MONITORING OF METHYL EUGENOL AND DDVP IN FRUIT NEAR FRUIT FLY TRAPS DURING A PEST TRAPPING PROGRAM

By

N.V. Carr, R. Gallavan and D. Tran

November, 1990

Environmental Hazards Assessment Program

STATE OF CALIFORNIA
Department of Food and Agriculture
Division of Pest Management, Environmental Protection and Worker Safety
Environmental Monitoring and Pest Management Branch
1220 N Street, Sacramento, California 95814

EH 90-10

ABSTRACT

In 1989, the California Department of Food and Agriculture undertook a monitoring program to determine if methyl eugenol and DDVP, two chemicals used in the Oriental Fruit Fly trapping program, could be detected in fruit growing near traps placed in trees. Methyl eugenol, a male attractant, and naled, an insecticide, were added to traps, and DDVP (also an insecticide) formed as a breakdown product of naled. Methyl eugenol is used as a food additive and occurs naturally in some fruit types. It is currently being evaluated for oncogenicity by the National Toxicology Program. DDVP is on the California Safe Drinking Water and Toxic Enforcement Act (Proposition 65) list of chemicals known to cause cancer. Both compounds had been detected in whole citrus fruit samples from trees containing traps in September 1988. Naled was not detected in any samples in 1988, so fruit was not analyzed for it in 1989. The 1989 monitoring was expanded to include apples and apricots as well as citrus (oranges).

Fruit samples were collected from trees containing Oriental Fruit Fly traps in Sacramento County between May and August, 1989. Traps were replaced (or rebaited) every 4-6 weeks during spring and summer. Sampling took place when traps were first replaced, and fruit was collected from trees in which traps had been in place at least 6 weeks. Samples were collected 4 h and 24 h after replacement of traps, at a distance of 31-61 cm from the traps, or from greater than 61 cm, if necessary. Fourteen trees of each fruit type were sampled.

For apples and apricots, the whole fruit was analyzed for methyl eugenol and DDVP. Neither chemical was detected in any apricot or apple samples. Methyl eugenol was found to occur naturally in orange rinds, so only the inner pulp of this fruit was analyzed. Methyl eugenol was detected in 14 of the 28 orange samples collected, at concentrations ranging from 28.8 ppb to 147.1 ppb. Since it was known to occur naturally in orange rinds, 14 control samples from trees not containing traps, but located in trapping areas, were collected and the inner pulp was analyzed. Methyl eugenol was detected in 8 of these 14 samples, at concentrations of 32.3 ppb to 289.0 ppb. Statistical tests performed on the data failed to find a significant difference between trapped and control samples.

DDVP was detected in 4 of the 28 orange samples collected from trapped trees, but was not detected in control samples. Concentrations ranged from 1.2 ppb to 1.6 ppb. Statistically, there was no difference between trapped and control samples. Although special precaution was taken to prevent sample contamination in the field and laboratory, the source of DDVP residues remains problematic. The DDVP found in orange pulp may have been due to ambient volatilization of DDVP from fruit fly traps or the result of sample contamination in the lab or field. Future research should determine the most likely source of these residues.

ACKNOWLEDGMENTS

Thanks to all EHAP field group personnel who assisted in this project with their usual outstanding cooperation and expertise. Special thanks to Dennis Eastly, Senior Agricultural Inspector, of the Sacramento County Agricultural Commissioner's Office, for his patient and willing cooperation. Thanks also to the Agricultural Inspector Aides working under him who placed traps and obtained sampling permission from property owners.

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.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In 1988, the California Department of Food and Agriculture (CDFA) conducted studies to determine environmental concentrations of methyl eugenol (4-allyl-1,2-dimethoxybenzene), naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), and dichlorvos (2,2-dichlorovinyl dimethyl phosphate; DDVP) from fruit fly trapping and eradication programs in the state (Turner et al., 1989). Methyl eugenol and naled are used in traps and bait stations, and DDVP is a breakdown product of naled. Methyl eugenol acts as a lure to attract male fruit flies into traps. This compound is also used as a food additive and occurs naturally in several fruit types including citrus. It is currently being evaluated for oncogenicity by the National Toxicology Program (Nelson, 1989). Naled is an organophosphate pesticide which works by direct contact. It degrades to DDVP, which is more volatile and acts as an insecticidal vapor within fruit fly traps. In January 1989, DDVP was added to the State of California Safe Drinking Water and Toxic Enforcement Act (Proposition 65) list of chemicals known to cause cancer. More detailed information on these chemicals and their environmental fate can be found in the report on CDFA's 1988 monitoring (Turner et al., 1989).

As a part of that monitoring, citrus fruit samples were collected in September 1988 from trees containing traps. Two of eight fruit samples collected contained both methyl eugenol and DDVP residues. Additional samples were collected in November 1988 but neither chemical was detected. Since this second sampling took place under cooler conditions which may have reduced volatilization of the chemicals, further monitoring of fruit during the spring and summer of 1989 was recommended to determine whether methyl eugenol and DDVP are absorbed by fruit in the vicinity of traps. The

objective was simply to determine if these chemicals would be found in fruit near traps; any evaluation of possible health effects is beyond the scope of this study. Since naled was not found during previous monitoring, samples were not analyzed for this compound.

MATERIALS AND METHODS

Trapping

The Oriental Fruit Fly trapping program uses a Jackson trap (Fig. 1) baited with methyl eugenol, which acts as a male attractant, and naled, in a dilute solution, which stuns rather than kills the flies. Naled degrades to DDVP which is an insecticidal vapor. The traps contain a cotton wick to which 5 ml of the solution of diluted Dibrom® (naled) in methyl eugenol is added by eyedropper. The solution contains 1% naled active ingredient. Flies are caught as they fall on to a sticky insert placed at the bottom of the trap. Traps are placed in fruit trees at a density of one to two per square mile in urban areas of Northern California from May through November. In the spring and summer traps are either replaced by new ones, or rebaited (more solution is added to the trap in the field) every 4-6 weeks. For this study, new traps were placed in the trees we sampled. No rebaiting took place for these trees, to eliminate the possibility of inadvertant contamination of fruit due to rebaiting. The pole used to place traps in trees was cleaned with alcohol and deionized water after each use.

