Journal of Agricultural and Food Chemistry

Volume 29, Number 2 March/April 1981

Worker Environment Research: Rapid Field Method for Estimation of Organophosphorus Insecticide Residues on Citrus Foliage and in Grove Soil

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Data for the development of a rapid field method for estimating levels of organophosphorus (OP) insecticide residues on agricultural crop foliage and in the dry mobile soil dust of groves and fields are reported. The field method for collecting foliage samples and removing the dislodgeable residues was patterned after the widely used Gunther laboratory method. The quantitative step is based on the reaction at 150 °C for 3 min of OP residues with 4-(*p*-nitrobenzyl)pyridine which reacts with a broad spectrum of OP compounds. The method is adaptable to kit availability for use by competent nonchemists for use in testing fields to ensure that residues have declined to a toxicologically established safe level. The results obtained by using a prototype field kit are compared with results obtained by a laboratory method using samples collected after a field application of parathion, malathion, and methidathion to citrus trees. One person can, exclusive of sample collection time, conduct 6, 12, and 24 tests in 30, 50, and 90 min, respectively.

After application of organophosphorus (OP) insecticide sprays to agricultural crops such as citrus, grapes, and peaches, persisting residues exist both on the surface of the target crop and on the soil surface of the field. Workers entering the treated field and engaging in tasks such as picking, fruit thinning, or pruning which involve extensive and prolonged contact with residue-bearing foliage might accumulate sufficient dermal absorption of toxic residues to exhibit symptoms of cholinesterase depression. Residues sorbed on mobile soil dust on the field surface are also a source of worker exposure. To minimize worker exposure to toxic residues, regulatory agencies have established worker exclusion times or "reentry intervals" to prohibit workers from entering treated fields until a specified number of days have elapsed after insecticide application to allow residues to dissipate to lower and, thus, safer levels. Rates of insecticide dissipation and conversion to their toxic oxygen analogues (oxons), however, can be variable. Some conditions which contribute to this variability are the nature and quantity of dust on the foliage, insecticide application methods, type of formulation used, amounts of heat, sunlight, humidity, and precipitation, and the nature of the agricultural crop. Thus, a rapid, portable field method for estimating levels of toxic residues prior to worker entry into a field is needed to help ensure safe working conditions. The reentry problem has been reviewed by Gunther et al. (1977).

Smith et al. (1977) reported a method for foliage whereby residues were directly transferred to a paper strip, paper chromatographed, and then reacted with 4-(*p*-nitrobenzyl)pyridine (NBP) to form a "blue" color. Comparison of the obtained color with a reference color chart gave an estimate of the insecticide level. Shortcomings of the method were that key components such as the above "transfer" device had to be custom-made, final judgment as to the color intensity was subjective, and the method was unworkable with foliage bearing high, but not unusual, levels of dust. Smith and Gunther (1978) reported a method for mobile soil dust wherein dust was extracted with hexane–acetone and the residues were reacted with NBP to form a "rose-red" color in solution. Comparison of the color with a reference color chart gave an estimate of the insecticide level.

Reported here is a further development of the above methods which is suitable for both dislodgeable foliar residues and soil dust residues. All key components are

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0021-8561/81/1429-0209$01.25/0

commercially available; glass and plastic ware are disposable after use; final measurements are numerical values obtained with a battery–operated spectrophotometer.

The final objective of the work reported herein is to assemble a commercially available field test kit which can be used by pest control operators, work crew foremen, or other reasonably competent personnel to quantitate the level of OP insecticide residues present in the grove environment. The test kit would be used to ensure that insecticide residue levels to which workers would be exposed are below a predetermined "safe" level which has been established toxicologically. The kit would contain all the materials and equipment required to conduct the tests. The field procedure will be simplified based on the results reported herein, and standard solutions will be available for testing the user's technique.

