Methods for the Comparative Analysis of Organophosphate Residues in Four Compartments of Needles of *Pinus ponderosa*

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The extent of interaction between vapor phase organophosphate pesticides and plant foliage is not known. Pesticide vapors may deposit onto foliage while bound to dust particles, sorb directly to the surface, sorb into the cuticle, or penetrate to the interior of the leaf. On the basis of these different modes of interaction, four compartments of leaves are identified as significant: water-dislodgable surface material; surfactant-soluble surface material; chloroform-soluble cuticular wax; and the remaining material after the removal of the first three compartments. Analytical methods have been developed for the comparative determination of organophosphate insecticides in these four compartments of *Pinus ponderosa* needles and of their oxons in the first three. Method recoveries and detection limits were determined with diazinon, methidathion, and chlorpyrifos and their respective oxons in extracts of untreated pines and were then field-tested with needles collected from ponderosa pines growing in the Arboretum of the University of California and from potted ponderosa pines placed in a peach orchard after application of a dormant spray containing diazinon.

Keywords: *Pesticides; organophosphates; ponderosa pine (Pinus ponderosa); needles, analysis*

INTRODUCTION

The accumulation of toxic compounds from the atmosphere into plant tissues has received much recent attention. Some of the focus of research in this area has been on the sorption into pine needles of chlorinated organics such as PCBs, dioxins, DDT and its metabolites (Calamari et al., 1994; Hauk et al., 1994; Kylin et al., 1994), and polynuclear aromatic hydrocarbons (Simonich and Hites, 1994). Other studies have looked at the accumulation of toxicants that may be injurious to the plant, such as nitrophenols (Hinkel et al., 1989). There is much interest in understanding the role foliage may play in the environmental fate of airborne residues of conventional and new pest control chemicals. There is also the need to ensure the safety of crops used for food that may be inadvertently exposed to agricultural chemicals that have origins beyond normal spray drift distances (Turner et al., 1989). Finally, exposure to semivolatile organics deposited from the air could affect sensitive species of plants and animals in nontarget ecosystems.

Over 14 million of pounds of organophosphate pesticides are applied annually in California's Central Valley (Cal EPA, 1993). While most of the pesticide lands on the target, for many chemicals over 90% of the material applied disappears within as little as 3 days (Spencer and Claith, 1990). Loss routes include absorption into the plant, soil, or water surface to which it was applied; degradation by hydrolysis, photolytic action, plant metabolism, or microbial metabolism; or volatilization and subsequent movement away from the site of application. There is much evidence that for chemicals of appreciable vapor pressure, which are applied to foliage or soil surfaces, volatilization can be a dominant fate process (Seiber and Woodrow, 1995; Taylor and Glotfelty, 1988; Taylor and Spencer, 1990), but where the bulk of these airborne residues wind up remains un-

explained and largely unexplored. Once in the air, residues may remain as a vapor, sorb to particles, or partition to droplets of water that will eventually form rain, snow, or fog (Seiber et al., 1983). These three atmospheric forms may all be moved far away from the site of application by wind. Such is the case in California's Central Valley, where winds draw air from the valley floor up the slopes of the Sierra Nevada mountains that bound the valley to the east and south (Zabik and Seiber, 1993).

In the Sierra Nevada mountains, pesticide residues are likely to contact plants such as the abundant conifer *Pinus ponderosa* (ponderosa pine). Because of the demonstrated uptake of organic vapors into needles, pine and spruce trees have been proposed as biomonitors of air quality. However, the mechanism and extent of the interaction between semivolatile organic vapors and the foliage of trees are poorly characterized. The needles of the ponderosa pine are quite long, averaging 10 in. in length, and may remain on the tree for 3–5 years. Mature trees range from 15 to 70 m in height (Munz and Keck, 1968), providing considerable needle surface area. Means of interaction between atmospheric pesticides and needles include contact with the cuticular surface and uptake through the stomata (Riederer, 1995). The cuticle may be viewed as interacting with pesticide residues in three ways. First, dust particles to which pesticide vapors are sorbed are filtered from the air and become deposited on the cuticle. The dust simply adheres to the needle surface, yet the pesticide itself need not enter the needle. Second, pesticide vapors may sorb to the lipophilic surface of the needle itself. Third, the vapors may partition into the lipophilic cuticular wax and may traverse the cuticle and sorb to the epidermal cells beneath the cuticle. Vapors that get taken up through the stomata may become sorbed to the hydrophilic mesophyll cells of the interior of the needle.

To elucidate the extent and means of interaction between organic vapors and pine needles, methods are

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Diazinon

Chlorpyrifos

Methidathion

Diazinon Oxon
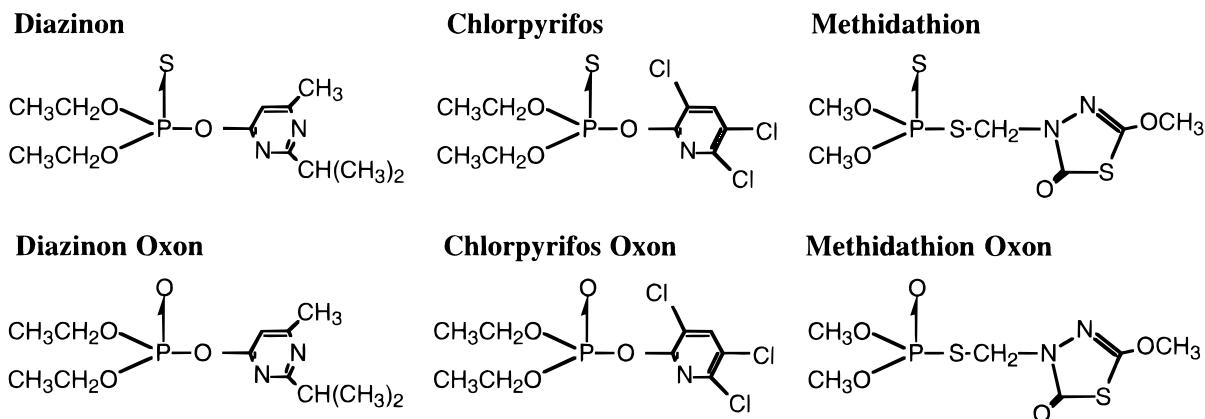
Chlorpyrifos Oxon

Methidathion Oxon

Figure 1. Structures of organophosphate insecticides and their oxons.