Sampling

Sampling took place in Sacramento county in late spring and summer of 1989, just after the first re-trapping (i.e., traps being replaced) of the

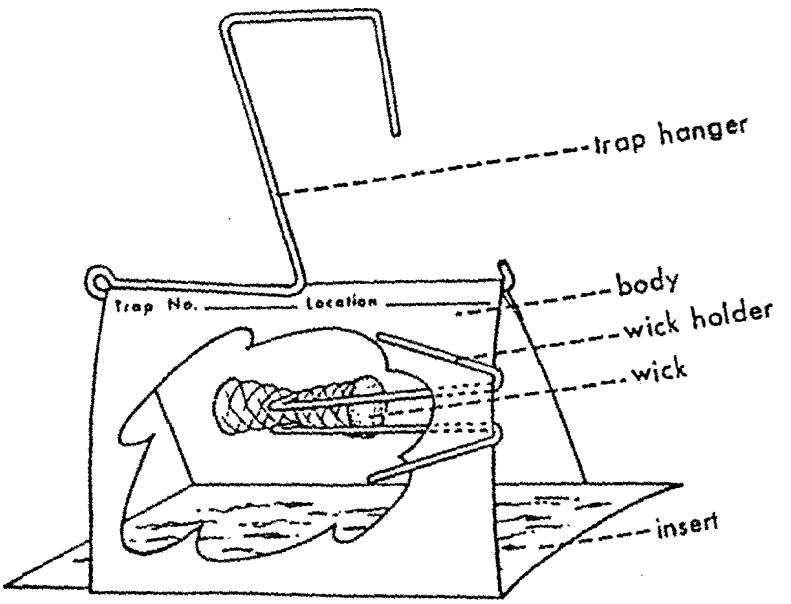

FIGURE 1. JACKSON TRAP

trees. Whole fruit was collected from trapped trees in scattered residential areas of the county (Fig. 2). Three fruit types were collected: oranges, apricots, and apples. Fourteen trees of each fruit type were sampled, and two control samples of each type were collected in the sampling areas from trees that did not have traps. After results for the first orange samples were obtained, 14 more orange control samples were collected in June 1989 to compare with samples from trapped trees (see Results and Discussion).

County agricultural staff selected trees and obtained permission from property owners to collect samples. Samples were collected when fruit was ripe: late May to early June for oranges, mid-June to early July for apricots, and mid-August for apples. Traps had been in place for at least six weeks prior to re-trapping. Sampling took place 4 hours and 24 hours after re-trapping. This sampling design was chosen to duplicate previous tests in September 1988 when chemicals were detected in fruit from trees in which traps had been freshly rebaited; air monitoring of bait applied for the eradication project that year detected both chemicals 4 h and 24 h after application (Turner et al., 1989).

A minimum of 500 g (fresh weight) of fruit was collected from each tree, at a distance of 31-61 cm from the trap, if possible. Occasionally, sufficient fruit was not available within this range, so it was taken from somewhat further than 61 cm from the trap. Fruit was collected with a fruit picker consisting of a rubber-coated wire basket on a pole. The fruit picker was rinsed with alcohol and deionized water between samples. Fruit was placed in 2-liter wide-mouth glass jars or plastic bags (when fruit was too large for jars), put on wet ice until delivered to the laboratory that same

