MONITORING OF THE 1990 GYPSY MOTH ERADICATION PROJECT

February 1991

Environmental Hazards Assessment Program

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EH 91-l

ABSTRACT

The California Department of Food and Agriculture (CDFA) conducted an eradication program for gypsy moth in spring, 1990 which treated two small areas with the insecticide diflubenzuron. CDFA's Environmental Hazards Assessment Program (EHAP) collected foliage, air, water, and tank mix samples to monitor environmental concentrations of diflubenzuron resulting from these treatments. Both the eradication and monitoring programs were similar to those conducted for the Gypsy Moth program in Los Angeles County in 1987. The objective this year was to monitor applications and compare results with those found in 1987, not to provide a detailed characterization of diflubenzuron degradation.

The two sites were each about 0.8 ha in size. One was located in Tiburon in Marin County and the other was in Roseville in Placer County. Each site received two applications of Dimilin® 25W (diflubenzuron, 25% a.i.) spaced at two week intervals in March-April 1990. The pesticide was applied with a ground spray rig and foliage was sprayed to the point of drip.

Foliage samples were collected 1 day prior to, and immediately after each application, and then 28 days after the final application. Concentrations of diflubenzuron were reported as µg/g (µg diflubenzuron/g dry weight of leaves) and µg/cm^2 leaf area. Concentrations ranged from none detected for background samples (collected prior to the first spray) to 18.31 µg/g immediately after the second application; and from none detected for background to 0.252 µg/cm^2 leaf area immediately after the second application. Samples collected 28 days after the second application showed sharp decreases in diflubenzuron concentration, indicating possible degradation over this period. However, no samples were collected during these 28 days to document a degradation trend.

Air samples were collected in the treated areas 1 day prior to (background), during, and 1 day after each application. Diflubenzuron was detected during three of the four applications at concentrations from 0.0106 to 0.0187 µg/m^3. Diflubenzuron was not detected in any background or 1 day post samples.

Water samples were collected from streams and water bodies in and near the treated areas. Samples were collected the day prior to, immediately after, and 7 days after each application. No diflubenzuron was detected in any samples.

One tank sample was collected during each application and analyzed to measure the actual concentration of diflubenzuron in the tank mix. Actual concentrations were very close to desired concentrations except for the first application in Tiburon when the tank mix was 33% of the desired concentration.

Environmental concentrations of diflubenzuron in 1990 were similar to or lower than those found in 1987. Foliage results perhaps indicate a trend for greater degradation in 1990 than in 1987. Air concentrations in 1990 were an order of magnitude lower than in 1987. In both 1990 and 1987, no diflubenzuron was detected in any water samples.

ACKNOWLEDGMENTS

Thanks to all EHAP field personnel who assisted in this project with their usual outstanding cooperation and expertise. Thanks also to the property owners who allowed us to collect samples on their property. Special thanks to Mark Lubinski, Associate Economic Entomologist and his spray crews from CDFA's Pest Detection/Emergency Projects Branch for their willing help and cooperation.

DISCLAIMER

The mention of commercial products, their source or use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such product.

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INTRODUCTION

The gypsy moth (Lymantria dispar) is a serious pest of trees in the eastern United States. The leaf-eating larvae (caterpillars) have been known to completely defoliate trees. The gypsy moth is not established in California, but it is periodically introduced into the State by egg masses "hitchhiking" on vehicles, outdoor furniture, or other household goods entering the state from infested areas. When an infestation is detected, the California Department of Food and Agriculture (CDFA) undertakes a program to eradicate this pest, including treatment of infested areas with pesticides. More information on the gypsy moth and CDFA's past eradication programs can be found in Loughner et al. (1987).

In 1989, gypsy moth egg masses, pupae, and adult moths were found in two areas of northern California: Tiburon in Marin County, and Roseville in Placer County. An eradication program was carried out in the spring of 1990 which included treating both areas with the insecticide diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea). This compound had been used in a previous gypsy moth eradication program in Los Angeles County (Marade et al., 1989). Diflubenzuron is a chitin inhibitor which kills the caterpillars by preventing them from molting into their next stage of growth. Caterpillars ingest it as they feed on treated foliage. It is a selective pesticide which has little effect on

non-plant feeding insects such as bees; it also has a low toxicity to mammals, birds, and fish (Loughner et al., 1987).

The Environmental Hazards Assessment Program (EHAP) of the CDFA monitored concentrations of diflubenzuron on foliage and in air and water in both treated areas. The objective was to monitor the applications and compare environmental levels of diflubenzuron with those found in the past, not to provide a detailed characterization of diflubenzuron degradation over time. Therefore, this year's monitoring was not as extensive as in the past (see Marade et al,, 1989), and no statistical analyses were performed.

MATERIALS AND METHODS

Site Description

Two sites were treated for gypsy moth infestations, one in Tiburon, Marin County, and one in Roseville, Placer County. The Tiburon site consisted of portions of a condominium complex and a motel property, at the southern end of Point Tiburon just off San Francisco Bay. The treated area was a ıt 7.8 ha in size and was flat, except for a hillside on the north side of the area. An artificial lagoon bordered the area to the east and a natural lagoon was located just outside the northwest boundary of the treated area. Vegetation consisted mainly of ornamental trees and shrubs; wild grasses and shrubs covered the hillside.

The Roseville site, also about 0.8 ha, consisted of five adjacent properties in a residential neighborhood in the Granite Bay area near Folsom Lake. A small stream, about 30 m to the east and downslope of the treated area, received flow from a small drainage ditch which carried water from the treated area. The site sloped gently to the east. Vegetation consisted of ornamental trees and shrubs with a few scattered native oaks.

Spray Program

Diflubenzuron was applied four times, twice to each site, at two-week intervals, during March and April 1990. A hydraulic ground-spray rig was used to spray the foliage to the point of drip. Diflubenzuron was applied at two different rates during the treatment program. A low amount was inadvertently sprayed for the first Tiburon application. Rather than the normal 2 oz, only 1 oz of Dimilin® 25W (0.25 oz active ingredient, a.i.) was added per 100 gal water to achieve a tank concentration of 0.00195% a.i. For the remaining three applications, 2 oz was added per 100 gal water for a concentration of 0.0039% a.i. Approximately 150 gal of tank mix was used for the first application in Tiburon and both Roseville applications; 200 gal was used for the second Tiburon application.

