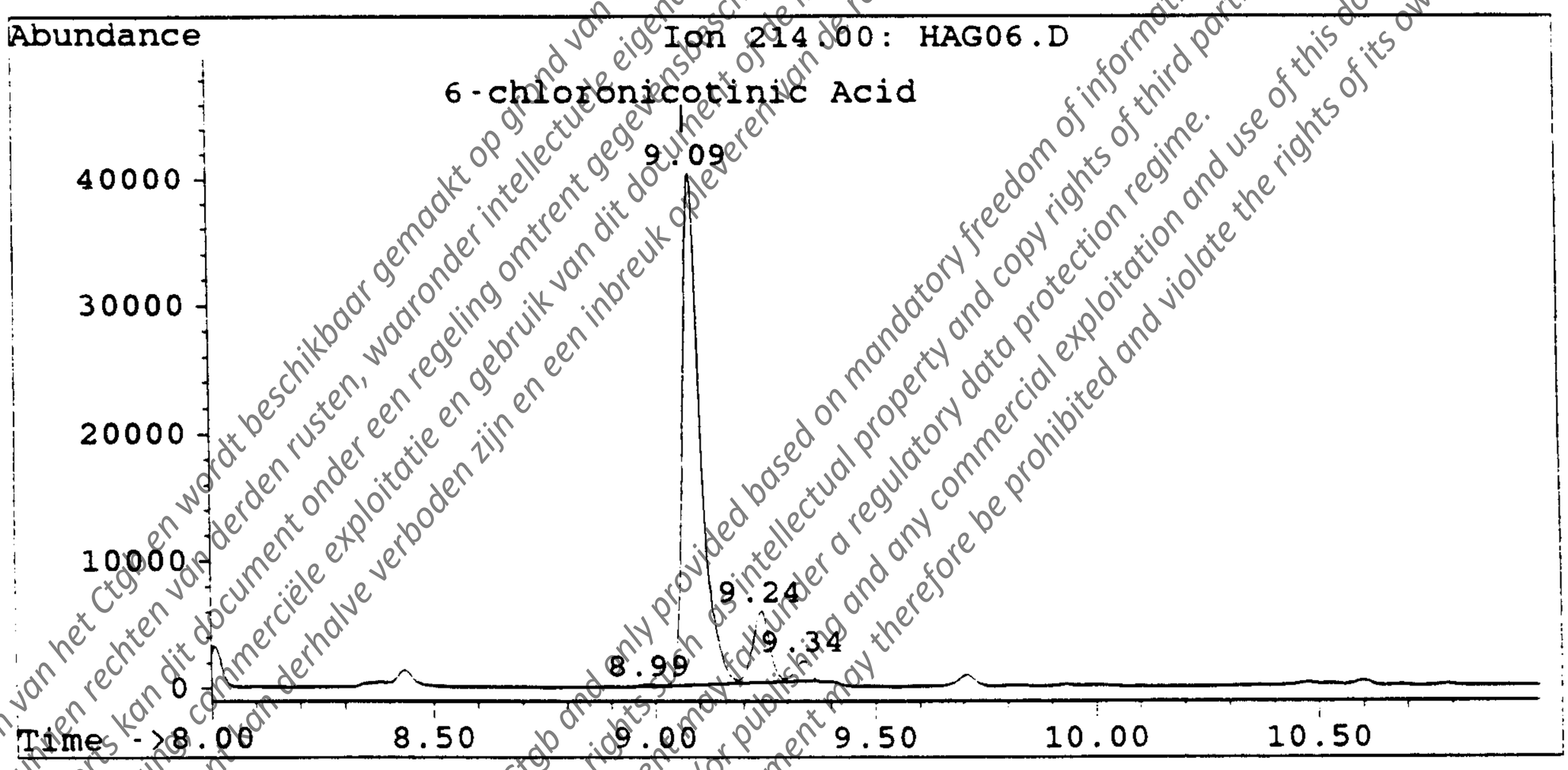
Ion 214.00: HAG04.D
93706A-1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	8.987	BV	0.035	6207	8.927	9.018
2	9.030	VV	0.034	2571	9.018	9.063
3	9.099	VV	0.045	3516	9.063	9.145
4	9.244	PV	0.038	150482	9.186	9.296
5	9.341	PBA	0.027	12283	9.296	9.352

Figure 4. Example Chromatogram - Control Alfalfa Extract (2.5 g matrix/mL)

MILES INCORPORATED
AGRICULTURE DIVISION
Residue Analysis Laboratory
Hewlett Packard GC/MSD

File: C:\CHEMPC\DATA\HAG06.D
Operator: CAL
Date Acquired: 6 Aug 92 12:25 pm
Method File: TOTAL2.M
Sample Name: 93706A-2
Date Analyzed Thu Aug 06 12:45:50 1992
ALS vial: 5



Ion 214.00: HAG06.D
93706A-2

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	8.988	PV	0.020	1766	8.895	9.020
2	9.089	PV	0.042	1115930	9.020	9.187
3	9.243	VV	0.041	151967	9.187	9.297
4	9.338	VBA	0.036	37206	9.297	9.369

Figure 5. Example Chromatogram - Fortified Alfalfa Control Extract (0.50 ppm of Each Analyte Mixed @ 2.5 g matrix/mL)