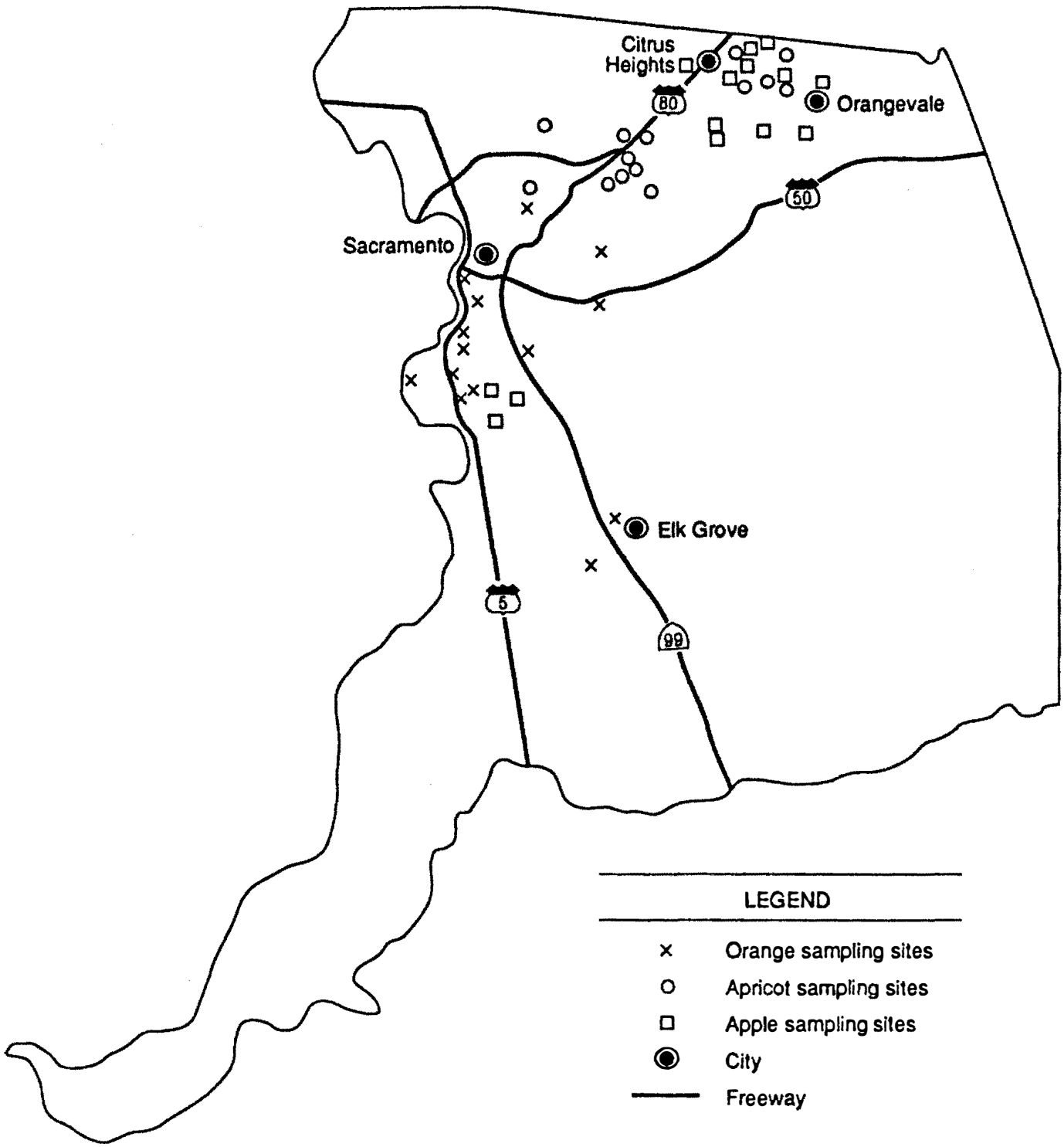

Figure 2. Fruit sampling sites in Sacramento County, methyl eugenol and DDVP monitoring, summer 1989.

day, then extracted immediately. A chain of custody record accompanied each sample from the time of container preparation through lab analysis. Ambient air temperature was recorded at each site when traps were replaced in trees and when samples were collected (4 and 24 hours after trapping).

Chemical Analysis

All samples were analyzed for methyl eugenol and DDVP by CDFA's Chemistry Laboratory Services in Sacramento. During method validation for chemical analysis, methyl eugenol was found to occur naturally in the rind of oranges at an average concentration of 170 ppb. Therefore, only the inner pulp of orange samples was analyzed. For apricots and apples whole fruits were analyzed. Minimum detection limits for all fruit were 28 ppb for methyl eugenol and 1 ppb for DDVP.

Analytical methods (see Appendix I) were the same as those used in the 1988 monitoring of Oriental Fruit Fly eradication and trapping programs (Turner et al., 1989). Positive samples were confirmed by high resolution gas chromatograph/mass spectrometer. Continuing quality control during analysis consisted of a blank matrix and blank matrix spike analyzed with each extraction set.

Statistical Analysis

Because it was expected that no positive samples (samples containing detectable residue) would be found, the study was designed with the objective of having enough samples to be able to conclude with certainty that methyl eugenol and DDVP were not present in fruit near traps. The population was conceived as consisting of fruit samples either containing or not containing detectable residue. In n trials (samples) the probability of finding x

positives follows the binomial distribution. Using binomial probabilities it was calculated that if 14 samples were taken and all observed to be negative, it could be stated with 95% confidence that the true probability of a positive sample in the population was less than 0.20 (20%). Although it would have been desirable to be able to conclude that the true probability was closer to 0, the number of samples required would have been impractical.

RESULTS AND DISCUSSION

Oranges

Results of analyses of orange pulp samples for methyl eugenol and DDVP are found in Table 1. For samples taken 4 h after re-trapping, methyl eugenol was detected in 9 of 14 samples at concentrations ranging from 28.8 ppb to 133.6 ppb. For oranges sampled 24 h after re-trapping, 5 of 14 samples contained methyl eugenol at concentrations of 32.6 ppb to 147.1 ppb. After these results were obtained, 14 more orange control samples were collected in the original sampling areas from random trees that had not been trapped, and the pulp was analyzed for methyl eugenol and DDVP. These results are presented in Table 2. Methyl eugenol was detected in 8 of 14 samples at concentrations of 32.3 ppb to 289.0 ppb. (The original two orange control samples were collected before re-trapping began, and the outer rind only of these samples was analyzed. Since the 14 additional samples had pulp rather than rind analyzed, the results for the first two controls are not included.)

DDVP residues were found in 2 of the 14 orange samples taken 4 h post-retrapping, and in 2 more samples taken 24 h post (Table 1). Concentrations

Table 1. Methyl eugenol and DDVP concentrations in orange pulp of samples from trees containing traps, 4 h and 24 h after re-trapping, Oriental Fruit Fly monitoring 1989.

Site	---Methyl eugenol--- 4 h post	24 h post	--------DDVP-------- 4 h post	24 h post
	--ppb (wet wt.)--		--ppb (wet wt.)--	
1	45.9	ND[1]	ND	ND
2	28.8	ND	ND	ND
3	ND	ND	ND	ND
4	ND	ND	ND	ND
5	129.5	77.6	ND	ND
6	38.9	50.7	ND	1.6
7	ND	ND	ND	ND
8	32.6	ND	ND	1.3
9	ND	ND	ND	ND
10	104.0	32.6	1.2	ND
11	133.6	ND	ND	ND
12	ND	ND	1.5	ND
13	53.6	147.1	ND	ND
14	33.8	40.0	ND	ND

[1]Not detected. Minimum detection limit was 28 ppb for methyl eugenol and 1 ppb for DDVP.

Table 2. Methyl eugenol and DDVP concentrations in orange pulp of control samples, Oriental Fruit Fly Monitoring 1989.