Due to wind conditions during the first application in Tiburon, only half the treatment area was sprayed on the first day. The second half was treated the next day with the remaining tank mix. Each remaining application took only one day, and proceeded according to schedule.

Study Design and Sampling Methods

Foliage

Two replicate foliage samples were collected at each site the day before, and immediately after each application (after the spray had dried), to measure the amount of diflubenzuron applied. Samples were also collected 28 days after the final application to determine if any dissipation of diflubenzuron had occurred. Samples collected prior to the first spray served as background samples. For each replicate, approximately 60 leaves were collected at random from treated trees and shrubs. In Tiburon, leaves were collected throughout the treated area, but in Roseville, due to logistics and cooperation of homeowners, one property was chosen to represent the application area and samples were collected from this property only. Leaves were snipped directly into glass mason jars which were then sealed, placed immediately on wet ice, and kept at 4°C until analyzed by the lab. As the study progressed and plants produced new foliage, an effort was made to collect older leaves which had been on the plants for the entire study period. Since the first Tiburon application was split into two days, samples were collected each day from foliage that had been treated that day.

Air

At each site, one air sample was collected 1 day prior to, during, and 1 day after each application. The sample collected the day before each spray served as background for that spray; samples collected during application periods measured diflubenzuron released into air from the spray; and the 1 day post samples measured diflubenzuron possibly

released from foliage by volatilization. Since the first Tiburon application occurred over two days, samples were collected during both application periods and the 1 day post was taken after the second application day. The duration of background and 1 day post sampling periods was 3 h. Application sampling periods coincided with the following spray periods: the first Tiburon application was 2 h 50 min on the first day and 3 h on the second day; the second application in Tiburon lasted 3 h; the first Roseville application was 2 h 30 min; and the second Roseville application was 2 h.

High volume air samplers were used which were calibrated to draw air at a rate of 1000 L/min through a glass fiber filter, trapping the pesticide onto the filter. Filters were placed immediately on dry ice and kept frozen until analyzed. Samplers were placed near buildings in the spray area where observers might be exposed to the pesticide, but at least several feet from treated foliage.

Water

Two replicate water samples plus one field blank were collected from the water bodies or streams in and near the application areas. Samples were collected the day prior to, immediately after, and 7 days after each application. Samples taken before the first application served as background samples; the others were taken to measure possible drift and/or runoff of diflubenzuron from the spray areas. In Tiburon, samples were collected from both lagoons adjacent to the application area. For the first application, samples were collected from the artificial lagoon the first day, because it was adjacent to the area treated; the natural lagoon was near the area sprayed the second day and so was sampled on that day. For the second Tiburon application, both lagoons were sampled

on the application day since the entire area was treated at once. In Roseville, samples were collected from the drainage ditch before it entered the stream east of the spray area.

Samples were collected in 1 L amber glass bottles, sealed, placed immediately on wet ice, and kept at 4°C until analyzed.

Tank Mix

One tank sample was collected for each application and analyzed to measure the actual diflubenzuron concentration of the tank mix. Samples were collected from the spray nozzle into pint mason jars, sealed, put in plastic bags, and placed on wet ice in a cooler separate from the other samples. Tank samples were kept at 4° C until analyzed.

A chain of custody record accompanied each foliage, air, water, and tank sample to document handling of the sample from the time of container preparation through lab analysis, and to record any special sample information.

Chemical Analysis and Quality Control

All samples were analyzed for diflubenzuron by CDFA's Chemistry Laboratory Service in Sacramento. Leaves were washed with a surfactant solution to recover dislodgeable residues. The surfactant solutions, glass fiber filters, and water samples were extracted with a 50/50 mixture of hexane and acetone. Extracts were analyzed by high pressure liquid chromatography. Detailed analytical methods are found in Appendix I. Quality control procedures included method validation for each matrix, and continuing quality control of one blank matrix spike per extraction set.

RESULTS AND DISCUSSION

Foliage

Leaves were analyzed for dislodgeable surface residue of diflubenzuron and reported by the lab as µg per sample, which was about about 60 leaves. After analysis, leaves were pressed, air-dried, weighed, and the total area of each sample was measured with an area meter. Results for concentrations of dislodgeable residue are reported on both a weight basis, µg diflubenzuron/g air-dry weight of leaves (Tables 1 and 2) and a surface area basis, µg diflubenzuron/cm^2 leaf surface area (Tables 3 and 4). Concentrations ranged from none detected (background samples) to 18.31 µg/g or 0.252 µg/cm^2 leaf area. The highest concentrations occurred immediately after the second application at each site. Since samples taken the day before Application 2 (Day 13 after Application 1) still showed diflubenzuron to be present, the high levels for the second application included residual from Application 1. In Tiburon, the tank concentration of diflubenzuron used for the second application was higher than for the first (see below), and a greater volume of tank mix was used for the second application (200 gal vs. 150 gal). These factors also contributed to the higher concentrations in Tiburon after Application 2.

Table 1. Diflubenzuron concentrations (µg/g) on foliage, Tiburon application site, Gypsy Moth monitoring, Spring 1990.

Sample period	Date	Foliar Residue Replicate 1	Foliar Residue Replicate 2
		---µg/g, air-dry weight---	
Background	26 March	ND[a]	ND
Application 1[b]			
Day 0	27 March	2.91	4.07
Day 0	28 March	1.40	1.35
Day 13	9 April	4.43	3.22
Application 2[c]			
Day 0	10 April	12.86	14.13
Day 28	8 May	1.56	1.77

[a]None detected. Minimum detection limit was 0.06 µg/g.

[b]Because of wind conditions, application area was treated on two consecutive days and leaf samples were taken both days. Tank concentration of diflubenzuron was 6.41 µg/g.

'Tank concentration of diflubenzuron was 40 µg/g.

Table 2. Diflubenzuron concentrations (µg/g) on foliage, Roseville application site, Gypsy Moth monitoring, Spring 1990.

Sample period	Date	Foliar Residue Replicate 1	Foliar Residue Replicate 2
		---µg/g, air-dry weight---	
Background	2 April	ND[a]	ND
Application 1[b]			
Day 0	3 April	11.43	10.35
Day 13	16 April	10.20	8.32
Application 2[c]			
Day 0	17 April	18.31	14.57
Day 28	15 May	5.52	4.57

[a]None detected. Minimum detection limit was 0.07 µg/g.