needed for analyzing separately the sites in and on the needle where the pesticides may reside. Often, the needle or leaf is analyzed as a whole (Hinkel et al., 1989; Bacci et al., 1990) or the wax compartment and the rest of the needle (Reischl et al., 1987; Strachan et al., 1994; Hauk et al., 1994). In this paper, we report extraction and analytical methods for the comparative analysis of organophosphate (OP) residues in four compartments of the pine needle. The insecticides examined include diazinon and its degradation product diazinon oxon, chlorpyrifos and chlorpyrifos oxon, and methidathion and methidathion oxon (Figure 1). The movement of vapors from the San Joaquin Valley area of heavy use to pine forests on the western slopes of the Sierra Nevada mountain range has been demonstrated (Zabik and Seiber, 1993). The first compartment consists of the residues that may be removed from the surface of the needle with a distilled water rinse, similar to the method developed to remove deposits of inorganic ions from plant surfaces (Bytnerowicz et al., 1987). This rinsate would include residues that may be mechanically removed from the surface such as those bound to dust particles and those that are poorly sorbed to the needle surface. The second compartment consists of surface residues dislodgable with a surfactant wash (Iwata et al., 1977). The third consists of residues sorbed by the cuticular wax. Soluble cuticular lipids are first removed from the needle by a chloroform rinse and then separated from coextracted analytes by solvent-induced precipitation. The fourth compartment, consisting of the remainder needle tissue and residues that may have entered the needle interior, is processed by methods used to analyze foodstuffs for organophosphate pesticide residues (*Pesticide Analytical Manual*, 1994).

These methods were applied to two sets of field-exposed samples. The first set was collected in the Arboretum located on the campus of the University of California, Davis. Since the campus is located in the agricultural Central Valley, the vegetation may be exposed at low levels to pesticide vapors and particles originating from the orchards and fields outside of the campus. These samples were analyzed for diazinon and chlorpyrifos, two organophosphates of broad usage in the Central Valley. A second application was to needles from a potted pine tree that was placed in a peach orchard 4 h after an application of a dormant spray containing diazinon. This was performed to measure, in a high-exposure situation, the rate and extent of uptake of diazinon vapors over a 3 week period and comparative residue distribution among the needle compartments.

MATERIALS AND METHODS

Materials. All reagents were of Resi-analyzed grade (J. T. Baker Inc., Phillipsburg, NJ, and Fisher Scientific, Fair Lawn, NJ). Standards of organophosphates utilized for spike recoveries were of 98% or greater purity. Organophosphates used include diazinon and diazinon oxon, methidathion and methidathion oxon (Ciba-Geigy Corp., Greensboro, NC), and chlorpyrifos and chlorpyrifos oxon (Dow Elanco, Indianapolis, IN). Solid phase extraction cartridges were 0.5 g, 6 cm^3, C$_{18}$ (Bond Elut Analytichem, Harbor City, CA). Florisil gel (mesh 60/100) (Fisher Scientific, Pittsburgh, PA) was conditioned by storing for at least 24 h prior to use at 130 °C. Spike and recoveries were performed on needles as the matrix. Pine needles used for blanks as well as for spiking were collected at a remote site 100 mi northeast of Sacramento, CA, in the Sierra Nevada foothills. Needles were removed by hand from a branch in the lower canopy of a ponderosa. The needles were immediately placed on dry ice, transported to the laboratory, and transferred to a −20 °C freezer. All samples were kept frozen until analysis. Three separate replicates of 10 g each were separated for dry weight determination by drying in a heated (60 °C) vacuum oven for 48 h.

Spiking. The samples were removed from the freezer and allowed to warm to room temperature. Three 10-g subsamples were removed. These subsamples were then treated as individual replicates of the entire sample and were handled identically. In addition to the spiked needle samples, one needle blank and one solvent blank were run concurrently with the extraction set for each compartment. Solvent blanks and solvents spiked with 0.1 μg of the spiking mixture were run to ensure that the extractions were performed without laboratory contamination. Those results are not reported here. Spiking of needle compartments was done in triplicate at levels of 10 and 50 ppb (0.1 and 0.5 μg/10 g needle sample). Spiking was performed by adding the spiking solution to the initial extract from each compartment.

Extraction. Extraction methods for all four compartments are summarized in Figure 2.

(i) Distilled Water Wash. After the needle samples were brought to room temperature, three 10-g samples were removed. Using gloved hands, the needles were held over a stainless steel funnel inserted into a 250-mL Erlenmeyer flask and sprayed with 200 mL of distilled, deionized (DI) water from a squirt bottle held approximately 12 cm away. While the spraying proceeded, the needles were turned and rotated so that all sides of the needles were equally sprayed. The DI water was then extracted using a C$_{18}$ Bond Elute solid phase extraction cartridge. The SPE cartridges were first prepared by conditioning with 1 column volume each of ethyl acetate, methanol, and distilled deionized water. After the sample was vacuum-filtered through the cartridge, it was then eluted with 10 mL of ethyl acetate. The volume of the extract was reduced to 0.5 mL by nitrogen stream evaporation. The sample was then ready for analysis.

