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Rapid Field Method for Estimating Organophosphorus Insecticide Residues on Crop Foliage

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It has been generally agreed that foliar dislodgable residues of organophosphorus (OP) insecticides are largely responsible for episodes of reentry agricultural worker illnesses, particularly in the harvesting of citrus fruits and grapes in California (summarized by GUNTHER et al. 1980). KNAAK et al. (1980) has proposed safe levels for three OP insecticides used on citrus based upon dermal toxicity studies. Thus, for these three insecticides, a grower could allow worker entry into a treated field prior to the expiration of the mandated reentry interval if evidence is provided that residues have dropped to a safe level. Currently, California Assembly Bill 1090 makes allowance for this testing procedure. What is needed is a rapid field method for use by the grower, such as presented herein. It is sensitive, simple, adaptable to kit availability, and reliable in the hands of non-chemists after a few practice runs.

MATERIALS

a) Leaf-punch sampler manufactured by Birkestrand Co., 2705 Lee Avenue, South El Monte, CA 91733. Sampler is available for excising 1.8- or 2.5-cm diameter leaf disks. See IWATA et al. (1977) for details of use.

b) 8-oz Sample collection jars for attachment to the leaf-punch sampler and screw cap lids for the jars.

c) Test-tube brush for cleaning above jars.

d) 500-mL Size bottles containing 20% (w/v) NaCl in water.

e) 50-mL Size screw-cap, graduated, polypropylene centrifuge tubes (e.g. Vanlab, catalogue no. 21008-500).

f) 500-mL Size glass bottles containing reagent grade hexane.

g) One 50-mL size and one 100-mL size polypropylene graduated cylinders.

h) 16 x 100 mm Screw-cap culture tubes marked to indicate a 5-mL volume.

i) Small vial containing NaCl crystals.

j) Disposable 5 3/4-in. long, glass, Pasteur, capillary pipets with pipet bulbs.

k) Sealed 1-mL size glass ampules each containing 0.5 mL of 2% (w/v) 4-(p-nitrobenzyl)pyridine plus 0.08% (w/v) oxalic acid, in acetone solution. Ampule A. (Larger size ampules with larger openings facilitate pouring.)

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l) Sealed 5-mL size glass ampules each containing 2.5 mL of 20% (w/v) triethylamine in acetone solution. Ampules should be stored in a cool dark place to retard triethylamine decomposition. Ampule B.

m) Sealed 1-mL size glass or plastic containers each filled with 1 mL of 12% (w/v) Na_2CO_3 plus 15% (w/v) NaCl in water. Ampule C.

n) Power inverter to convert 12 v DC to 115 v AC with a 200 watt output and 20 amp fuse (e.g. Model 12-115, Nucleonic Products Co., Canoga Park, CA 91303). Wire and clamps for attaching the inverter to a vehicle battery are needed.

o) Aluminum block heating unit thermostatted at 150°C for use in heating above 16-mm OD diameter culture tubes (e.g. COD Reactor model 16500-10, Hach Chemical Co., Loveland, CO 80537).

p) Battery-operated spectrophotometer utilizing 16-mm OD diameter culture tubes as cells and measuring at 560 nm (e.g. Model DR/2, Hach Chemical Co.; or Bausch and Lomb mini-spectronic 20 with disposable cells).

q) Sealed 5-mL size glass ampules each containing 5 µg of parathion (Ampule D), methidathion (Ampule E), or azinphosmethyl (Ampule F) in 5 mL of hexane.

PROCEDURES

1) Attach inverter to 12 v battery and plug in heating unit to the inverter.

2) With the sample collection jar attached to the leaf punch sampler, collect 40 leaf disks from the foliage to be sampled. Samples should be collected from portions of the plant where residues would be expected to be highest such as plant surfaces which would receive the most residue during application.

   Thus, for citrus trees, the present recommendation is to collect 3 samples for each 10-acre plot. Assuming a uniform spray coverage for the plot, a "center" row is selected for sample collection. The first 5 trees at the end of the row are omitted and samples are collected from the next 10 trees. Two leaf punch samples are collected from each between-row sides of the trees at approximately the 4-ft level from each of the 10 trees to give a 40-leaf-disk sample. The sampling is repeated twice to give a total of 3 replicate samples for analysis.

3) Remove the jar from the sampler, add 40 mL of NaCl solution using the 100-mL graduated cylinder for measurement, cap the jar securely, and shake vigorously for 30 sec.

4) Remove the cap and decant 30 mL of the leaf wash water into the graduated polypropylene centrifuge tube.

5) Add 15 mL of hexane to the centrifuge tube using the 50-mL graduated cylinder for measurement. Cap the tube securely and agitate moderately for 20 sec. Vigorous shaking will cause formation of an emulsion.

6) Remove the cap and using a disposable pipet with attached bulb, transfer 5 mL of the upper hexane phase into the culture tube. Avoid adding leaf fragments or other debris and use the mark on the culture tube as a guide for the volume measurement.