[b]Tank concentration of diflubenzuron was 40 µg/g.

'Tank concentration of diflubenzuron was 39 µg/g.

Table 3. Diflubenzuron concentrations (µg/cm²) on foliage, Tiburon application site, Gypsy Moth monitoring, Spring 1990.

Sample period	Date	Foliar Residue Replicate 1	Foliar Residue Replicate 2
		µg/cm²	
Background	26 March	ND[a]	ND
Application 1[b]			
Day 0	27 March	**0.048**	**0.065**
Day 0	28 March	**0.019**	**0.027**
Day 13	9 April	**0.054**	**0.044**
Application 2[c]			
Day 0	10 April	**0.178**	**0.180**
Day 28	8 May	**0.022**	**0.025**

[a]None detected. Minimum detection limit was 0.001 µg/cm².

[b]Because of wind conditions, application area was treated on two consecutive days and leaf samples were taken both days. Tank concentration of diflubenzuron was **6.41** ppm.

[c]Tank concentration of diflubenzuron was 40 ppm.

Table 4. Diflubenzuron concentrations (µg/cm²) on foliage, Roseville application site, Gypsy Moth monitoring, Spring **1990.**

Sample period	Date	Foliar Residue Replicate 1	Foliar Residue Replicate 2
		µg/cm²	
Background	2 April	ND[a]	ND
Application 1[b]			
Day 0	3 April	**0.167**	**0.147**
Day 13	16 April	**0.124**	**0.143**
Application 2[c]			
Day 0	17 April	**0.252**	**0.244**
Day 28	15 May	**0.072**	**0.053**

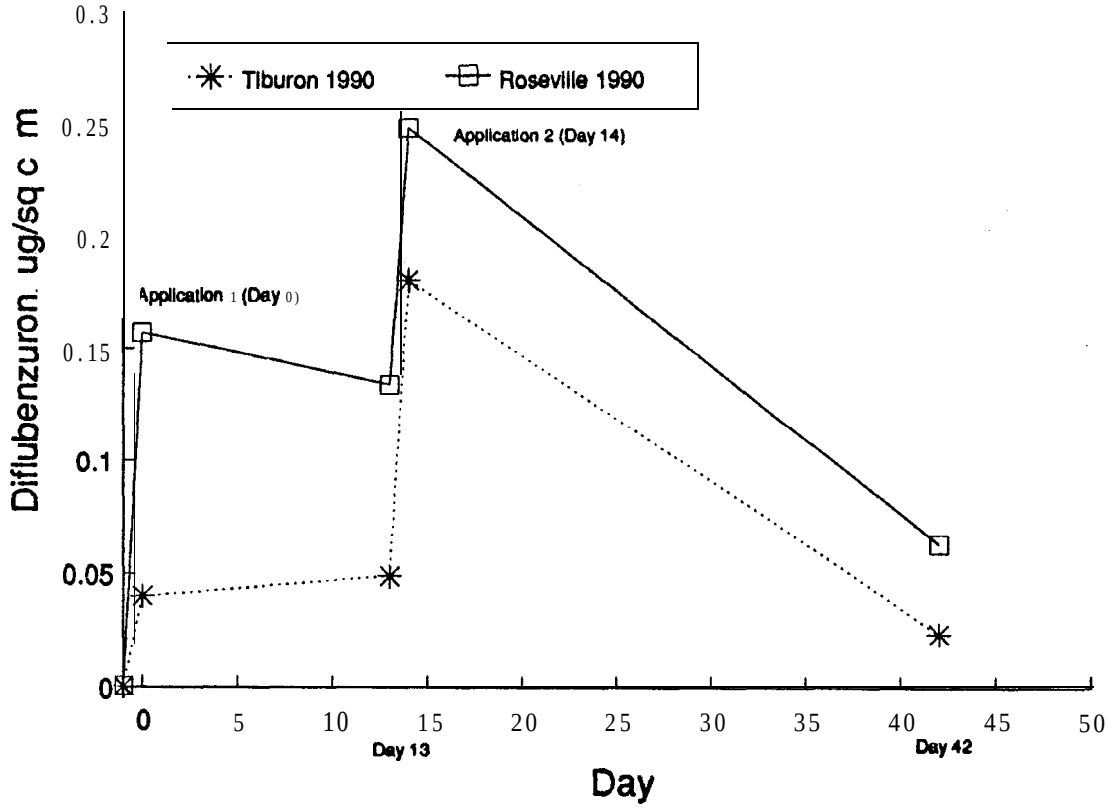
[a]None detected. Minimum detection limit was **0.001** µg/cm².

[b]Tank concentration of diflubenzuron was 40 ppm.

[c]Tank concentration of diflubenzuron was 39 ppm.

In comparison, concentrations found in diflubenzuron monitoring during the **1987** program ranged from none detected for background samples to **19.14** µg/g or **0.218** µg/cm^2 for samples collected **21** days after the second application (Marade et al., **1989).** Figure 1 compares the concentrations (µg/cm^2, mean of 2 replicates at each site) found at the 2 properties sampled in Los Angeles County in 1987 (also treated during March-April) with concentrations (mean of 2 replicates at each site) found in Tiburon and Roseville in 1990. In general, the range of concentrations were similar for the 2 years, but trends over time were different. The 1990 results showed sharp decreases in diflubenzuron concentrations between the second application and **28** days post-application, but no samples were collected during these **28** days to document the variation with time. In **1987,** residues tended to increase over time and degradation was not observed until **28** days after the second application. These results were attributed to high variability between samples (Marade et al., **1989).**

The drop in concentration on leaves observed in 1990 may have been due to several factors: degradation of diflubenzuron; dilution of concentration as leaves increased in size and mass over the study period; and washing off of leaves by rain and/or sprinklers (1 rainstorm occurred during the study period and sprinklers were observed in operation at both sites). This last factor may have little influence because diflubenzuron has a high attraction for the leaf surface and exhibits rainfastness (Dobroski et al., **1985).**