MATERIALS AND METHODS

Sampling of Foliage. The method of Gunther et al. (1973) was used to collect samples each consisting of 40 leaf disks of 2.54-cm diameter. A commercially available leaf punch sampler (Birkestrand Co., South El Monte, CA 91733) was used; see Iwata et al. (1977) for details of its use.

Removal of Residues from Foliage. The 8-oz (240-mL) collection jar containing the leaf disks was removed from the sampler. By use of a plastic variable-volume dispenser with a 500-mL reservoir, 20 mL of 20% (w/v) aqueous NaCl solution was added to the sample. The jar, capped tightly, was shaken vigorously for 30 s. The cap was removed and 15 mL of the leaf wash was decanted into a 50-mL graduated polypropylene screw-cap centrifuge tube. After addition of 15 mL of hexane, the tube was tightly capped and moderately shaken for 20 s; vigorous shaking caused difficult emulsions. The hexane and aqueous layers were allowed to separate; tapping the tube or gently swirling the contents hastens layer separation. The cap was removed and, with the tube inclined at ~30° off of horizontal, 10 mL of the hexane phase was removed while avoiding transfer of leaf fragments. The hexane was placed in a prepared reaction tube containing NBP which is described below.

Sampling of Soil Dust. Spencer et al. (1977) recommended collection of dust samples by vacuuming with a portable vacuum cleaner through a 100-mesh screen placed over the soil surface. As this technique requires a generator to power the vacuum cleaner, the following method was used for a field method. A metal template with 4 × 4.5 in. (10.2 × 11.4 cm) opening (18 in.^{2}) was placed on the soil surface to be sampled. The surface for sampling was selected at the dripline of the citrus tree as this represented the area of highest insecticide concentrations and greatest worker activity (Spencer et al., 1977; Elliott et al., 1977). The template opening was placed against the collection edge of the rigid polyethylene dustpan, and the exposed soil surface was swept with a nylon fiber antimagnetic brush to collect the loose dust. The process was repeated at the eight cardinal points of a tree to obtain a composite sample representing 1.0 ft^2 (0.093 m^3). The sample was placed in a 10-mesh screen nested on a 100-mesh screen and catchpan to discard extraneous debris. The screens were vigorously shaken for 1 min to obtain the dust in the catchpan for residue analysis.

Standard measuring spoons of the type used for domestic cooking were used to obtain subsamples for residue analysis. Thus, a sandy loam soil delivered by level $^1/_4$-, $^1/_2$-, and 1-tsp sizes were 1.5 ± 0.1, 2.9 ± 0.1, and 6.0 ± 0.1 g, respectively. Thus, for estimation of residues in soil dust, a value of 1.5 g was used for a level $^1/_4$ tsp of dust.

Removal of Soil-Dust Residues. A measured volume of sieved dust was placed in a 50-mL screw-cap polypropylene centrifuge tube; then 0.25 mL of 20% NaCl solution and 20 mL of acetone–hexane (15:85) were added. The tube was capped and vigorously shaken for 20 s. The aqueous and organic layers were allowed to separate. An aliquot of the hexane solution was removed and placed in a reaction tube: start with 5 mL, but less may be required if residue levels are high.

Reaction Tube Preparation. To an 18 × 150 mm test tube was added 100 μL each of acetone solutions of 10% (w/v) 4-(*p*-nitrobenzyl)pyridine and 0.4% (w/v) oxalic acid. From 25 to 50 grains of NaCl crystals were added to serve as boiling "chips"; omission will result in superheating of the hexane followed by sudden boilover when the tube is used. Tubes may be used immediately or after the solvent has evaporated; crystals are deposited on the lower half of the tube when the solvent has evaporated.