(ii) Surfactant Wash. Immediately after the distilled water wash, the needles were placed in an Erlenmeyer flask, to which
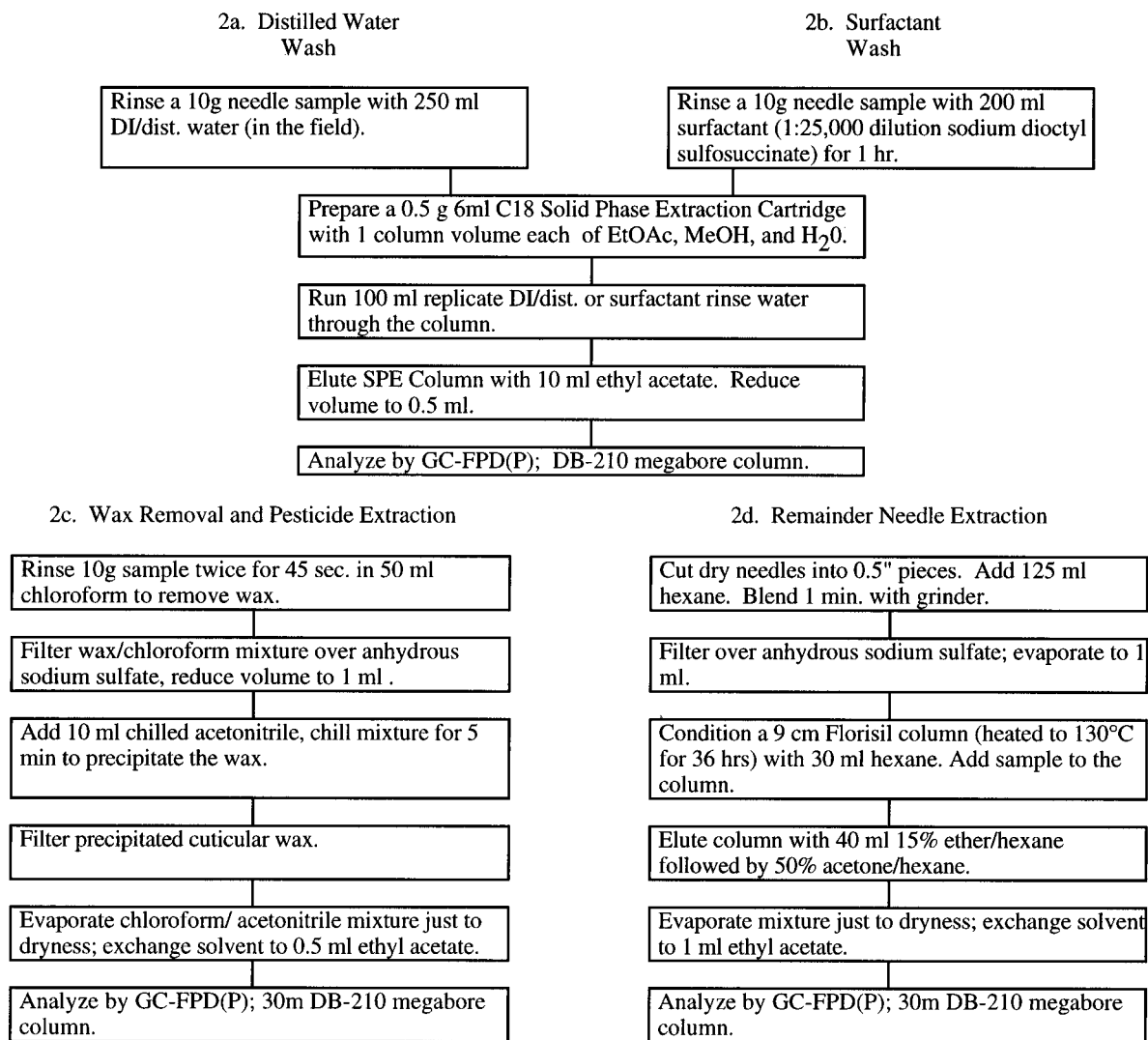
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2a. Distilled Water Wash

Rinse a 10g needle sample with 250 ml DI/dist. water (in the field).

2b. Surfactant Wash

Rinse a 10g needle sample with 200 ml surfactant (1:25,000 dilution sodium dioctyl sulfosuccinate) for 1 hr.

Prepare a 0.5 g 6ml C18 Solid Phase Extraction Cartridge with 1 column volume each of EtOAc, MeOH, and H_2O.

Run 100 ml replicate DI/dist. or surfactant rinse water through the column.

Elute SPE Column with 10 ml ethyl acetate. Reduce volume to 0.5 ml.

Analyze by GC-FPD(P); DB-210 megabore column.

2c. Wax Removal and Pesticide Extraction

Rinse 10g sample twice for 45 sec. in 50 ml chloroform to remove wax.

Filter wax/chloroform mixture over anhydrous sodium sulfate, reduce volume to 1 ml .

Add 10 ml chilled acetonitrile, chill mixture for 5 min to precipitate the wax.

Filter precipitated cuticular wax.

Evaporate chloroform/ acetonitrile mixture just to dryness; exchange solvent to 0.5 ml ethyl acetate.

Analyze by GC-FPD(P); 30m DB-210 megabore column.

2d. Remainder Needle Extraction

Cut dry needles into 0.5" pieces. Add 125 ml hexane. Blend 1 min. with grinder.

Filter over anhydrous sodium sulfate; evaporate to 1 ml.