Site	Methyl eugenol	DDVP
	ppb (wet wt.)	ppb (wet wt.)
61	107.0	ND[1]
62	289.0	ND
63	47.5	ND
64	ND	ND
65	83.3	ND
66	41.0	ND
67	175.2	ND
68	ND	ND
69	ND	ND
70	ND	ND
71	32.3	ND
72	ND	ND
73	53.1	ND
74	ND	ND

[1]Not detected. Minimum detection limit was 28 ppb for methyl eugenol and 1 ppb for DDVP.

ranged from 1.2 ppb to 1.6 ppb. No DDVP was detected in any of the orange control samples.

Nonparametric statistical procedures are appropriate for analyzing data including both quantitative and nonquantitative (ND) values. The data have some ordinal properties which would allow ranking but it is not clear how to rank ND observations. Therefore, simple tests were chosen that did not require complete ranking. More powerful tests exist but they were considered inappropriate for data that could not be completely ranked. Tests were performed for the following comparisons, for both chemicals: control samples vs. 4 h post samples; control samples vs. 24 h post samples; and 4 h post samples vs. 24 h post samples. These tests found no significant differences between any of the groups. A description of these statistical analyses can be found in Appendix III.

In summary, methyl eugenol was detected in a high proportion (22 of 42) of orange samples, a fruit in which it occurs naturally, from both trees with and without traps. DDVP was detected in 4 of 42 orange samples, at concentrations of 1.2 ppb to 1.6 ppb, but only from trees containing traps, both 4 h and 24 h after trap placement.

Apricots and Apples

Apricot and apple samples were collected in the same manner as orange samples, and the whole fruit analyzed for methyl eugenol and DDVP. Neither chemical was detected in any samples, including control samples. Therefore, it can be stated with 95% confidence that for either fruit the probability of finding a sample containing detectable methyl eugenol or DDVP is less than 20%.

Quality Control Results

Results for quality control analyses are found in Appendix II. Method validation results 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 ± 2 SD) for accuracy. Initially, method validation analyses for methyl eugenol were done with a minimum detection limit of 50 ppb (Tables II-1, II-2, and II-3). Later, as procedures improved, the minimum detection limit dropped to 28 ppb (Tables II-4 and II-5). The minimum detection limit for all DDVP analyses was 1.0 ppb (Tables II-6 through II-9).

Continuing quality control during analyses included blank matrix spikes (fruit matrix spiked with a known amount of analyte) analyzed with each extraction set (Tables II-10 through II-15). Percent recovery fell outside control limits for DDVP in apricot in four out of five extraction sets (Table II-13), and for methyl eugenol in apple in two out of four extraction sets (Table II-14). No corrective action was taken. All other continuing quality control analyses fell within their respective control limits.

Temperature Data

Since temperature influences the volatility of methyl eugenol and DDVP, air temperature was measured when traps were placed in trees and when samples were collected. Ambient air temperatures during the summer 1989 sampling ranged from 17°C to 35°C (Table 3). In comparison, in September 1988 the maximum air temperature on the day fruit samples were collected was 28°C; the maximum on the day fruit samples were collected in November 1988 was 12°C. Therefore, temperatures during the 1989 sampling were similar to those during the September 1988 fruit sampling, when both chemicals were detected in

citrus fruit, and on average much higher than during the November 1988 sampling, when neither chemical was detected.

SUMMARY AND CONCLUSIONS

The objective of this study was to determine if methyl eugenol and DDVP residues occur in fruit near fruit fly traps.

Methyl eugenol residues were not detected in any apple or apricot samples. According to the study design, it can be stated with 95% confidence that for either fruit the probability of finding a sample containing detectable methyl eugenol is less than 20%.

Methyl eugenol was detected in oranges from trapped trees, but also in oranges from untrapped (control) trees. Statistical tests failed to find significant differences in methyl eugenol concentrations between control trees, trees 4 hours after trapping, and trees 24 hours after trapping. However, because of the nature of the data the statistical power of the tests was low, and only very large differences between the groups could have been detected. As previously mentioned, methyl eugenol was found to occur naturally in orange rinds. It seems probable that methyl eugenol found in orange pulp from trapped trees occurred naturally. It cannot be determined if it came from fruit fly traps, since it was found in similar concentrations in oranges from untrapped trees, and was not detected in apples or apricots near traps.

No DDVP residues were detected in any apple or apricot samples, and again, there was 95% confidence that the probability of finding DDVP in an apple or apricot sample was less than 20%. DDVP was detected in 4 orange

Table 3. Ambient air temperatures during fruit sampling, Oriental Fruit Fly monitoring 1989.

Fruit Type		Traps Deployed	4 h post	24 h post
		----------Temperature, °C----------		
Orange:	range	20-25	24-32	18-28
	average	22	29	24
Apricot:	range	18-30	23-35	21-31
	average	23	30	25
Apple:	range	20-31	28-36	17-31
	average	26	32	24

samples, at concentrations of 1.2 ppb to 1.6 ppb. No significant differences were found between untrapped trees and trees 4 hours after trapping, and between untrapped trees and trees 24 hours after trapping. However, the statistical power of these tests is extremely low. Given the sample sizes used, only very large differences between groups could have been detected statistically.

A previous study had found residues of methyl eugenol and DDVP in citrus fruit in September 1988, but not in November 1988. The present study was conducted during the spring and summer when ambient air temperatures were similar to those in September 1988, and higher than during the November 1988 sampling. Since the chemicals were found only during the warmer seasons, it is possible that warmer temperatures increased the potential for both methyl eugenol and DDVP volatilization and absorption by oranges. Nevertheless, because methyl eugenol occurs naturally in orange pulp, it cannot be presumed to have volatilized from traps to be absorbed by fruit. In addition, the source of DDVP residues in orange pulp is questionable even though special precaution was used to prevent sample contamination in the field and laboratory.