Reaction. Two 0.25 in. (6.4 mm) thick aluminum plates about 6 × 7.5 in. (15 × 19 cm) and two 2 × 3 × 4 in. (5.1 × 7.6 × 10.2 cm) aluminum blocks each having six 23.4 mm diameter holes ($^{59}/_{64}$-in. drill bit used) and three 8 mm diameter holes ($^5/_{16}$-in. drill bit used) placed between the larger holes were required; all holes were drilled to a depth of 1.6 in. (4.1 cm). The large holes accommodated the reaction tubes for hexane evaporation and heating, and the small holes served as thermometer wells and for use in moving or positioning the heated blocks with needle-nosed pliers. Ethylene glycol was placed in the holes to serve as a heat transfer medium: sufficient amounts were added so that when a reaction tube or thermometer was in place, the glycol was not forced out of the holes, with allowance for expansion of the liquid upon being heated in the block. Loss of glycol must be replenished. Ethylene glycol is inexpensive, readily available, nontoxic, not readily flammable, and easily washed away with water for cleaning of the block.

One 0.25-in. aluminum plate was placed on each burner position of a Coleman two-burner gasoline camp stove. The aluminum blocks were placed on the plate over the main burner which was then lit. With the thermometer in place, the blocks were brought up to 150–160 °C. The burner valve was closed to extinguish the flame and the reaction tubes were placed in the aluminum blocks to boil off the hexane; ~1.5 min was required. Each tube was removed from the blocks ~3 s after hexane vaporization visibly ceased. No more than 0.1–0.2 mL should condense back into the tube. No open flames must be present as the hexane vapors produced will ignite. In this regard, a propane camp stove is preferred since there is no afterburn as with a gasoline camp stove after the fuel valve is closed.

After the hexane evaporation, additional heating of the dry reaction tube is required. The blocks were reheated to 150 °C. Adjustment of the burner could maintain this temperature within a degree. The block temperature was lowered, if needed, by a momentary lateral transfer using pliers to the unheated adjacent aluminum plate. When the block temperature could be maintained within 1 °C of 150 °C, the reaction tubes were placed in the holes and heated for 3 min.

Color Development. This step could be conducted in a shaded area as the developed color is slowly degraded by light. Color development should be limited to no more than four tubes at a time and absorbance measurements conducted expeditiously.

Two separate base solutions needed were 20% (w/v) triethylamine in acetone and 12% (w/v) Na_2CO_3 in 15% (w/v) aqueous NaCl. To the 150 °C heated reaction tube

was added 2.5 mL of the triethylamine solution and then 1.0 mL of the Na_2CO_3 solution. The tube was gently oscillated to mix the reactants. When the mixture was allowed to stand, two clear phases result. Triethylamine should be protected from air and sunlight as it is subject to air oxidation. Reagent blank values increase with increased darkening of the originally colorless triethylamine solution. The limit of usefulness is reached when the reagent blank gives an absorbance value of 0.1 or higher.

Quantitation. The upper organic phase was decanted into a spectrophotometric cell and the absorbance at 560 nm was determined. The values reported herein were obtained by using a Beckman Model DB spectrophotometer in the laboratory and a rechargable battery-operated Bausch & Lomb Spectronic Mini 20 grating spectrophotometer in the field. Spectrophotometric readings were converted into micrograms of OP compound per cm^2 (leaves) or into ppm (soil) values using previously prepared standard curves or by calculation.

Field Testing. Orange trees were sprayed at the highest rate likely to be made under agricultural practice in California. Formulations used were Phoskil 25WP (parathion), Malathion 25WP, and Supracide 40WP and 2EC (methidathion). Low-volume applications were made with a Kinkelder sprayer equipped with an air tower. Rates were 7.2 lb (3.3 kg) of active ingredient (AI) parathion, 12 lb (5.4 kg) of AI malathion, or 4.8 lb (2.2 kg) of AI methidathion per 100 gal (3.8 hL) per acre (0.41 ha). Dilute full-coverage applications were made manually using spray mixtures of 0.38 lb (0.17 kg) of AI parathion, 0.63 lb (0.29 kg) of AI malathion, or 0.25 lb (0.11 kg) of AI methidathion per 100 gal (3.8 hL) of water.