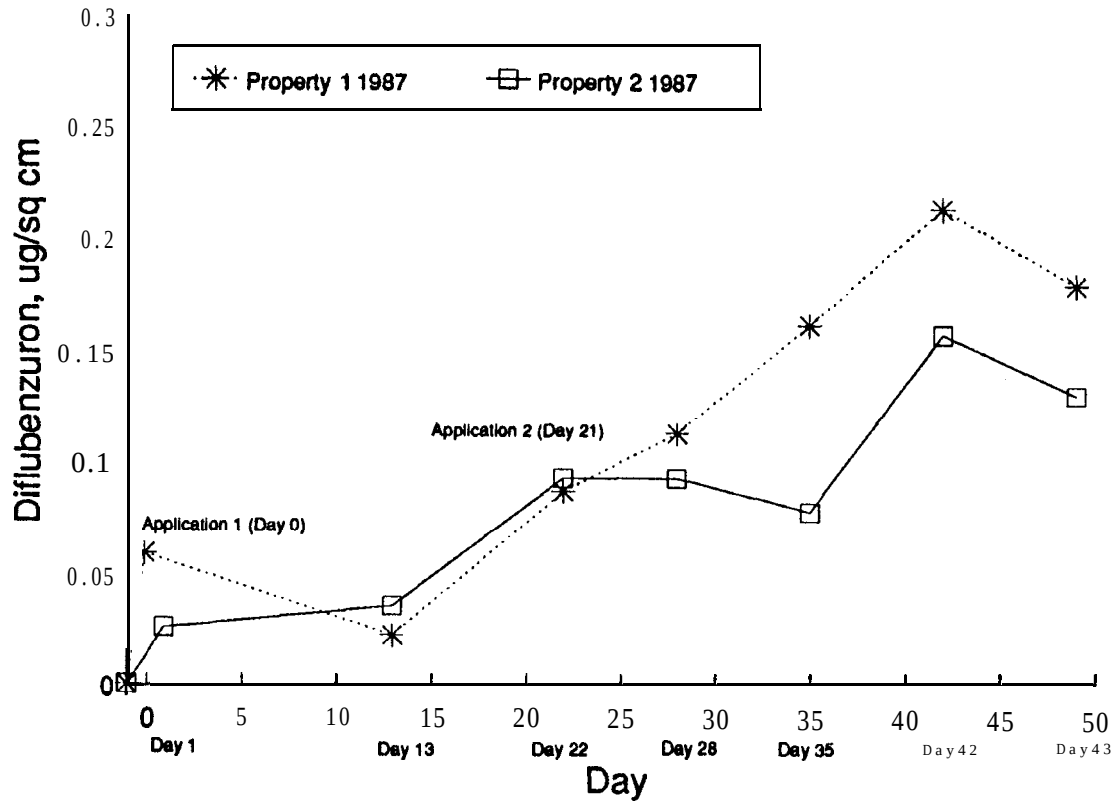


Figure 1. Comparison of 1990 and 1987 Gypsy Moth monitoring data for foliar residue of diflubenzuron, µg/sq cm leaf area. Treatments occurred at 2 week intervals in 1990 and 3 week intervals in 1987.

Air

Results were reported by the lab as µg per sample, then converted to µg/m^3 air by dividing results by the total volume of air sampled (1000 L/min x min sampler ran). Diflubenzuron was detected during three of the four applications at concentrations ranging from 0.0106 to 0.0187 µg/m^3 (Tables 5 and 6). No residues were detected in any background or 1 day post samples; minimum detection limit was 0.001 µg/m^3 initially then increased to 0.002 µg/m^3. In 1987, diflubenzuron was detected during, immediately after, and 24 h after application for both spray events, but not in any background samples. Concentrations ranged from none detected to 0.769 µg/m^3 with the highest concentrations occurring during application periods; minimum detection limit was 0.002 µg/m^3 (Marade et al., 1989). The lower concentrations found in the 1990 monitoring, compared to the 1987 monitoring, may be due to the smaller size of the treated areas and therefore fewer tank loads of pesticide applied; weather conditions; density of vegetation; or other factors.

Water

As in 1987, no diflubenzuron was detected in any water samples collected during the 1990 monitoring period. The minimum detection limit was 0.5 ppb.

Table.5. Diflubenzuron concentrations in air ($\mu g/m^3$), Tiburon application site, Gypsy Moth monitoring, Spring **1990.**

Sample period	Date	Diflubenzuron concentration
		---$\mu g/m^3$ ---
Background	**26** March	ND[a]
Application 1[b]		
Day 0	27 March	ND
Day 0	28 March	ND
Day **1**	29 March	ND
Day **13 (1** d prior Appl. 2)	9 April	ND
Application 2[c]		
Day 0	10 April	**0.0106**
Day 1	**11** April	ND

[a]None detected. Minimum detection limit was 0.001 $\mu g/m^3$ for samples collected through 29 March, and 0.002 $\mu g/m^3$ thereafter.

[b]Because of wind conditions, application area was treated on two consecutive days and air samples were taken both days. Tank concentration of diflubenzuron was 6.41 ppm.

'Tank concentration of diflubenzuron was 40 ppm.

Table 6. Diflubenzuron concentrations in air ($\mu g/m^3$), Roseville application site, Gypsy Moth monitoring, Spring **1990.**

Sample period	Date	Diflubenzuron concentration
		---$\mu g/m^3$---
Background	2 April	ND[a]
Application 1		
Day 0	3 April	**0.0187**
Day 1	4 April	ND
Day **13 (1** d prior Appl. 2)	16 April	ND
Application 2[c]		
Day 0	**17** April	0.0150
Day 1	18 April	ND

[a]None detected. Minimum detection limit was 0.001 $\mu g/m^3$ for the background sample, and 0.002 $\mu g/m^3$ for all others.

[b]Because of wind conditions, application area was treated on two consecutive days and air samples were taken both days. Tank concentration of diflubenzuron was **40** ppm.

'Tank concentration of diflubenzuron was 39 ppm.