This study was designed to sample fruit with the highest possibility of containing residues of methyl eugenol and DDVP from traps, based on the 1988 results. This dictated sampling fruit from trees with freshly replaced traps. Even if the methyl eugenol and DDVP residues found were due to vapor absorption by fruit, it still is unknown whether the residues were the result of long or short term exposure, or both, since the fruit had been exposed to traps for at least 6 weeks prior to trap replacement.

Future research should be directed toward determining whether DDVP can actually be absorbed by fruit or if sample contamination has played a role in these results.

REFERENCES

Nelson, L. 1989. Methyl eugenol exposure in oriental fruit fly eradication project. California Dept. Food and Agric. Memorandum to R. Magee, p. 2.

Turner, B., N. Miller, D. Tran, and S. Powell. 1989. The environmental monitoring of methyl eugenol, naled, and dichlorvos during a pest trapping and eradication program. California Dept. Food and Agric. 21 p.

APPENDIX I:

ANALYTICAL METHODS

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4998/4999

Original Date:??
Supercedes: NEW
Current Date:9/14/1988
Method #:

METHYL EUGENOL ON VEGETATION

SCOPE:

This method is used to determine Methyl Eugenol on vegetation samples.

PRINCIPLE:

The vegetation is blended with dry ice, then extracted with hexaxe . The extract is concentrated to a final volume in hexane and transfered to a silica sep-pak .Elute the analyte with a mixture of chloroform and hexane.The Methyl Eugenol is analyzed by GC using a FID detector .

REAGENTS AND EQUIPMENT

Hexane , chloroform ,pesticide quality or equivalent .
Sodium sulfate , anhydrous.
Graduated test tube.
Silica sep-pak .
Working standard,prepared from stock standard
10 ml syringe.
Dry ice
Mason jars: 2 quart, 1 pint sizes
Boiling flasks: 500 ml, 250 ml
Separatory funnels: 500 ml.
Filter funnels: 90 mm
Aluminum foil
Whatman filter paper: #1, 18.5 cm
G10 gyratory shaker
Cuisinart food processor
Buchi rotovapor
Meyer N-evap analytical evaporator
GC Varian 3700 with FID

ANALYSIS :

1. Blend the entire sample with dry ice in a cuisinart, then store in a freezer to allow the carbon dioxide to evaporate. (Do not cap the container too tight.)
2. Weigh 50g of the sample into a pint size jar. Add 75ml hexane to the jar and shake for 20 minutes .
3. Filter the sample through #1 filter paper into a 500 ml separatory funnel. Rinse the jar and the filter paper several times with 70ml hexane .
4. If the water is present at the bottom of the seperatory funnel , drain the water layer into a beaker and discard . Taking care not to lose any of the solvent .
5. Drain the solvent layer through sodium sulfate into a 500 ml boiling flask.
6. Rinse the sodium sulfate well with 70ml hexane.
7. Evaporate the contents of the flask to near dryness by using a rotary evaporator .
8.Transfer the extract to a graduated test tube with 10 ml of hexane .

Evaporate the final volume to 2ml by using a nitrogen evaporator.
SILICA SEP-PAK CLEAN UP:
1.Take 1ml out of 2ml of the final volume and transfer it to a 10 ml syringe which was connected to a 4ml hexane washed silica sep-pak .
2.Add 7.0 ml of the mixture chloroform : hexane (75:25) to the 10 ml syringe.
3.Force the mixture through the sep-pak at a constant flow rate (3ml/min) by pressing the plunger consistently .
4.Discard the first two milliliters of the mixture .
5.Collect the last five milliliters of the mixture into a graduated test tube and concentrate to the final volume of 2mls.Mix the test tube for 10 seconds by using a vortex mixer.
6.Analyze by gas chromatography .

DESORPTION COEFFICIENT:

EQUIPMENT CONDITIONS:

Gas chromatograph : Varian 3700
- -Initial temp : 100°C
- -Initial time : 5 min
- -Prog. rate : 10°C/ min
- -Final time : 0.0 min
- -Final temp : 190°C
- -Injector temp: 210°C
- -Detector temp:260°C
- -Hydrogen flow rate : 30ml/min
- -Air flow rate : 350ml/min
- -Helium flow rate : 30ml/min

Detector type : Flame ionization detector
Column : DB WAX 15m
Retention time : 8.5 min

CALCULATIONS:

Results are reported as PPM

DISCUSSION:

Spike level :258.65ug/50g grapefruit

	% recovery
Spike 1	74.0
Spike 2	68.2
Spike 3	82.6
Spike 4	77.4
spike 5	61.4

Because methyl eugenol evaporates quickly, sample should be extracted and analyzed as soon as possible .

WRITTEN BY: DUC TRAN

TITLE: Agricultural chemist I

REVIEWED BY : CATHERINE COOPER

Catherine Cooper

TITLE: Agricultural Chemist III

APPROVED BY : GEORGE TICHELAAR

George Tichelaar

TITLE: Principle Agricultural Chemist

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4998/4999

Original Date:??
Supercedes: NEW
Current Date:2/4/88
Method #:

DDVP/NALED

SCOPE:
This method is for the determination of DDVP and NALED from resin tubes and from vegetation.

PRINCIPLE:
DDVP and NALED have been collected from the air into resin tubes, and a mixture of hexane:acetone(50:50) is used to extract DDVP and NALED from the tubes. Vegetation samples have also been collected, and hexane is used to extract DDVP and NALED from them. The concentration of DDVP and NALED is determined by GC using a TSD detector.