At each of 12 sampling dates spanning 1 day preapplication to 62 days postapplication, four sets of 40-disk leaf punch samples were taken for each of the eight treatments. Two sets of samples were processed in the field by the field method described herein, yielding results of 20 tests within 1.75 h of termination of sampling. The other two sets were analyzed by gas chromatography after processing samples by the method of Iwata et al. (1977).

Spray Distribution. For determination of the areas on citrus trees of the highest dislodgeable foliar residues after application of sprays, trees were treated with Supracide 40WP with both an oscillating boom and an airblast sprayer with an air tower. Three replicate 40-disk samples were collected at the outer periphery of treated trees at the 1.5-, 4-, and 6-ft (0.5-, 1.2-, and 1.8-m) levels at the north, south, east, and west sides of the tree.

RESULTS AND DISCUSSION

The field method for collecting foliage samples and removing the dislodgeable residues was patterned after the laboratory method of Gunther et al. (1973) which is currently used by a significant number of research groups. Thus, the field method should give data that can be more readily compared to the large store of data in the technical literature which have been obtained by the laboratory method. Use of a commercially available leaf punch sampler simplifies sample collection and gives samples of known surface area. The aqueous leaf wash used, followed by a hexane partitioning step, gives extracts quite free of coextractives; thus, results are more reliable and better minimum detectability is achieved. The quantitative step is based on the use of 4-(*p*-nitrobenzyl)pyridine (NBP) which was shown by Getz and Watts (1964) to react with a broad spectrum of organophosphorus compounds. The selection of NBP was based on its reactivity, unlike other possible reagents, to both P=O and P=S phosphoric acid esters. This consideration was important due to the formation in the field of toxic oxygen analogues (oxons) from parent P=S insecticides. One person familiar with the field method described herein can, exclusive of sample collection time, conduct 6, 12, and 24 tests in 30, 50, and 90 min, respectively.

The extent of the reentry problem is difficult to assess on a worldwide scale. The problem has received the most attention in California due to documented episodes of poisonings involving mostly citrus and grapes in California. The following are the OP insecticides used in California for crops (citrus, grape, nectarine, peach, and apple) for which reentry intervals are assigned: azinphosmethyl, carbophenothion, demeton, dialifor, diazinon, dimethoate, dioxathion, ethion, malathion, methidathion, methyl parathion, mevinphos, monocrotophos, naled, oxydemeton-methyl, parathion, phosalone, phosmet, phosphamidon, and trichlorfon (California Department of Food and Agriculture, 1977). These are the compounds the analyst might encounter in the field. Assigned reentry intervals vary with both the insecticide and the crop to account for both the insecticide toxicity and special features of the crop. For example, citrus leaves may accumulate more foliar dust than peaches since citrus is a nondeciduous tree. Reentry intervals have not been assigned for monocrotophos and trichlorfon.

The data given below were obtained for use in selecting the final components and optimum conditions for use in a field kit for estimating OP insecticide residues on foliage and in soil dust. Initial discussion centers on various aspects of the reaction tube. Then, discussion relates to application to dislodgeable foliar residues and soil-dust residues. Finally, data collected by using a prototype field kit are compared to results obtained with the widely used laboratory method of Gunther et al. (1973).

Reaction Tube Preparation. For convenience, the reaction tubes charged with NBP and oxalic acid are best prepared prior to sample collection in the field. The recommended concentrations and proportions of reagents were obtained empirically (Papadopoulou et al., 1978). The procedure directs that 100 μL of each reagent solution be added to the reaction tube. If the NBP is omitted, no color will form. If oxalic acid is omitted, a color will develop but results are erratic. Addition of an extra dose of either or both reagents above that specified will, in general, give values ~20% below values obtained by the specified proportions. Since "no color" can be interpreted as "no determinable residues present" and low erratic values can be attributed to low field residues and normal field sample variations, standard solutions must be available to check the prepared tubes.