7) Add from 10-25 salt crystals to the tube; then add the contents of Ampule A. Place the tube in the 150°C heated block and note when the last traces of solvent have boiled off. With multiple tubes the evaporation time will differ slightly from tube to tube so it is recommended that each tube be removed from the heating block as soon as the evaporation for the particular tube is completed. Avoid open flames which will ignite the solvent vapors.

8) Return the tube to the heating block and heat for precisely 3 min. Remove the tube and allow it to cool to air temperature.

9) This step must be conducted in a shaded area as the color which is developed is slowly destroyed by light. Add to the cooled tube the contents of Ampule B and shake gently to dissolve all solid material except the salt crystals. Add the contents of Ampule C and shake gently. Wait two min to allow the resulting light emulsion to clear and proceed to the next step.

10) Place the tube in the spectrophotometer and measure the absorbance of the solution at the 560-nm wavelength setting. The spectrophotometer should be pre-checked for zero adjustments as directed in the operating instructions.

11) Record the value obtained on the meter along with the description of where, when and by whom the sample was collected.

The leaf punch sampler can be wiped clean with cleaning tissue moistened with water. The sample jars should be washed with soap and water using the test-tube brush provided, rinsed with clean water, and allowed to drain and dry. Dispose of the caps, centrifuge tubes, pipets and culture tubes; do not reuse.

For checking the procedure, the analyst should add 5 mL of hexane to each of two culture tubes (reagent blanks) and add the contents of Ampule D (or E or F) to each of another two culture tubes and then proceed starting with step 7 above. The average absorbance of the two solutions using Ampule D (or E or F) less the average value obtained for the reagent blanks should be 0.18±0.01 (or 0.39±0.01 for E or 0.20±0.01 for F).

RESULTS

Table 1 gives the absorbance values obtained after reaction of a number of OP insecticides and their oxygen analogues (oxons). Different compounds yield different absorbance values for the same weight of material; these differences must be taken into account in using the field method. The values given in Table 1 should be

TABLE 1. Absorbance of Solutions after Reaction of Compounds with 4-(p-Nitrobenzyl)pyridine at 150°C for 3 Minutes.

Compound	Micrograms of compound[a]					Absorbance Unit per µg[b]
	5	10	15	20	25	
azinphosmethyl	0.18	0.36	0.54	0.83	0.99	0.040
azinphosmethyl oxon	0.29	0.58	0.77	1.3	1.6	0.064
carbophenothion	0.14	0.26	0.32	0.42	0.54	0.021
chlorpyrifos	0.19	0.38	0.57	0.73	0.95	0.037
chlorpyrifos oxon	0.13	0.25	0.34	0.43	0.58	0.022
demeton	0.08	0.20	0.25	0.34	0.35	0.014
diazinon	0.06	0.13	0.15	0.20	0.27	0.010
dimethoate	0.26	0.55	0.80	0.96	-	0.050
dimethoate oxon	0.24	0.45	0.66	0.83	1.2	0.045
dioxathion	0.16	0.33	0.46	0.54	0.62	0.025
EPN	0.13	0.24	0.33	0.44	0.51	0.020
ethion	0.14	0.31	0.39	0.53	0.71	0.027
malathion	0.21	0.39	0.58	0.78	1.1	0.042
malathion oxon	0.25	0.47	0.54	0.66	0.84	0.031
methidathion	0.38	0.80	-	-	-	0.078
methidathion oxon	0.35	0.65	1.0	1.7	1.8	0.076
mevinphos	0.47	0.91	1.6	1.8	-	0.095
naled	0.16	0.32	0.37	0.59	0.67	0.027
parathion	0.20	0.35	0.56	0.70	0.91	0.036
parathion oxon	0.13	0.28	0.39	0.51	0.63	0.025
phenthoate	0.19	0.35	0.58	0.80	0.91	0.037
phenthoate oxon	0.20	0.37	0.49	0.62	0.77	0.030
phosphamidon	0.23	0.40	0.71	0.90	-	0.046
trichlorfon	0.20	0.39	0.54	0.84	0.92	0.038

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[a] Absorbance values are means of duplicate sample determinations and were corrected for reagent blank values. Data from GUNTHER et al. (1980); values should be redetermined or verified for the particular set of components and reagents adopted for use.

[b] Slopes of the standard curves were obtained using in addition to the absorbance values for 5, 10, 15, 20 and 25 µg given in this table, the absorbance values obtained for 1 and 2 µg of compound.

redetermined or verified for the particular set of components and reagents adopted for use. Table 2 gives the recovery efficiency for several insecticides and their oxons from the aqueous leaf wash by hexane partitioning. The efficiency differs for each compound owing to their differing solubilities in water; these differences must also be taken into account in using the field method as discussed next.