REAGENTS AND EQUIPMENT:
Hexane/Acetone, 1:1 mixture
Hexane
Methanol
Sodium sulfate, anhydrous
Dry ice
XAD-2 resin
Clean vegetation
1 pint Mason jars
2 quart Mason jars
500 ml brown bottles
90 mm stemmed filter funnels
500 ml separatory funnels
250 ml separatory funnels
500 ml boiling flasks
15 ml conical test tubes
Aluminum foil
Whatman filter paper, #1, 18.5 cm
Cuisinart food processor
Sonicator
Gyratory shaker
Rotary evaporator
Nitrogen evaporator
Vortex mixer
Cutting board and knife
GC (Varian 3700 TSD, Autosampler)
Stock standard
Working standard

ANALYSIS:
(I) Air Samples

1) **Transfer the glass wool and/or resin from the sample hi-vol resin jar to a 500 ml brown bottle with 1:1 hexane/acetone, rinsing the resin jar. Add enough 1:1 hexane/acetone to the brown bottle to reach a final solvent**

volume of 150 ml. Seal the bottle with aluminum foil and a lid.
2) Sonicate the sample for 15 minutes.
3) Shake the sample on the gyratory shaker for 1 hour at 220 rpm.
4) Pour the solvent through a 90 mm funnel containing filter paper and 70 grams of sodium sulfate. Collect the sample in a 500 ml boiling flask.
5) Add 100 ml of 1:1 hexane/acetone to the brown bottle and shake for 1 hour at 220 rpm.
6) Transfer the entire contents of the brown bottle (solvent and resin) to the filter funnel and combine the extracts in the 500 ml boiling flask. Rinse the funnel contents well with about 20ml of 1:1 hexane/acetone mixture.
7) Evaporate the contents of the boiling flask to near dryness on the rotary evaporator with the setting on 40.
8) Transfer the contents of the boiling flask to a 15 ml conical test tube with 10 ml of methanol.
9) Concentrate the sample to 4 ml on the nitrogen evaporator. Mix the contents of the tube for 10 seconds with the vortex mixer.
10)Analyze by gas chromatography.

(II) Vegetation Samples

1) Weigh the sample and cut it into small pieces with a knife.
2) Blend the sample in a Cuisinart blender with dry ice.
3) Transfer to a 2 quart Mason jar, cover lightly with aluminum foil and a lid, and place in a freezer overnight to allow the dry ice to sublime.
4) While still frozen, stir the sample and weigh out a 100 g aliquot into a 1 pint Mason jar. Add 150 ml of hexane to the jar and shake on a gyratory shaker for 30 minutes at 220 rpm.
5) Filter the sample through #1 filter paper into a 500 ml seperatory funnel. Rinse the jar and the filter paper several times with a total of 70ml of hexane.
6) If the water is present in the 500ml separatory funnel, drain the bottom water into a beaker and discard .
7) Drain the solvent layer through sodium sulfate into a 500 ml boiling flask.
8) Rinse the sodium sulfate well with 50 ml of hexane .
9) Rotoevaporate the contents of the boiling flask to near dryness.
10)Transfer the sample to a 15 ml test tube with 10 ml of methanol.
11)Reduce the volume to 4 ml on the nitrogen evaporator.
12)Mix the sample on the vortex mixer.
13)Analyze by gas chromatography.

DESORPTION COEFFICIENT:

EQUIPMENT CONDITIONS:
GC condition :
Column :Methyl Silicone .53 mm x 10M .
Carrier gas : Helium 7psi .
Detector : TSD .
Bead : 5.6 , Hydrogen : 29psi .

Tem. Progam :	Initial	:	100	5min.
	Rate	:	20	1min.
	Final	:	170	4min.
Retention time		:	DDVP approx. 4min.	
			Naled approx. 10min.	

CALCULATIONS:

$$\text{NG/CUBIC METER} = \frac{(A)(\text{ng standard})(1000)(\text{final volume mls})}{(B)(\text{total cubic meter of air})(\text{ul sample injected})}$$

A =area sample or peak heigh sample
B =area standard or peak heigh standard

$$\text{PPB} = \frac{\text{Final volume (ul)} \times \text{Amount of STD (ng)} \times \text{Peak height of sample}}{\text{Volume of sample injected(ul)} \times \text{Sample weight(g)} \times \text{Peak height of STD}}$$

DISCUSSION:
Recovery : Since Naled could be converted quickly to DDVP ,the percent recovery of Naled is proportional to the time .To determine the concentration of Naled , the sample should be analyzed within 24 hours with fresh working standards .

REFERENCES:

WRITTEN BY: DUC TRAN
Duc Tran
TITLE: Agricultural Chemist I

REVIEWED BY: CATHERINE COOPER
Catherine Cooper
TITLE: Agricultural Chemist III

APPROVED BY: GEORGE TICHELAAR
George C Tichelaar
TITLE: Principal Agricultural Chemist

APPENDIX II:

RESULTS FOR METHOD VALIDATION AND CONTINUING QUALITY CONTROL ANALYSES

Table II-1. Method Validation Data (% Recoveries) for the 1989 Methyl Eugenol Study: Orange Rind.

Analyte: Methyl Eugenol
Matrix: Orange Rind
Detection Limit: 50 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 05/16/89

EHAP Sample #	Lab Sample #	Results (ppm)	Spike Level (ppm)	Recovery %	$\bar{X}$	SD	CV (%)
1	3146	0.40	0.48	83.3			
2	3145	0.37	0.47	78.7			
3	3144	0.33	0.47	70.2			
4	3143	0.42	0.48	87.5			
5	3142	0.36	0.47	76.6	79.3	6.59	8.31
6	3141	1.98	2.38	83.2			
7	3140	1.87	2.38	78.6			
8	3139	1.82	2.36	77.1			
9	3138	1.88	2.38	79.0			
10	3137	1.67	2.37	70.5	77.7	4.61	5.93
OVERALL:					78.5	5.43	6.91

$\bar{X}$	SD	LWL	UWL	LCL	UCL
79	5.4	74	84	68	90

* The mean background level of methyl eugenol (0.17 ppm) in orange rind has been subtracted from each spike result.