Reaction Tube Storage Stability. It would be convenient if large batches of charged reaction tubes could be prepared and stored for use as needed. Tubes were stored at 22 and 43 °C for up to 12 weeks to check their stability. When stored tubes were used in the determination of parathion in the range 2.5–10 μg, no adverse effects due to storage were evident when corrections for higher, also stored, reagent blanks were made. Absorbance values after reaction (150 °C for 3 min) with 10 μg of parathion using tubes stored for 0, 2, 5, 7, 8, 10, and 12 weeks were 0.25, 0.28, 0.22, 0.24, 0.28, 0.26, and 0.28, respectively, for the 22 °C storage temperature and 0.23, 0.28, 0.21, 0.22, 0.27, 0.24, and 0.24, respectively, at the 43 °C storage temperature. Tubes stored at 43 °C were visibly yellow in color after 7 weeks, and the color intensity increased with increased storage time. For the 0- to 12-week storage interval, reagent blank absorbance values were 0.04, 0.02, 0.07, 0.06, 0.09, 0.09, and 0.08, respectively for the 43

°C stored tubes. After reaction with parathion, the 10- and 12-week stored tubes were visually off-color due to the strong yellow background present; however, spectrophotometric measurements were unaffected. Use of stored tubes is satisfactory as the resulting higher absorbance values will err on the side of safety.

Effect of Storage after Hexane Removal. Hexane extracts containing the insecticide residues are placed in the reaction tubes and then the hexane is boiled off. Some time could elapse until the subsequent heating step is conducted. Thus, different sets of reaction tubes containing parathion were heated at 150 °C for 3 min after 1, 24, 48, 120, or 144 h had elapsed after hexane removal. The absorbance values for reaction of 8 μg were 0.20, 0.20, 0.20, 0.20, and 0.19, respectively, with increasing time, and the corresponding slopes of the standard curves generated by using 2, 4, 6, and 8 μg were 0.025, 0.027, 0.025, 0.025, and 0.025 absorbance unit/μg, respectively, for the five storage times. The data show that the 150 °C reaction step can be delayed for up to 6 days without affecting the final results. However, storage time should be minimized to avoid any uncertainties.

Reaction Conditions. Sawicki et al. (1963) reported that alkylation of NBP in acetophenone solution gave optimum results when heated at 180 °C for 3.5–15 min; their general procedure used 3.5 min. They reported that heating for longer than 5 min produced a darker reagent blank and heating at lower temperatures, in general, gave lower sample absorbance readings. Getz and Watts (1964) in their study with OP compounds in a slightly basic solution used a temperature–time combination of 175–180 °C and 3 min. Turner (1974) studied the reaction of OP compounds with NBP in aqueous ethanol heated at reflux and reported an optimum time of ~15 min.

For the field method, use of a solvent for the NBP–OP compound reaction was considered undesirable in view of potential complications that could introduce errors. Parathion, used as a model compound, was reacted at various heating times and temperatures to determine optimum conditions for field use; no solvent was used for the reaction step.

One approach explored was the use of a boiling water bath for heating the reaction tubes. This was considered convenient as water is readily discarded after use. All other liquids were considered unsafe for use by untrained personnel. A boiling water bath was attractive in that a constant temperature could be maintained without careful attention. Shortcomings were the potential for scalding in case of an accidental spill and the requirement that the camp stove flame must be extinguished before hexane vaporization could be initiated. In the latter case, addition of the reaction tubes to the boiling water will immediately lower the temperature of the water sufficiently that hexane evaporation from the tubes is inefficient. By use of the two six-hole aluminum blocks preheated to 150–160 °C and in the absence of camp stove flame, rapid evaporation of 12 tubes each containing 10 mL of hexane was achieved within 1.5 min.

Table I gives data on the effect of 100 °C heating time on the reaction of parathion with NBP from 5 to 40 min. Increased heating time up to 30 min increased absorbance values. A 30-min heating was used for further testing with other OP compounds. Heating time beyond 30 min is excessive for a rapid field method.