Tank

Results for tank samples are found in Table 7. For the first Tiburon application, **1** oz. of Dimilin® 25WP (25% a.i.) was added per 100 gal water. For all other applications, 2 oz. was used per **100 gal** water. Actual concentrations were between **100%** and **103%** of theoretical, except for the first application in Tiburon, in which the tank concentration was only 33% of the theoretical concentration of **0.00195%.**

Quality Control

Results for method validation and continuing quality control analyses are found in Appendix II. Method validation results (Tables II-l, 11-2, II-3) include mean percent recovery ($\bar{X}$) and standard deviation (SD). These data were used to calculate the upper/lower warning limits (mean ± SD) and upper/lower control limits (mean ± 2SD) for accuracy. Minimum detection limits (MDLs) varied slightly as the study progressed. For glass fiber filters, the MDL ranged 0.2-0.4 µg/sample; for foliage, **0.36-1.0** µg/sample; and 0.3-0.5 ppb for water. The MDL for each analysis is noted in each appropriate table.

Continuing quality control results are found **in** Tables 11-4, 11-5, and 11-6. Blank matrix spikes (glass fiber filters, water, and leaves spiked with a known amount of pesticide) were analyzed with each extraction set, Percent recovery fell **outside** of the **set control limits** for one glass fiber filter set, one water set, and two foliage sets. No corrective action was taken, All other continuing quality control analyses fell within their respective control limits.

Table 7. Tank concentrations of diflubenzuron (%), Gypsy Moth monitoring, Spring 1990.

Application	% Diflubenzuron	% Relative to Theoretical Concentration[a]
Tiburon Application 1	0.000641	33
Tiburon Application 2	0.0040	103
Roseville Application 1	0.0040	103
Roseville Application 2	0.0039	100

[a]Theoretical tank concentration was 1 oz. of 25% a.i. wettable powder per 100 gallons water, or 0.00195%, for Tiburon Application 1; and 2 oz. per 100 gallons water, or 0.0039%, for all other applications.

SUMMARY AND CONCLUSIONS

This study monitored environmental levels of diflubenzuron resulting from the 1990 Gypsy Moth eradication project. Two small sites, one in Marin County and one in Placer County, each received two applications of diflubenzuron sprayed on foliage with a ground rig. Foliage, air, water and tank samples were collected for each application.

Diflubenzuron concentrations on leaves ranged from none detected for background samples to 18.31 µg/g after the second application, and from none detected to 0.252 $\mu g/cm^2$ after the second application. Samples collected 28 days later showed much lower concentrations, indicating possible degradation of diflubenzuron over time. However, samples were not collected during the 28 days period to document a degradation trend.

Air sampling was conducted in the treated areas 1 day prior to, during, and 1 day after each application. Diflubenzuron was detected only during application periods, at concentrations ranging from 0.0106 to 0.0187 $\mu g/m^3$.

Water samples were collected from water bodies in and adjacent to the treated areas 1 day before, the day of, and 7 days after each application. No diflubenzuron was detected in any samples.

Samples of the tank mixes used for each application were collected and the concentration of each mix determined. Actual concentrations were very close to the theoretical, except for the first application in Tiburon which was only 33% of the desired concentration.

The range of concentration of diflubenzuron on foliage was similar to that found in the monitoring of the 1987 gypsy moth eradication

program. In 1990 however, samples collected 28 days after the final application showed a sharp decrease in diflubenzuron concentration, perhaps giving a clearer indication of degradation over this period than was seen in 1987. The maximum air concentration detected in 1990 was an order of magnitude less than the maximum found in 1987, and no diflubenzuron was detected after the application, as it was in 1987. These differences between the 2 years may be due to a variety of factors, including differences in weather conditions, density of trees and shrubs, and size of treated areas. The 1990 water results were the same as in 1987: no diflubenzuron was detected in any samples. Overall, environmental concentrations of diflubenzuron from the 1990 gypsy moth eradication project were similar to or lower than those found in 1987.

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APPENDIX I:

ANALYTICAL METHODS FOR DIFLUBENZURON

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
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Original Date:??
Supercedes: NEW
Current Date: May 9, 1990
Method #:

Dimilin in Dislodgeables

SCOPE:

This method is for the analysis of dimilin residues that are dislodgeable from leaves. The sensitivity of the method ranges from 0.36 ug per sample to 1.0 ug per sample, depending on matrix,

PRINCIPLE:

Dimilin is dislodged from leaf surfaces by shaking in water containing a few drops of 2% Sur-Ten solution. Dimilin is then salted out of the extract with sodium chloride and partitioned into ethyl acetate. Ethyl acetate is exchanged for acetonitrile and the extract analyzed by reverse phase HPLC using UV detection.

REAGENTS AND EQUIPMENT:

Ethyl Acetate (Residue grade)
Acetonitrile (HPLC grade)
Water (HPLC grade)
Jars, Mason or Kerr
Sur-Ten solution, (Aerosol OT 75%, aqueous, American Cyanamid), adjusted to a 2% stock by addition of distilled water)
Micropipette (40-200 ul) (Finnpipette Digital, Labsystems)
Tumbler(Dayton Electric Manufacturing Co.)
Sodium chloride (Reagent, A.C.S.)
Sodium sulfate, anhydrous (Reagent, A.C.S)
Separatory funnels (1 liter)
Funnels, glass
Filter paper, Whatman #1, 15 cm
Boiling flasks, flat-bottomed (500 ml)
Beakers (600 ml)
Rotary evaporator (R110, Büchi/Brinkmann)
Graduated conical centrifuge tubes (15 ml)
Nitrogen evaporator (Myers N-EVAP, Organomation Associates)
Syringes, glass (5 or 10 ml) (Multi-fit, Becton & Dickinson)
Filters, nylon (2u) (Acrodisc, Gelman Sciences)

ANALYSIS:

1. Weigh uncovered mason jar containing approximately 60 leaves (30-40 g). Each sample will require a total of 300 ml of distilled water to which 260 ul of 2% Sur-Ten stock solution has been added, e.g with a micropipette.

(This is equivalent to 3-4 drops of 2% Sur-Ten stock per 100ml of distilled water), Add 100 ml of water spiked with 2% surten stock to the sample and spin on a tumbler at 60 rpm for 20 min.

2. Repeat the above extraction two more times using 100 ml of water spiked with 2% Sur-Ten stock each time. Finally, rinse leaf sample with 100 ml of distilled water, shaking vigorously by hand, Combine all extracts in a 1 L separatory funnei.