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-2. Method Validation Data (% Recoveries) for the 1989 Methyl Eugenol Study: Orange Inner Pulp.

Analyte: Methyl Eugenol
Matrix: Orange Inner Pulp
Detection Limit: 50 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 05/18/89

3180	0.41	0.47	87.2			
3181	0.42	0.47	89.4			
3182	0.41	0.48	85.4			
3183	0.39	0.47	83.0			
3184	0.34	0.48	70.8	83	7.3	8.8

$\bar{X}$	SD	LWL	UWL	LCL	UCL
83	7.3	76	90	68	98

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-3. Method Validation Data (% Recoveries) for the 1989 Methyl Eugenol Study: Apricot.

Analyte: Methyl Eugenol
Matrix: Apricot
Detection Limit: 50 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 05/18/89

Lab Sample #	Results (ppm)	Spike Level (ppm)	Recovery %	$\bar{X}$	SD	CV (%)
3178	0.46	0.46	76.1			
3177	0.40	0.47	85.1			
3176	0.45	0.47	95.7			
3174	0.37	0.47	78.7			
3175	0.45	0.48	93.8	86	8.8	10

$\bar{X}$	SD	LWL	UWL	LCL	UCL
86	8.8	77	95	68	104

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-4. Method validation data (% recoveries) for the 1989 methyl eugenol study: orange inner pulp.

Analyte: Methyl Eugenol
Matrix: Orange Inner Pulp
Detection Limit: 28 ppb

LAB: CDFA
Chemist: Duc Tran
Date: 07/24/89

Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
120	347.2	480.7	72.2			
121	391.2	475.9	82.2			
122	353.2	481.9	73.3			
123	323.0	476.2	67.8			

$\bar{X}$	SD	LWL	UWL	LCL	UCL
74	5.2	69	79	64	84

• Results based on using a clean-up procedure.

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-5. Method validation data (% recoveries) for the 1989 methyl eugenol study: apple.

Analyte: Methyl Eugenol
Matrix: Apple (Red Delicious)
Detection Limit: 28 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 07/24/89

Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
161	413.9	476.3	86.9			
162	400.0	466.7	85.7			
163	400.2	466.9	85.7			
164	358.2	462.9	77.4			
165	374.9	463.1	81.0	83.3	4.0	4.8

$\bar{X}$	SD	LWL	UWL	LCL	UCL
83	4.0	79	87	75	91

* Results based on using a sep-pak clean-up procedure.

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-6. Method Validation Data (% Recoveries) for the 1989 Methyl Eugenol / DDVP Study: Orange Rind.

Analyte: DDVP
Matrix: Orange Rind
Detection Limit: 1 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 05/31/89

Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
3335	2.08	2.39	87.0			
3334	2.08	2.39	87.0			
3333	2.06	2.38	86.6			
3332	2.05	2.39	85.6			
3331	1.55	2.28	68.0	82.8	8.32	10.0
3330	10.14	11.80	85.9			
3329	9.19	11.79	78.0			
3328	9.49	11.87	80.0			
3327	10.03	11.95	83.9			
3326	8.98	11.83	75.9	80.7	4.13	5.11
OVERALL:				81.8	6.29	7.69

$\bar{X}$	SD	LWL	UWL	LCL	UCL
82	6.3	76	88	69	95

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-7. Method Validation Data (% Recoveries) for the 1989 Methyl Eugenol / DDVP Study: Orange Inner Pulp.

Analyte: DDVP
Matrix: Orange Inner Pulp
Detection Limit: 1 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 05/22/89

Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
3256	1.88	2.39	78.7			
3255	1.68	2.34	71.8			
3254	1.87	2.33	80.3			
3253	1.80	2.39	75.3			
3252	1.60	2.38	67.3	74.7	5.27	7.05
3251	8.29	11.79	70.3			
3250	8.79	11.95	73.6			
3249	9.02	11.72	77.0			
3248	9.79	11.70	83.7			
3247	9.96	11.91	83.6	77.6	5.98	7.70
OVERALL:				76.2	5.53	7.27

$\bar{X}$	SD	LWL	UWL	LCL	UCL
76	5.5	71	82	65	87

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-8. Method Validation Data (% Recoveries) for the 1989 Methyl Eugenol Study: Apple.

Analyte: DDVP
Matrix: Apple
Detection Limit: 1 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 06/07/89

Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
3447	10.31	11.70	88.2			
3448	10.65	12.08	88.2			

$\bar{X}$	SD	LWL	UWL	LCL	UCL
84	4.7	79	89	75	93

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-9. Method Validation Data (% Recoveries) for the 1989 Methyl Eugenol Study: Apricot.

Analyte: DDVP
Matrix: Apricot.
Detection Limit: 1 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 06/07/89

Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
3469	10.24	12.06	84.9			
3470	10.21	11.81	86.5			
3468	9.64	11.81	81.6			
3471	8.28	11.66	71.0			
3467	9.94	11.88	83.7	81.5	6.16	7.55

$\bar{X}$	SD	LWL	UWL	LCL	UCL
82	6.2	76	88	70	94

LWL/UWL (lower warning limit/ upper warning limit) = mean +/- SD.
LCL/UCL (lower control limit/ upper control limit) = mean +/- 2 SD.

Table II-10. Continuing quality control data for the 1989 methyl eugenol study : orange inner pulp.

Analyte: Methyl Eugenol
Matrix: Orange Inner Pulp
Detection Limit: 28 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 07/24/89

EHAP Sample #	Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
3 - 10	3506	460.0	480.0	95.8			
19 - 30	3571	378.1	470.3	80.4			
11 - 18	3550	338.2	477.6	70.8			
181 - 187	7	372.0	477.6	77.8			
188 - 194	41	339.9	475.2	71.5	79.3	10.1	12.8

Table II-11. Continuing quality control data for the 1989 methyl eugenol study: orange inner pulp.