Condition a 9 cm Florisil column (heated to 130°C for 36 hrs) with 30 ml hexane. Add sample to the column.

Elute column with 40 ml 15% ether/hexane followed by 50% acetone/hexane.

Evaporate mixture just to dryness; exchange solvent to 1 ml ethyl acetate.

Analyze by GC-FPD(P); 30m DB-210 megabore column.

Figure 2. Summaries of analytical methods for pine needle compartments.

200 mL of a 1:25000 dilution of the surfactant sodium dioctylsulfosuccinate in distilled water was added. The flask was place on a table rotary shaker and agitated continuously for 1 h. The sample was then filtered through a C_{18} solid phase extraction cartridge in the same manner as above.

(iii) Soluble Cuticular Lipid. After the surfactant wash, the needles were removed from the flask and allowed to air-dry until all of the residual water had evaporated from the needle surfaces. The needles were placed back into the bottom of a flask, and 50 mL of chloroform was added. The flask was shaken by hand for 45 s, after which time the chloroform was filtered over anhydrous sodium sulfate into a round-bottom flask. This step was repeated with a second aliquot of 50 mL of chloroform, which was combined with the first. The volume of chloroform was then reduced by rotary and nitrogen evaporation to 0.5 mL and transferred to a 40-mL graduated test tube. Chilled acetonitrile (10 mL) was added to the tube, and after brief mixing, the tube was placed on ice for 5 min. The resulting mixture was then filtered through Whatman No. 1 filter paper which was prewetted with chilled acetonitrile. The filtered wax was dried, weighed, and discarded. The eluate was evaporated just to dryness by nitrogen evaporation and brought up to 0.5 mL final volume with ethyl acetate.

(iv) Remainder Needle. After the wax was removed from the needles, the needles were removed from the flask and allowed to desiccate until they became fairly brittle. They were then cut into 1-cm pieces and placed in a flask. Hexane (130 mL) was added to the flask, and the needles were ground for 1 min with an Ultra Turrex (IKA Works, Cincinnati, OH) tissue grinder. The macerated needles were filtered over anhydrous sodium sulfate. The hexane was then rotary-evaporated to 1 mL. A 9 cm × 15 mm diameter Florisil column was prepared by conditioning it with 30 mL of hexane, after which the sample was added. The organophosphate residues were eluted from the column by 40 mL of 15% ether/hexane followed by 40 mL of 50% acetone/hexane. The eluate was evaporated just to dryness by rotary evaporation and brought up to 1-mL final volume with ethyl acetate.

Gas Chromatographic Analysis. Final extracts of all four compartments in ethyl acetate were analyzed by gas chromatography using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Wilmington, DE), equipped with a flame photometric detector with a phosphorus filter, and a Hewlett-Packard 7673A automatic sampler. The column was a 30-m DB-210 megabore column (J&W Scientific, Folsom, CA) (0.53 mm i.d., 1-μm film thickness). Conditions were as follows: injector temperature, 220 °C; detector temperature, 255 °C; initial oven temperature, 100 °C; initial hold time, 1 min; ramp A, 20 °C/min; temperature A, 170 °C; ramp B, 5 °C/min; final temperature, 235 °C; final hold time, 0 min. Data were collected and integrated using a Hewlett-Packard 3396A integrator. Confirmation of the samples was done using a Hewlett-Packard 5890 gas chromatograph, equipped with a 30-m DB-210 capillary column (J&W Scientific) (0.25 mm i.d., 0.25-μm film thickness), and a Hewlett-Packard 5710 mass selective detector in selective ion mode. The ions selected were as follows: for diazinon, m/z 152, 179, and 304; for diazinon oxon, m/z 217, 273, and 288; for chlorpyrifos, m/z 314, 316, and 351; for chlorpyrifos oxon, m/z 270, 272, and 298; for methidathion, m/z 125, 145, and 302; and for methidathion oxon, m/z 145, 229, and 286. GC conditions were identical to the primary GC method.

Organophosphate Residues in Pine Needles

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Table 1. Percent Recoveries of Organophosphate Residues From Four Pine Needle Compartments

compound	amt spiked, μg	distilled water			surfactant wash			soluble cuticular lipid			remainder needle		
diazinon	0.1	**87**	(7)[b]	26[c]	**103**	(2)	8	**81**	(9)	36	**89**	(4)	18
	0.5	**74**	(4)	34	**105**	(3)	10	**71**	(8)	45	**80**	(13)	46
diazoxon	0.1	**143**	(9)	60	**64**	(11)	57	**135**	(4)	42	**115**	(1)	17
	0.5	**99**	(5)	10	**50**	(33)	116	**95**	(7)	18	**84**	(16)	47
chlorpyrifos	0.1	**72**	(2)	33	**81**	(7)	33	**88**	(12)	36	**83**	(7)	30
	0.5	**52**	(8)	64	**75**	(8)	64	**69**	(6)	42	**68**	(20)	73
chlorpyroxon	0.1	**69**	(4)	38	**133**	(1)	34	**74**	(7)	40	**0**		
	0.5	**50**	(9)	69	**124**	(4)	31	**68**	(2)	35	**0**		
methidathion	0.1	**95**	(8)	22	**113**	(29)	71	**91**	(3)	15	**57**	(11)	66
	0.5	**89**	(5)	21	**109**	(4)	34	**61**	(36)	112	**53**	(29)	105
methidoxon	0.1	**114**	(9)	31	**108**	(2)	13	**110**	(3)	17	**0**		
	0.5	**78**	(12)	45	**83**	(2)	21	**77**	(4)	30	**0**		

[a] Needle and solvent blanks run with each compartment spike showed no contamination or incurred residues. [b] Numbers in parentheses are percent relative standard deviations. [c] Total error for the method.