3. Add 50 g of sodium chloride to the Sur-Ten extracts, and shake the separatory funnel to dissolve.

4. Add 100 ml of ethyl acetate to the separatory funnel and shake vigorously for 1 minute. Drain the aqueous layer into a 600 ml beaker and reserve. Pour the ethylacetate layer out of the top of the separatory funnel through a 30 g be'd of sodium sulfate (held in filter paper) into a 500 ml boiling flask.

5. Return the aqueous layer to the separatory funnel and repeat the extraction three more times using 75 ml of ethyl acetate each time,

6. Wash the funnel containing sodium sulfate 'twice with 25 ml of ethyl acetate, to recover any adsorbed dimilin.'

7. Evaporate extracts to -3 ml on rotary evaporator at high vacuum, add 20 ml of acetonitrile, and evaporate to -3 ml'again.

8. Transfer the extracts to 15 ml graduated centrifuge tubes, washing the flask three times with -2 ml of acetonitrile. Reduce the volume to 1.5 ml using nitrogen evaporation, and make up to 3.0 ml with HPLC-grade water,

9. Filter extracts by passing them through 0.2 um disposable nylon filters using glass syringes to introduce samples and apply pressure. Submit filtered extracts to HPLC analysis.

10. Obtain weight of mason jar tare for each sample (temporarily remove the leaves and pour out any water'before weighing),

EQUIPMENT CONDITIONS:

Instrument : Perkin Elmer Series' 4 Chromatograph, with ISS autosampler
Column: Du Pont Zorbax ODS, 4.6 mm x 25 cm x 5 um
Detector: Kratos Spectraflow, Model 757
Wavelength: 254 nm
Range: (W Lamp): 0.01 absorbance units
Attenuation (Integrator): 2^4
Chart Speed: 0.5 cm/min
Column Temperature: 35°C
Injection Volume: 40 ul (autosampler)
Gradient:

Time(min)	Flow (ml/min)	% Acetonitrile	% Water
6 (EQUIL)	1.5	50	50
8	1.5	50	50
3	1.5	80	20

Retention time of Dimilin: 5.8 -7.4 minutes depending on column condition

It is helpful to include a "zero" command slightly before the peak is to emerge, since there is a good deal of early-eluting W-absorbing material.

CALCULATIONS:

Parts per billion of dimilin:

$$PPB = \frac{(\text{peak area sample})(\text{ng std. injected})(\text{sample fin volume ML})(1000\ \text{ul/ml})}{(\text{Peak area standard})(\text{ul sample injected})(\text{g of sample})}$$

DISCUSSION:

The minimum detection limit of dimilin using this method was 0.36 ug per sample (4.8 ng in a 40 ul injection volume) at a signal to noise ratio of 4.5:1 as determined during method validation. When leaf samples were run, there were many more interferences present and the working MDL was adjusted to 1.0 ug/sample. Average percent recoveries for validation spikes were as follows:

Spike Level	Average % Recovery ± S.D	n
3 ug	88.54 ± 2.19	5
30 ug	83.34 ± 2.27	5
100 ug	88.66 ± 3.47	5
200 ug	88.30 ± 3.91	5

It was found that addition of sodium chloride was very helpful in obtaining satisfactory recoveries of dimilin from aqueous Sur-Ten solution. In the absence of salt, solubility of dimilin in Sur-Ten solution is substantial, and extraction with dichloromethane gives poor recoveries, while extraction with ethyl acetate alone gives intractable emulsions.

REFERENCES:

The extraction of dislodgeable residues is based on "Captan Analysis for Sampling Methods Evaluation" by Mercedita del Valle. The LC analysis is based on "Dimilin" by Vincent Quan.

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Sylvia Richman

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REVIEWED BY: Catherine Cooper

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TITLE: Agricultural Chemist III

APPROVED BY: S. Mark Lee, Ph.D.

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Original Date:Dec. 4, 1987
Supercedes: ?? ,
Current Date:March 7, 1990
Method #: ??

DIMILIN IN GLASS FIBER FILTER

SCOPE:

This method describes the analysis of Dimilin in glass fiber filter from high volume air sampler?

PRINCIPLE:

Dimilin is extracted from filter with hexane/acetone(50/50, v/v), The analysis is by HPLC using a reversed phase column and W detector.

REAGENTS AND EQUIPMENT:

1 Hexane (Optima, Fisher)
2 Acetone (Pesticide Grade, Fisher)
3 Sodium sulfate, anhydrous
4 Acetonitrile (Optima, Fisher)
5 Glass syringe, 10 ml, with Luer lock
6 Acrodisc filter (0.2 micron)
7 Bottle, glass (1 gallon)
8 Graduated cylinder (2 liter)
9 Graduated cylinder (100 ml)
10 Bottle, amber glass (75 mm diameter, 145 mm high, with 49mm opening)
11 Plastic cap for the amber glass bottle
12 Centrifuge tubes, 15 ml, with .1 ml divisions, with ground glass opening
13 Glass funnel
14 Filter paper, 18.5 cm, Whatman No.1
15 Flat bottom flask, 500 ml.
16 Stopper, 24/40, ground glass
17 Ground glass stoppers, No. 13
18 Pasteur pippettes, borosilcate glass, 9 inches long
19 Suction bulb, for the Pasteur pippette
20 Erlenmeyer flask, 250 ml
21 Sonicator
22 Rotovapor-RE, Buchi
23 Test tube holding rack
24 Vibrating mixer for test tubes
25 N-Evap, Organomation

ANALYSIS:

(1) The sample (8 in x 10 in glass fiber filter) is folded into a 2 in x 1.5 in rectangle and put into a wide mouth amber bottle with 100 ml of hexane/acetone (50/50, v/v), Sonicate for 30 minutes.

(2) The solvent is decanted through 50 gm of anhydrous sodium sulfate held by filter paper in a glass funnel into a 500 ml receiving flask.

(3) Repeat (1) and (2) two more times, passing the solvent through the same anhydrous sodium sulfate in the glass funnel.

(4) The sodium sulfate is washed with 30 ml of hexane/acetone (50/50, v/v).

5) The combined solvent is concentrated in vacuum to 1-2 ml. About 5 ml of ACN is added as a keeper.

(6) The extract is transferred to a graduated centrifuge tube (15 ml) with a small suction bulb and a 9 in Pasteur pippette. The flask is washsd twice more with 2 ml hexane/acetone (50/50, v/v). Each wash is transferred to the same graduated centrifuge tube,

(7) The centrifuge tube is stoppered. The content is mixed by placing on a vibrating mixer for about 15 seconds.