Use of aluminum heating blocks and heating for a short time at a high temperature had the advantage of yielding even higher absorbance values in a shorter time. Table I gives data on the effect of heating at 140, 145, 150, 155, and 160 °C for 2, 3, 4, and 5 min on the reaction of NBP with parathion. A temperature–time combination of 150 °C for 3 min was selected as the working conditions based on these results. The data indicate that variations of a few degrees or a few seconds from 150 °C for 3 min are unlikely to cause significant error.

Table I. Effect of Temperature and Heating Time on the Reaction of Parathion with NBP

temp, °C	time, min	absorbance (560 nm)[a] 2.5 μg	5 μg	7.5 μg	10 μg	slope[b]
100	5	0.03	0.07	0.11	0.12	0.012
	10	0.05	0.09	0.13	0.16	0.015
	15	0.05	0.10	0.17	0.20	0.021
	20	0.06	0.12	0.16	0.21	0.020
	25	0.05	0.12	0.18	0.24	0.022
	30	0.06	0.12	0.20	0.25	0.026
	35	0.06	0.11	0.18	0.24	0.024
	40	0.05	0.10	0.16	0.23	0.024
140	2	0.08	0.17	0.23	0.31	0.030
	3	0.09	0.18	0.25	0.34	0.033
	4	0.09	0.18	0.26	0.37	0.037
	5	0.07	0.18	0.25	0.32	0.033
145	2	0.08	0.16	0.25	0.34	0.035
	3	0.10	0.18	0.28	0.35	0.034
	4	0.09	0.17	0.26	0.36	0.036
	5	0.08	0.14	0.25	0.29	0.030
150	2	0.09	0.16	0.22	0.35	0.034
	3	0.09	0.17	0.27	0.35	0.035
	4	0.08	0.16	0.23	0.31	0.030
	5	0.08	0.16	0.22	0.26	0.024
155	2	0.07	0.16	0.21	0.28	0.027
	3	0.08	0.16	0.25	0.31	0.031
	4	0.07	0.11	0.19	0.32	0.033
	5	0.09	0.15	0.17	0.22	0.016
160	2	0.08	0.16	0.22	0.31	0.030
	3	0.08	0.16	0.22	0.28	0.026
	4	0.09	0.12	0.21	0.28	0.026
	5	0.07	0.14	0.21	0.23	0.022

[a] Values were corrected for reagent blank values. [b] Slope of the standard curve; correlation coefficients were 0.97–0.99.

Effect of Storage after NBP Reaction. After the 150 °C reaction is completed, it is possible that some time might elapse before the two base solutions are added to produce the final color. Thus, different sets of tubes were heated by using parathion for 3 min at 150 °C, and after 1, 24, 48, or 72 h had elapsed, the base solutions were added. The absorbance values for reaction of 8 μg were 0.24, 0.21, 0.23, and 0.21, respectively, with increasing time, and the corresponding slopes of the standard curves generated by using 2, 4, 6, and 8 μg were 0.030, 0.026, 0.028, and 0.026 absorbance unit/μg, respectively. The data show that at least 3 days may elapse prior to color development without affecting the final results.

Color Development. In this step, the organic base solution (triethylamine) must be added before the aqueous base solution (carbonate). Otherwise, an erroneously low reading for the insecticide concentration will result. The standard curve for parathion with the organic base added first gave a slope of 0.036 (r = 0.999) whereas when the aqueous base was added first, the slope was 0.023 (r = 0.994). This would result in parathion values of 64% of actual levels. As the colored species is insoluble in water, it is mandatory that the acetone base solution be given the first opportunity to dissolve the NBP–OP compound reaction product. Initial addition of the aqueous base prevents access of the organic solvent to the NBP–OP compound reaction product due to the poor mixing situation existent in a hand-held test tube.