Field Sampling. Pine needles from the UC Davis Arboretum were collected in June 1994 by removing them by hand from ponderosa pine trees and immediately transporting them to the laboratory and extracting them according to the methods described above. A second sampling was done with a potted ponderosa pine tree, approximately 1.5 m tall, placed in a 1.6-ha peach orchard at the Wolfskill experimental farm near Winters, CA, 4 h after a dormant spray of diazinon had been applied. The application was made in mid January 1994, at a rate of 9 kg/ha active ingredient. Also included in the spray were copper sulfate and petroleum oil. Needles from the potted tree were collected by hand every 24–48 h over the next 3 weeks. The distilled water wash was performed on site by holding the needles that had been removed over a funnel placed in a large amber bottle, and spraying the needles according to the method described above. The needles and the distilled water rinse were immediately transported to the laboratory and extracted according to the methods described above.

RESULTS AND DISCUSSION

Recovery Experiments. Results of the recoveries of spiked samples by compartment are in Table 1. Recoveries of the parent compounds were quantitative (80–110%) for all compartment extracts over at least one of the spiking levels, except for chlorpyrifos from the distilled water wash, for which recoveries were 72% and 52%, and methidathion from the remainder needle compartment, for which recoveries were 57% and 53%. Methidathion is the most polar of the parent OPs and is the least soluble in hexane, perhaps explaining the low extractability of methidathion from the remainder needle compartment and low recovery from the Florisil column. Two of the oxons were not recovered at all (chlorpyroxon) or were recovered poorly (methidoxon) from the remainder needle compartment. This was likely due to incomplete extraction and/or loss during Florisil cleanup (*Pesticide Analytical Manual*, 1994). Diazoxon was the exception, providing recovery from remainder tissues comparable to the parent diazinon.

For the parent compounds the total error (McFarren et al., 1979) averaged 33% for the distilled water compartment, 34% for the surfactant wash compartment, 48% for the soluble cuticular lipid compartment, and 56% for the remainder needle compartment. Total error for the oxons averaged 42% for the distilled water compartment, 45% for the surfactant wash compartment, and 30% for the soluble cuticular lipid compartment. Total error below 100% is considered acceptable for trace analytical methods (McFarren et al., 1979). For diazoxon, the total error averaged 32% for the remainder needle, while chlorpyroxon and methidathion oxon were poorly recovered as noted above.
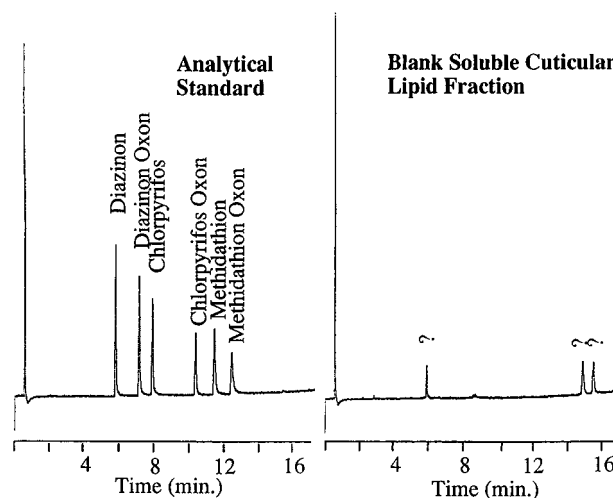

Figure 3. Gas chromatograms of an analytical standard and unknown peaks in a blank soluble cuticular lipid fraction.

Chromatograms of samples extracted according to these methods contained few interferences (Figure 3). Only three peaks of unknown composition consistently eluted from each of the four compartments. Only one peak was a potential interference, eluting just after diazinon. However, in all of the spiked fractions, diazinon was adequately integrated separately from this interfering peak (Figure 4). For field samples, all peaks of interest eluted without interference from unknown peaks (Figure 5). Peaks of interest were confirmed in 25% of the field samples by GC/MS.