KNAAK et al. (1980) proposed toxicologically safe levels for dislodgable foliar residues on citrus of 0.09, 0.6, and 3.1 μg/cm^2 for parathion, methidathion, and azinphosmethyl, respectively. If 0.09 μg/cm^2 of parathion is present, then a 40-leaf-disk sample (2.54-cm diameter) representing 400 cm^2 of leaf surface contains 36 μg of parathion. The 30-mL aqueous wash used represents 75% of the 40 mL of salt solution added. Thus, 27 μg is transferred to the polypropylene tube. Addition of 15 mL of hexane and use of 5 mL for subsequent reaction then represents 9 μg of parathion. Correcting for an 80% recovery (Table 2) from the wash water means 7.2 μg is actually transferred to the culture tube. If 1.0 μg of parathion gives an absorbance of 0.036 unit (Table 1, slope of the standard curve), then 7.2 μg should give a value of 0.26. Thus, any value above 0.3 absorbance unit obtained for a field sample can then be regarded as signalling an unsafe condition for worker entry into any parathion-treated citrus grove.

The procedure must be slightly modified for use with the much higher levels recommended for methidathion and azinphosmethyl. For both compounds the amount of aqueous wash added to the sample must be increased to 100 mL. The procedure is further changed and for methidathion, 15 mL of the aqueous wash is decanted into the polypropylene tube; for azinphosmethyl, 10 mL is decanted. The amount of hexane added is now 30 mL; 5 mL is removed for reaction. Using a recovery value of 90% for methidathion and 100% for azinphosmethyl (Table 2), the amount transferred to the reaction tube is 5.4 μg methidathion and 20 μg azinphosmethyl. Using the Table 2 values of 0.078 for methidathion and 0.040 for azinphosmethyl, the absorbance values are 0.42 for methidathion and 0.80 for azinphosmethyl. Thus, any value above 0.4 absorbance unit obtained for a field sample for methidathion and above 0.8 absorbance unit for azinphosmethyl can be regarded as signalling an unsafe condition.

The examples given above for the three compounds do not take into account the values that are obtained for untreated samples, about 0.05 to 0.10 absorbance unit. Also, to thoroughly check the method, the OP compound dissolved in salt water should be shaken with a leaf sample to determine overall recovery as lipophilic compounds such as parathion, solubilized in the water after desorption from the dust particles, can partition into the citrus leaf wax and be lost for the analytical method. For example, using the equipment and procedures described herein, the procedure was tested by adding to the 40-leaf-disk sample, 40 mL of NaCl solution containing 36 μg of parathion or 100 mL of NaCl solution containing 240 μg of methidathion or 1240 μg of azinphosmethyl. For parathion,

TABLE 2. Recovery of Compounds after Fortification of Aqueous Leaf Washes.

Insecticide	Recovery (%)[a/] Fortification (μg) 7.5	15	22.5	24	30	36	Value for Calculations[b/]
azinphosmethyl	110	110	98	-	100	-	100
azinphosmethyl oxon	19	19	21	-	22	-	20
carbophenothion	90	110	110	-	110	-	100
chlorpyrifos	110	100	110	-	100	-	100
dimethoate	21	14	13	-	11	-	10
dioxathion	120	85	-	93	-	97	90
EPN	54	68	-	73	-	77	70
ethion	88	100	-	92	-	77	80
malathion	82	110	86	-	98	-	90
malathion oxon	100	97	-	89	-	86	90
methidathion	97	85	93	-	110	-	90
parathion	87	83	82	-	77	-	80
parathion oxon	64	82	94	-	86	-	80
phosphamidon	33	28	-	33	-	30	30

[a/] To 15 mL of the aqueous leaf wash in the polypropylene tube was added the indicated amounts of compound and 15 mL of hexane. Data from GUNTHER et al. (1980).

[b/] These values would serve to indicate recovery efficiency for the water-hexane partitioning.

methidathion, and azinphosmethyl, the overall recoveries were 48, 80, and 101%, respectively, and the absorbance values (four replicates) after correction for reagent blanks were 0.17±0.02, 0.29±0.03, and 0.73±0.08, respectively. Thus, absorbance values for the three compounds used to signal unsafe conditions could be values above 0.1, 0.2 and 0.5, respectively.

If a mixture of OP compounds is applied, the results can be interpreted in terms of the most toxic component of the mixture. If the spray history of the grove is unknown, then the results can be interpreted in terms of the most toxic OP insecticide used for the crop in question.

DISCUSSION

In practiced hands, this method requires approximately 30, 50, and 90 min for 6, 12 and 24 samples, respectively, after collection of the foliar samples. The method as outlined will respond positively to levels of 0.04 $\mu g/cm^2$ of leaf surface and up of parathion, methidathion, and azinphosmethyl. As illustrated in Table 1, the method will respond to at least 24 OP compounds. Full details validating each step of the method will be published elsewhere. Development of this method was supported by the Environmental Protection Agency. It is expected that a portable field kit for this method will shortly be on the market. Also, with slight modification, this field method is adaptable to analyzing soil residues on the orchard or vineyard floor: details will be published elsewhere.

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