Color Stability. Addition of the base solutions to the

150 °C heated reaction tubes develops the final magenta color. As this color is unstable and shortly begins to fade, the absorbance of the solution at 560 nm should be measured expeditiously within 3 or 4 min after addition of the base solutions. Measurements were taken 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h after addition of base to the parathion–NBP reaction product to illustrate this decrease in absorbance with time. With 10 μg of parathion, absorbance values were 0.29, 0.28, 0.25, 0.21, 0.18, and 0.17, respectively, with a half-life of 2.9 h. Measurements were made on different tubes to avoid the effects of pouring a single solution more than once. The decrease in absorbance was a first-order kinetic process; the half-life values obtained with a number of samples varied between 1.5 and 3.0 h under laboratory fluorescent lighting conditions. Turner (1974) reported that the stability of the color varies with the pesticide used. Half-life values calculated from his data obtained after addition of tetraethylenepentamine in acetone to NBP adducts with malathion, dichlorvos, tetrachlorvinphos, and fenchlorphos were 1.7, 1.2, 2.0, and 1.5 h, respectively. He recommended that exposure of the colored solution to bright (direct) sunlight be avoided, as confirmed in the present study.

Wavelength. The wavelength, molar absorptivity, and half-life of the colored species formed upon addition of the base solution to the OP–NBP adduct can vary greatly depending upon the nature of the base solution used. The procedure reported here can readily measure 1 μg of OP compound and allows sufficient time for absorbance measurement. Absorbance data between 535 and 580 nm for solutions prepared by using azinphosmethyl, malathion, methidathion, and parathion and their corresponding oxons and carbophenothion, dimethoate, ethion, and trichlorfon were obtained. These compounds include *O*-ethyl and *O*-methyl esters, phosphorodithioates, phosphorothioates, and phosphates, and a phosphonate. The spectra for all compounds had broad peaks with the maxima between 555 and 560 nm. For example, parathion reacted with NBP gave absorbance values of 0.53, 0.55, 0.56, 0.57, 0.58, 0.58, 0.57, 0.55, 0.53, and 0.50 starting at 535 nm and measuring at 5-nm increments up to and including 580 nm. The 560-nm wavelength was selected as a working wavelength for the method although any wavelength between 550 and 565 nm would give equally satisfactory results.

Reaction with OP Compounds. Table II gives data for the reaction of NBP with a number of OP insecticides and many of their oxygen analogues which may be present in environmental field samples. Values were obtained after heating for 30 min using a boiling water bath and also after heating for 3 min using a 150 °C heating block. The data in Table II indicate that with the exception of two compounds, heating for 30 min at 100 °C gave lower absorbance unit values per microgram of material than heating for 3 min at 150 °C. The use of a water bath for a field method was abandoned based on these results coupled with the excessive time required and the necessity of maintaining boiling water for this period.

Except for azinphosmethyl oxon, the oxygen analogues produced absorbance values of 60–80% of those obtained from an equivalent weight of the parent insecticide; this is contrary to the results of Getz and Watts (1964) but their reaction conditions were different (solvent used). The insecticides and their oxons listed represent a number of phosphates and thio- and dithiophosphates; EPN and trichlorfon are phosphonates. Demeton and carbophenothion have sulfide side chains; in this regard, Getz and Watts (1964) reported similar reactivity for carbophenothion, its sulfoxide, and its sulfone under their reaction conditions. Diazinon was found to be the least responsive to the NBP test procedure: this was due to the formation of a reddish color rather than the purple color obtained for the other OP compounds; this anomaly was also reported by Watts (1965). No interferences were obtained from up to 250 μg of dicofol, a hydroxyl-containing acaracide, and up to 200 μg of carbaryl, a heavily used carbamate insecticide which is highly persistent on citrus foliage (Iwata et al., 1979).