Limits of quantitation (LOQ) by compartment, for the method validation study, are given in Table 2. These are calculated on the basis of a 10-g sample size (wet weight) and an analyte signal 3 times the detection limit which is, in turn, at least 3 times the background (Keith et al., 1985). For all of the organophosphates except methidathion oxon, and all of the compartments except the remainder needle compartment, the detection limit was 0.05 ng/μL and the LOQ was 7.5 ppb (wet weight) (18 ppb dry weight). For methidathion oxon the relative response of the detector was half that of the other compounds, and therefore the LOD and LOQ for this compound were twice that of the other compounds. For the remainder needle compartment, the final extract contained more coextractives and thus was concentrated to twice the volume of the extracts of the other compartments; the LOQ for the remainder needle is twice that of the other compartments for each OP. The oxons of methidathion and chlorpyrifos were not recoverable from the remainder needle compartment at any spiking
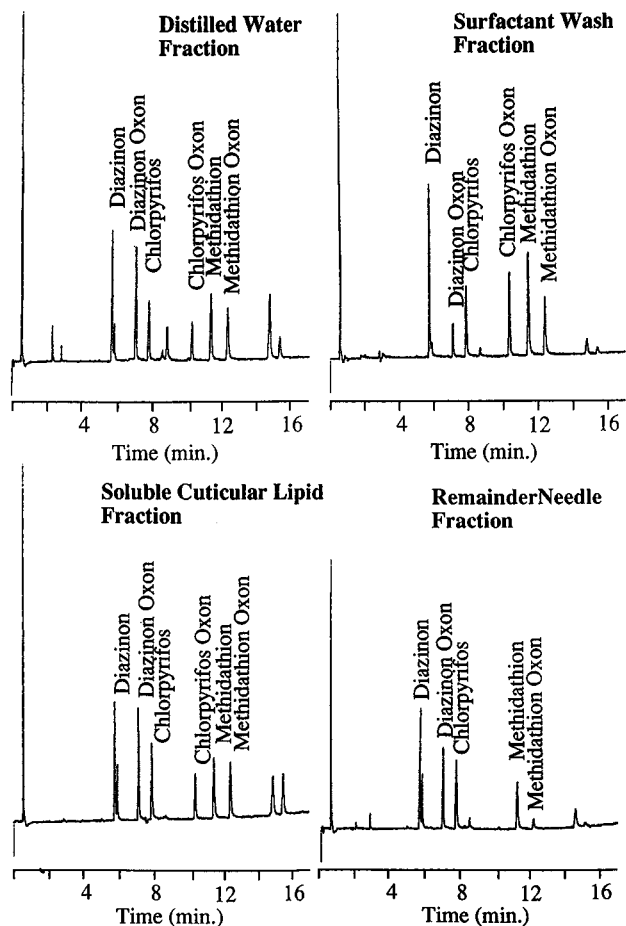
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Figure 4. Gas chromatograms of 10 ppb spiked fractions of pine needle extracts.
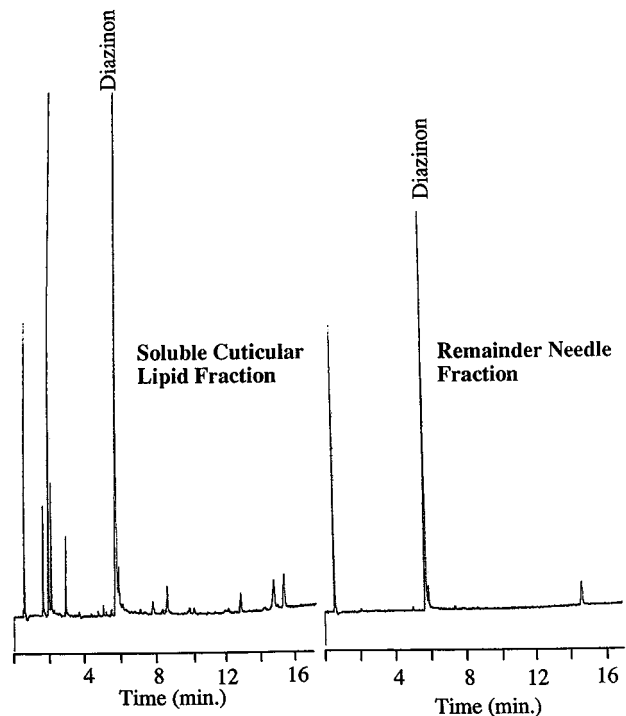

Figure 5. Sample chromatograms of fractions from pine needles collected from the Wolfskill peach orchard.

level, and thus LOQs were not reported for these combinations. LOQs based on the dry weight equivalent of the needles were calculated using a measured value of 41% dry weight (Table 2). These low LOQs were considered necessary for the monitoring of the

Table 2. Limits of Quantitation for Method Validation Study Based on Needle Wet Weight (Dry Weight)[a]

	compartment			
	distilled water	surfactant wash	soluble cuticular lipid	remainder needle
diazinon	7.5 (18)	7.5 (18)	7.5 (18)	15 (30)
diazoxon	7.5 (18)	7.5 (18)	7.5 (18)	15 (30)
chlorpyrifos	7.5 (18)	7.5 (18)	7.5 (18)	15 (30)
chlorpyroxon	7.5 (18)	7.5 (18)	7.5 (18)	NR[b]
methidathion	7.5 (18)	7.5 (18)	7.5 (18)	15 (30)
methidoxon	15 (37)	15 (37)	15 (37)	NR

[a] All values are in ng/g or ppb. [b] Not recovered.

pesticides in ambient or remote samples. Field sample sizes were approximately 25 g. The LOQs, therefore, were lower than those for the method validation study. The LOQs for the field samples are stated in Figures 6–8.

Field Samples. This approach to compartmental analysis of pine needles offers a consistent means for comparing and contrasting residue distribution among samples collected under differing conditions of exposure to airborne residues. It is not expected to cleanly separate residues in each compartment, because there may be some redistribution of residues during the sequential extraction steps. With these advantages and limitations in mind, there may be trends in compartmental distribution resulting from application of the methods to field samples which can indicate the likely mode of initial contact (e.g. particulate matter vs vapor), the likely duration of contact, and events that may intervene between contact and sampling, such as rainfall washoff. For verification of this analytical objective, we examined two sets of field-collected needles that were assumed to have contacted airborne pesticide residues.

These samples were analyzed to check method performance and to get an idea of how incurred residues might be distributed among the four compartments. For these samples of needles from the UC Davis Arboretum, for which low levels of ambient contamination were expected, residues of diazinon resided in the remainder needle, while chlorpyrifos was found in the remainder needle as well as in the soluble cuticular lipid (Figure 6). This latter difference may be due to the lower polarity of chlorpyrifos, favoring its stabilization in the wax compartments. The total residues were low, ranging from 12 to 39 ppb (dry weight of needle). The general absence of residues on the surface of the needles may be due to rain that may have washed residues from the surface or to the migration of residues through the needle surface into the interior parts of the needle over the duration of exposure or to loss of the surface residues by photolytic action, degradation, or revolatilization. The source of residues in the Arboretum pines is assumed to be transported vapors from areas surrounding campus. There are no fields or orchards that receive OP treatments within a 0.5 mi of the location of the Arboretum trees. Therefore, exposure by direct spray drift is unlikely.