(8) After washing the barrels of the N-Evap with 5 ml acetone the centrifuge tube containing the extract is put under the apparatus. A gentle stream of nitrogen is introduced. Evaporate until about 2 ml is left, Adjust the volume to 10 ml with ACN.

(9) The centrifuge tube is stoppered; the content is mixed by placing on a vibrating mixer for about 15 seconds.

(10) Before HPLC analysis the sample is passed through a 0.2 micron filter using a Luer lock glass syringe.

EQUIPMENT CONDITIONS:

HPLC: Perkin-Elmer Series 4 with ISS-100 automatic sampler

Column: Beckman Ultrasphere ODS, 5 micron particle size, 4.6 mm x 15cm
no guard column

Detector:UV (Varian 2550)

Wavelength: 254 nm

Injection Volume: 40 ul

Gradient Profile:

Time(mim)	Flow(ml/min)	ACN	H_2O
0 - 8	1.5	50	50
8 - 10	1.5	60	40
10 - 11	1.5	80	20
11 - 17	1.5	50	50

Retention time: about 6.1 minutes

CALCULATIONS:

For glass fiber filter:

$$\frac{(\text{std,ng/ul})(\text{sample peak ht})(\text{vol injected,std})(\text{final vol,ml})(1000\ \text{ul/ml})}{(\text{std peak ht})(\text{vol injected,sample})(1000\ \text{ng/ug})}$$

= ug of Dimilin

RECOVERY

Spike, ug	% Recovery, mean	S.D
3.0 (n=5)	101	2.68
10.0 (n-5)	102	2.91
30.0 (n=5)	101	6.69

WRITTEN BY: Vincent Quan

TITLE: Agricultural Chemist II

APPROVED BY: Catherine Cooper

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TITLE: Principle Investigator

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Original Date:
. Supercedes: NEW
Current Date: February 5, 1990
Method #:

Dimilin in Surface Water

SCOPE:

This method is for the analysis of Dimilin in surface water. The sensitivity of the method is 0.3 ppb at a signal to noise ratio of 4.5 to 1..

PRINCIPLE:

Dimilin is extracted from water samples with methylene chloride. The methylene chloride extract is dried with sodium sulfate, and the solvent removed by rotary evaporation and exchanged for acetonitrile, The **aceto-nitrile** extract is then analyzed by reverse phase HPLC using W detection at 254 **nm.**

REAGENTS AND **EQUIPMENT:**

1. Methylene chloride (Residue grade--EM Science Omnisolve)
2. Acetonitrile (HPLC grade)
3. Water (HPLC grade)
4. Sodium sulfate, anhydrous (Reagent, A.C.S.)
5. Separatory funnels (1 liter)
6. Funnels, glass
7. Filter paper, **Whatman #1**
8. Boiling flasks, flat-bottomed (500 ml)
9. Rotary evaporator **(R110, Büchi/Brinkmann)**
10. Graduated Conical centrifuge tubes (15 ml)
11. Nitrogen Evaporator (Myers N-EVAP, Organomation Associates Inc.)

ANALYSIS :

1. Surface water (500 ml, is measured out and transfered to a 1 liter separatory funnel.

2. Extract water sample with 100 ml of methylene chloride, shaking gently for 1 minute. After standing, break up remaining bubbles manually with a glass rod or pipette, and drain the methylene chloride layer through a glass funnel containing a bed of sodium sulfate (30 g) in a paper filter. Collect the filtrate in a 500 ml boiling flask. Do not drain the interface **layer** as this hydrates sodium sulfate and slows filtration.

3. Repeat the extraction twice, using 80 ml of methylene chloride, On the last extraction, drain the interface layer into the sodium sulfate funnel, where water and organic matter will be retained.

4. Wash the funnel containing sodium sulfate three times with 25 ml of methylene chloride to recover any adsorbed dimilin.

5. Evaporate extracts to -8 ml on rotary evaporator, add -5 ml of acetonitrile as "keeper" and remove remaining methylene chloride by further rotary evaporation to constant volume.

6. Transfer the extract with a pasteur pipet to graduated centrifuge tube. Rinse boiling flask three times with -2ml aliquots of acetonitrile, adding these rinses to the centrifuge tube. Adjust the volume of extract by nitrogen evaporation to 2 ml if low levels (1 ppb) are expected, otherwise to 4 ml. Analyze extract by reverse phase HPLC, using UV detection. .

EQUIPMENT CONDITIONS:

Instrument: Perkin Elmer Series 4 Chromatograph, with ISS autosampler.
Column: Du Pont Zorbax ODS, 4.6 mm x 25 cm., 5 u particle size
Detector: Kratos Spectraflow Model 757
Wavelength: 254 nm
Range (W Lamp): 0.01 absorbance units
Attenuation (Integrator): 2^4
Chart Speed: 0.6 cm/min
Injection Volume: 40 ul (autosampler)
Gradient:

Time (min)	Flow (ml/min)	% Acetonitrile	% Water
6 (EQUIL)	1.5	50	50
8	1.5	50	50
3	1.5	80	20

Retention time of Dimilin: 7.7 minutes

CALCULATIONS:

Parts per billion of dimilin:

$$PPB = \frac{\text{(peak area sample)(ng std. injected)(sample fin. volume ml)(1000 ul/ml)}}{\text{(Peak area standard)(ul sample injected)(g of sample)}}$$

DISCUSSION:

The minimum detection limit of dimilin using this method is 0.3 ppb (4.8 ng in a 40 ul injection volume) at a signal to noise ratio of 4.5 : 1. The mean recoveries and standard deviations for 5 replicates at each of the three levels below are:

	1 ppb	3 ppb	5 ppb
$\bar{X}$	95 ± 4.7	92 ppb ± 2.3	88 ppb ± 2.8

REFERENCES:

This method is based on " Dimilin", by Vincent Quan and the water extraction protocol is drawn from "Atrazine, Bromacil, Diuron, Prometon, Simazine in Water" by Karen Hefner

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Table I-I. Method validation (% recoveries) for the 1990 Gypsy Moth monitoring.