Analyte: DDVP
Matrix: Orange Inner Pulp
Detection Limit: 1 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 07/24/89

EHAP Sample #	Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
3 - 10	3506	9.57	12.01	79.7			
19 - 30	3571	8.11	11.79	68.9			
11 - 18	3550	9.48	11.97	79.2			
181 - 187	7	8.00	11.97	66.8			
188 - 194	41	9.55	11.91	80.2	75.0	6.54	8.73

Table II-12. Continuing quality control data for the 1989 methyl eugenol study: apricot.

Analyte: Methyl Eugenol
Matrix: Total Apricot
Detection Limit: 28 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 07/24/89

EHAP Sample #	Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
35-42	3573	450.5	499.3	90.2			
37,45-47,50	3599	419.8	478.7	87.7			
31-33,44,49	3601	456.7	470.0	97.2			
53-56	38	412.3	481.6	85.6			
57-60	59	454.7	481.6	94.4	91.0	4.76	5.23

Table II-13. Continuing quality control data for the 1989 methyl eugenol study: apricot.

Analyte: DDVP
Matrix: Total Apricot
Detection Limit: 1 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 07/24/89

EHAP Sample #	Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD	CV (%)
35-42	3573*	8.57	12.51	68.5			
37,45-47,50	3599*	8.12	11.99	67.7			
31-33,44,49	3601*	8.10	11.78	68.8			
53-56	38*	7.80	12.10	64.5			
57-60	59	9.10	12.10	75.2	68.9	3.89	5.65

*Samples fell below the lower control limit set for DDVP in Apricot at 70%.

Table II-14. Continuing quality control data for the 1989 methyl eugenol study: apple.

Analyte: Methyl Eugenol
Matrix: Total Apple
Detection Limit: 28 ppb

Lab: CDFA
Chemist: Duc Tran
Date: 07/24/89

EHAP Sample #	Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD
61-67	451**	434.20	472.22	91.9		
65, 68-9, 71-76	516	382.00	477.6	80.0		
77-78, 80-87	514*	353.00	476.2	74.1		
58, 70, 79, 89, 90	519	394.40	475.90	82.9	82.2	7.42

*Samples fell below the lower control limit set for Methyl Eugenol in Apple at 75%.
**Samples fell above the upper control limit set for Methyl Eugenol in Apple at 91%.

Table II-15. Continuing quality control data for the 1989 methyl eugenol study: apple.

Matrix: Total Apple
Detection Limit: 1 ppb

Chemist: Duc Tran
Date: 07/24/89

EHAP Sample #	Lab Sample #	Results (ppb)	Spike Level (ppb)	Recovery %	$\bar{X}$	SD
61-67	451	9.29	11.84	78.5		
65, 68-9, 71-76	516	9.00	11.98	75.1		
77-78, 80-87	514	9.17	11.94	76.8		
58, 70, 79, 89, 90	519	7.97	11.86	67.3	74.4	4.95

APPENDIX III:

STATISTICAL TESTS

Statistical Tests for Results from Orange Samples

One-tailed tests were used for control samples vs. 4-hour-post samples, and control samples vs. 24-hour-post samples. A two-tailed test was used for 4-hour-post vs. 24-hour-post samples since the direction of observed differences was not predicted. The degrees of freedom associated with each χ^2 test is 1.

Untrapped (control) vs. 4-hour-post

The comparisons of control trees to trapped trees are comparisons of independent samples. A median test (Siegel, 1956) was used to test whether the two samples could have come from populations with the same median concentration of methyl eugenol. The null hypothesis of equal medians could not be rejected (χ^2 adjusted for continuity = 0.0, $p > 0.05$). A chi-square test of whether the two sample sets came from populations with the same proportion of NDs (Siegel, 1956) also failed to find significant difference (χ^2 adjusted for continuity = 0.0, $p > 0.05$). The Kolmogorov-Smirnov two-sample test (Siegel, 1956) was used to test whether there were any differences between the two distributions (i.e., differences of location of the mean, dispersion, skewness, etc.). It too was non-significant (KD = 2, $p > 0.05$).

For DDVP, Fisher's Exact Test (Siegel, 1956) was used to determine whether the two sample sets came from populations with the same proportions of NDs. The null hypothesis of no difference could not be rejected ($p > 0.05$).

Untrapped (control) vs. 24-hour-post

Because so many of the 24-hour-post samples were ND, it was not possible to statistically compare the medians of the two groups. The chi-square test of whether the two sample sets came from populations

with the same proportion of NDs (Siegel, 1956) failed to find significant difference (χ^2 adjusted for continuity = 0.574, $p > 0.05$). The Kolmogorov-Smirnov two-sample test (Siegel, 1956) for any differences between the two distributions (i.e., differences of location of the mean, dispersion, skewness, etc.) was non-significant (KD = 3, $p > 0.05$).

For DDVP, Fisher's Exact Test (Siegel, 1956) was used to determine whether the two sample sets came from populations with the same proportions of NDs. The null hypothesis of no difference could not be rejected ($p > 0.05$).

4-hour vs. 24-hour-post trapping

Comparisons of the two time points are comparisons of paired samples, since the same trees were sampled at both times. A sign test (Siegel, 1956) was done to test the null hypothesis that the 4-hour and the 24-hour samples from each tree are equally likely to have the higher concentration of methyl eugenol. The null hypothesis could not be rejected ($p > 0.05$). No statistical test was done on the DDVP data. The observations gave no evidence of a difference (two trees increased, two decreased, the rest were ND on both occasions).

REFERENCE

Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co. New York, NY, 312 p.