A second application of the methods was to needles from a potted pine tree that was placed in a peach orchard 4 h after an application of a dormant spray containing diazinon to orchard trees. This represented a high-exposure test of the method. It also provided an opportunity to measure the compartmental concentrations of diazinon over a 3 week interval. Residue levels of parent diazinon in the needles ranged from over 3600 ppb after 1 day in the orchard to less than 600 ppb (dry weight) 3 weeks later (Figure 7). In contrast to the
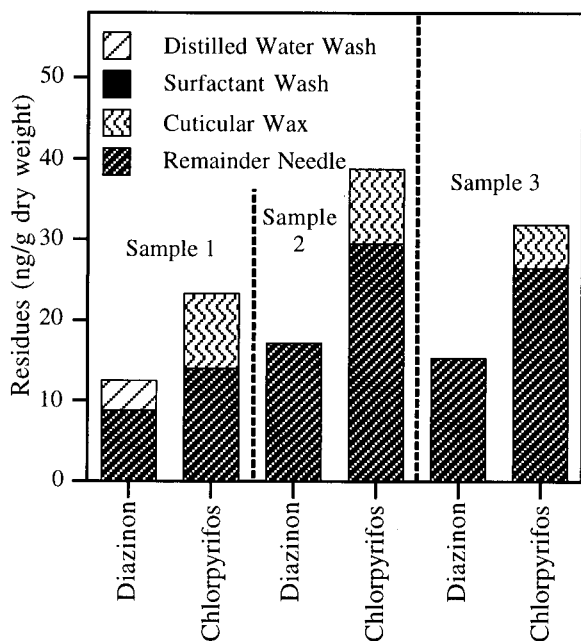
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Figure 6. Pesticide residues in ambient samples of Ponderosa pine needles collected at UC Davis Arboretum. [LOQ for diazinon and chlorpyrifos in these samples was 7.3 ppb (dw) for the remainder needle compartment and 3.7 ppb (dw) for the other compartments.]
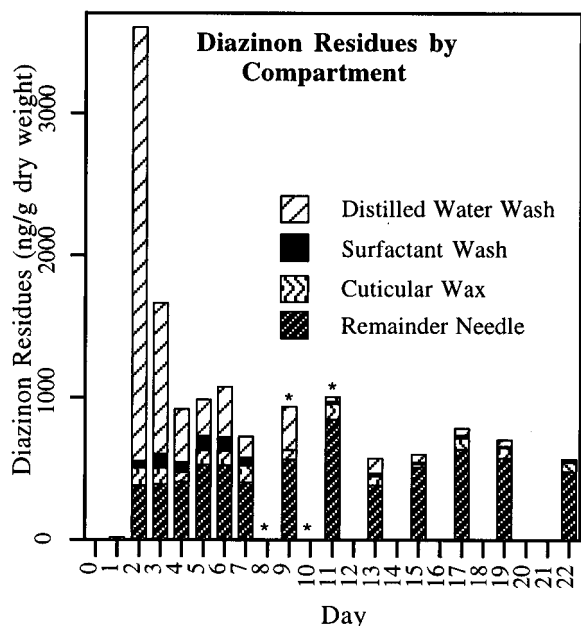

Figure 8. Diazinon oxon concentrations by compartment for a pine tree placed in a peach orchard after dormant spray application. [LOQ for diazoxon in these samples was 7.3 ppb (dw) for the remainder needle compartment and 3.7 ppb (dw) for the other compartments. * Indicates days on which it rained in the orchard.]

Figure 7. Diazinon concentrations by compartment for a pine tree placed in a peach orchard after dormant spray application. [LQQ for diazinon in these samples was 7.3 ppb (dw) for the remainder needle compartment and 3.7 ppb (dw) for the other compartments. * Indicates days on which it rained in the orchard.]

Arboretum samples, a large fraction of the total initial residue was found on the surface of the needle, in the distilled water compartment. This may have been a result of the short time between exposure and sampling, limiting the opportunity for penetration or translocation. Or it may also be due, in part, to settling of particulate matter on the needle surface. The subsequent decline of the parent from the surface compartment may be due to resuspension of dust particles from the surface, conversion of the parent to the oxon on the surface, revolatilization of surface residues, or the washing effect of rain that fell midway through the experiment. Also
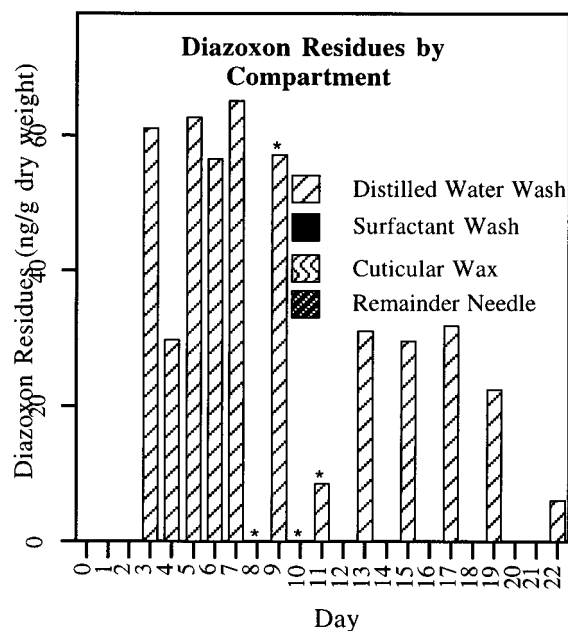
of interest is the rather constant level of diazinon in the interior compartments (soluble cuticular lipids and remainder needle) over the 3 week period. This indicates that the interior of the needle may retain the pesticide residues long after the vapor levels in the air diminish and the surface residue has been depleted by one or more of the mechanisms mentioned above. Concentrations of the oxon of diazinon reached a peak of 63 ppb (dry weight) 1 week after spraying and appeared only in the distilled water (surface) wash (Figure 8). Oxons are much more water soluble than the corresponding thions, perhaps preventing them from becoming sorbed in the less polar cuticular wax (Bowman and Sans, 1979). Air samples taken simultaneously to these needle samples showed measurable levels of diazinon oxon (Aston and Seiber, 1996), indicating that the presence of oxons on the surface of the needles could be due either to sorption of the oxon vapors or to the oxidation of sorbed diazinon vapors after deposition on the surface. Conversion to oxons on the surface of leaves has been shown to occur rather significantly for other OPs (Woodrow et al., 1977). The constant level of diazinon oxon in the surface compartment may reflect the balance between the normal breakdown of the oxon and its formation from the parent compound.