Study: 93
Chemical: Dimilin
MDL: 0.25 ug/sample
Date of Report: 2/28/90

Matrix Sample Type: Glass Fiber Filters
Lab: CDFA
Chemist: Vince Quan

Lab Sample #	Results (ug)	Spike Level (ug)	Recovery %	$\bar{X}$	SD	CV (%)
2432	2.9	3	97			
2433	3.1	3	103			
2434	3.1	3	103			
2435	3.1	3	103			
2436	3.0	3	100	101	2.68	2.65
2437	10.1	10	101			
2438	10	10	100			
2439	10.2	10	102			
2440	10.7	10	107			
2441	10	10	100	102	2.92	2.86
2442	29	30	97			
2443	31	30	103			
2444	28.8	30	96			
2445	30	30	100			
2446	33.7	30	112	102	6.43	6.30
			OVERALL:	102	4.05	3.97

$\bar{X}$	SD	LWL	UWL	LCL	UCL
102	4.05	98	106	94	110

LWL/UWL (lower warning limit/ upper warning limit) = mean $\pm$ SD.
LCL/UCL (lower control limit/ upper control limit) = mean $\pm$ 2 SD

Table II-2. Method validation (% recoveries) for the 1990 Gypsy Moth monitoring.

Study: 93
Chemical: Dimilin
MDL: 0.3 ppb
Date of Report: 2/28/90

Matrix Sample Type: Water
Lab: CDFA
Chemist: Sylvia Richman

Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
1943	0.98	1.0	98			
1944	0.97	1.0	97			
1945	0.87	1.0	87			
1946	0.93	1.0	93			
1947	0.98	1.0	98	95	4.7	5.0
1948	2.67	3.0	89			
1949	2.79	3.0	93			
1950	2.85	3.0	95			
1951	2.79	3.0	93			
1952	2.73	3.0	91	92	2.3	2.5
1953	4.35	5.0	87			
1954	4.35	5.0	87			
1955	4.25	5.0	85			
1956	4.50	5.0	90			
1957	4.60	5.0	92	88	2.8	3.2
			OVERALL:	92	4.2	4.6

$\bar{X}$	SD	LWL	UWL	LCL	UCL
92	4.2	88	96	84	100

LWL/UWL (lower warning limit/ upper warning limit) = mean $\pm$ SD
LCL/UCL (lower control limit/ upper control limit) = mean $\pm$ 2 SD

Table II-3. Method validation (% recoveries) for the 1990 Gypsy Moth monitoring.

Study: 93
Chemical: Dimilin
MDL: 0.36 - 1 .Oug/sample
Date of Report: 3-29-90

Matrix Sample Type: Dislodgeable Vegetation
Lab: CDFA
Chemist: Sylvia Richman

Lab #	Results ug/sample	Spike Level ug/sample	Recovery %	$\bar{X}$	SD	c v %
2720	2.61	3.0	87			
2725	2.58	3.0	86			
2730	2.67	3.0	89			
2735	2.67	3.0	89			
2740	2.75	3.0	92	89	2.3	2.6
2721	24.7	30	82			
2726	25.5	30	85			
2731	25.1	30	84			
2736	25.7	30	86			
2741	24.0	30	80	83	2.4	2.9
2722	84.5	100	85			
2727	91.1	100	91			
2732	86.0	100	86			
2737	88.0	100	09			
2742	92.9	100	93	89	3.4	3.8
2743	178.0	200	89			
2746	173.0	200	87			
2733	168.0	200	84			
2738	175.0	200	88			
2743	189.0	200	95	89	4.0	4.5
			OVERALL:	87	3.7	4.3

$\bar{X}$	SD	LWL	UWL	LCL	'JCL
87	3.7	80	94	76	98

LWUUWL (lower warning limit/ upper warning limit) = mean + SD
LCU UCL (lower control limit/ upper control limit) = mean + 2SD

Table II-4. Continuing quality control data for the 1990 Gypsy Moth monitoring.

Study: 93
Analyte: Dimilin
MDL: 0.2 ug/sample
Date of Report: 4/13/90

Sample Type: Glass Fiber Filters
Lab: CDFA
Chemist: Vince Quan

Extraction Set #	Lab Sample #	Results (ug)	Spike Level (ug)	Recovery %	$\bar{X}$	SD	CV (%)
107-8	2801	9.3*	10	93			
109-l 1	2799	9.7	10	97			
112-17	3025	9.6	10	96			
118-19	3294	9.7	10	97			
				OVERALL:	96	1.9	2.0

* Result fell below the lower control limit set for Dimilin at 94%.

Table II-5. Continuing quality control data for the 1990 Gypsy Moth monitoring.

Study: 93
Analyte: Dimilin
MDL: 0.3 PPB
Date of Report: 4/13/90

Sample Type: Water
Lab: CDFA
Chemist: Sylvia Richman

Extraction Set #	Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
1, 3-4, 6-7, 9	2796	2.79	3	93			
31-6, 49-51, 61-2	3028	2.60	3	87			
2, 5, 8-12, 25-30	3027	2.93	3	98			
37-8, 55-7, 67-9	3296	2.58	3	86			
39-42, 65-66	3298	2.58	3	86			
43-5	3313	2.87	3	96			
19-24	3315	2.19 *	3	73			
13-18	3347	2.71	3	90			
				OVERALL:	89	7.0	8.8

*Result fell below the lower control limit set for Dimilin at 84%.

Table II-6. Continuing qualiiy control data for the 1990 Gypsy Moth monitoring.

Study: 93
Analyte: Dimilin
MDL: 0.36 - 1.0 ug/sample
Date of Report: 4/13/90

Sample Type: Dislodgeable Vegetation
Lab: CDFA
Chemist: Sylvia Richman

Extraction Set#	Lab Sample #	Results (ug)	Spike Level (ug)	Recovery %	$\bar{X}$	SD	c v (%)
71-74	2964	28.9	30	96			
75-76	2966	27.9	30	93			
77-80	2969	28.4	30	95			
85-86	3318	35.4 *	30	118			
81-84	3319	34.5 *	30	115			
87-88	3345	26.3	30	88			
89-90	3439	28.6	30	95			
91-92	3521	27.6	30	92			
				OVERALL: 99		11	11

* Results fell above the upper control limit set for Dimilin at 98%.