The method for the analysis of residues in the distilled water wash compartment does not distinguish between analytes that are removed directly from the needle surface and analytes that may be sorbed to dust. Surface residues measured on samples collected in the field must be regarded as potentially contaminating both needle-surface-sorbed and dust-particle-sorbed residues. Applying the distilled water wash method consistently allows comparisons to be made between field samples even if absolute values are not known. Methods are needed to quantitate dust-sorbed residues and to determine what proportion of the residues on needle surfaces is in this fraction. For example, in a heavy dust situation, it might be advantageous to filter

the rinsate, for separate collection of dust, before Bond Elute extraction.

This compartmentalized approach to the analysis of organophosphate residues in pine needles has revealed interesting trends in residue distribution. When there was a short-term exposure of the needles to high concentrations of pesticide vapors, as occurred in the peach orchard, a significant portion of those vapors was on the surface. The bulk of these residues did not migrate into the interior compartments of the needle but rather degraded or revolatilized or mechanically removed relatively rapidly. It thus appears that residues that are on the surface, especially those removable by a distilled water wash, are quite transient and perhaps not a good indicator of exposure that has occurred over a longer duration. In the needles sampled from the Arboretum, where the exposure was to low ambient levels of pesticide residues probably for a long period of time, the surface contained practically no residues.

Most models being developed to describe the interaction between organic vapors and plant foliage assume partitioning into either one or two compartments of the needle. These surface-retained residues would surely cause an overestimation of the amount of residue that could be adsorbed into the needle in a one-compartment model or into the soluble cuticular lipids in models that describe the needle by the two compartments: cuticular wax and remainder needle. When one attempts to determine bioconcentration factors between airborne vapors and pine needles, an initial judgment must be made whether to include residues on the surface that are not truly sorbed by the needle. Because of the transient nature of surface residues, it could be argued that they not be included in calculations of bioconcentration factors.

CONCLUSIONS

The methods presented give fair to good recovery of parent organophosphate and oxon residues in extracts of all four needle compartments, with the exception that methidathion oxon and chlorpyroxon were not recoverable from the remainder tissue. Recoveries ranged from 113% to 52% for the parent organophosphates and from 143% to not recovered (remainder needle tissue) for the oxons. Total error was acceptable (<100%) for 19 of the chemical−compartment combinations. Total error was >100% or indeterminate in five combinations. Good recovery coupled with low quantitation limits (18−37 ppb on a dry weight basis) for the parent compounds makes these methods sensitive enough for application to needle residues in ambient exposure situations. The method included confirmation by GC/MS.

This comparative approach to the compartmental analysis of pesticide residues in needles has revealed distinctions between surface residues and interior residues. Field samples show that the relative distribution of residues between the compartments may depend on such factors as concentration and form of the pesticide in the air and length of time that elapses between initial exposure of the plant and subsequent sampling of the needles. Surface residues removed by the distilled water wash appear to be of a transient nature. These residues vary in concentration more than the residues in the soluble cuticular lipid compartment or the remainder needle compartment and show relatively rapid dissipation as the airborne exposure is decreased. The residues in the distilled water compartment may include those bound to dust particles and favors those that are very soluble in water (such as oxons). The low water solubility of many parent organophosphates such as chlorpyrifos and diazinon might indicate that they are probably bound to particles when they are found on the needle surface, particularly in low-level (ambient) exposure situations. It may be argued that residues sorbed to surface dust particles are not truly taken up by the pine needle, because the dust can be resuspended by wind. Similarly, loosely sorbed residues resulting from high-level vapor exposure may revolatilize or be degraded by photooxidation rather rapidly. Thus, the surface fraction should not be included in calculations measuring uptake or accumulation of airborne pesticides into needles or leaves. The oxon degradation products of the OP insecticides apparently exist only on the surface of the needles as evidenced by their appearance only in the distilled water compartment in the peach orchard samples. Because these oxons can be readily removed by water, they are also not good indicators of contamination in an ambient situation where rain might completely remove them prior to sampling. Thus, the best indicator of ambient contamination of pine forests by airborne OPs probably lies in the cuticular wax and remainder needle tissue analysis, where parent OPs appear to predominate.

The varied distribution of the OP residues in the four compartments with differing exposure conditions demonstrates an advantage of this analytical approach over whole needle analysis. Further study is needed to determine the fate of the residues in the needle compartments. Application of these methods to other classes of atmospheric pollutants might further the effort to use plants as biomonitors of atmospheric quality. It may be possible to sample only one compartment, for example the soluble cuticular lipid compartment, to get an idea of the amount of residue present in the whole needle or even the whole tree. Finally, nontarget plant contamination may be controlled more effectively once the interaction with airborne pollutants is better understood. Some nontarget contamination, such as on food crops contaminated by dust, might be remediated by a relatively simple water washing procedure.

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