Table II. Reactivity of OP Compounds with NBP

compd	150 °C for 3 min (slope A)[a]	100 °C for 30 min (slope B)[a]	(slope B)/(slope A)
azinphosmethyl	0.040	0.032	0.80
azinphosmethyl oxon	0.064	0.049	0.76
carbophenothion	0.021	0.015	0.71
chlorpyrifos	0.037	0.046	1.2
chlorpyrifos oxon	0.022	0.020	0.91
demeton	0.014	0.0079	0.56
diazinon	0.010	0.0085	0.85
dimethoate	0.050	0.046	0.92
dimethoate oxon	0.045	0.036	0.80
dioxathion	0.025	0.027	1.1
EPN	0.020	0.011	0.55
ethion	0.027	0.020	0.74
malathion	0.042	0.037	0.88
malathion oxon	0.031	0.030	0.93
methidathion	0.078	0.076	0.97
methidathion oxon	0.076	0.059	0.78
mevinphos	0.095	0.076	0.80
naled	0.027	0.020	0.74
parathion	0.036	0.026	0.72
parathion oxon	0.025	0.013	0.52
phenthoate	0.037	0.030	0.81
phenthoate oxon	0.030	0.023	0.77
phosphamidon	0.046	0.023	0.50
trichlorfon	0.038	0.030	0.79

[a] Slope of the standard curve (absorbance unit per microgram) obtained by using 1, 2, 5, 10, 15, 20, and 25 μg of compound; duplicate determinations were made by using each amount of compound, and values were corrected for values obtained with the reagent blank. Correlation coefficients for the standard curves were 0.98–0.99.

The 10-fold differences in the range of reactivity of the compounds listed in Table II must be taken into account in using the field method for estimating residue levels. This problem is discussed later.

Dislodgeable Residues. One sample consists of 40 leaf disks (2.5-cm diameter) collected from the trees of interest. The leaf disk sample is then shaken with 20 mL of 20% aqueous NaCl solution to remove surface residues from the leaf disks. A 15-mL aliquot of the aqueous leaf wash is removed and partitioned with 15 mL of hexane. A 10-mL aliquot of the hexane extract is removed and analyzed for OP residues. Data on the recovery of various OP compounds added to 15 mL of aqueous NaCl solution and to 15 mL aliquots of leaf washes obtained from residue-free citrus leaves are given in Table III. The data indicate a wide range in recovery efficiency reflecting the corresponding wide range in solubilities in water of the OP compounds. These differences must be taken into account in estimating residue levels in the field. This problem is discussed later.

Soil-Dust Residues. One sample consists of sweepable dust collected from the eight cardinal points of a tree each comprising $^1/_8$ ft^2 of soil surface at the dripline area. The 1-ft^2 sample is sieved through a 100-mesh screen to obtain soil fines. A level $^1/_4$-tsp household measuring spoon of dust is removed for residue estimation and placed in a 50-mL polypropylene tube with a screw cap; the weight

Table III. Recovery of Compounds after Fortification of 20% NaCl Solutions and Aqueous Leaf Washes

compd	recovery, %[a] NaCl solution	leaf wash
azinphosmethyl	120 ± 18	110 ± 12
azinphosmethyl oxon	29 ± 5	20 ± 2
carbophenothion	100 ± 6	100 ± 8
chlorpyrifos	110 ± 13	100 ± 5
dimethoate	18 ± 8	32 ± 27
dioxathion	97 ± 9	97 ± 21
EPN	68 ± 5	66 ± 9
ethion	86 ± 11	93 ± 9
malathion	86 ± 10	88 ± 15
malathion oxon	100 ± 17	99 ± 12
methidathion	98 ± 7	93 ± 10
parathion	74 ± 7	80 ± 7
parathion oxon	98 ± 18	80 ± 11
phosphamidon	50 ± 6	49 ± 30

[a] Mean ± standard deviation. Fortifications were conducted by adding 1.5, 3, 7.5, 15, 22.5, 24, 30, and 36 μg (minimum of five levels used for each compound) to 15 mL of either 20% NaCl solution or 20% NaCl leaf washes and proceding with the described method. Reaction was at 150 °C for 3 min.

Table IV. Effect of the Use of Different Ratios of Salt Water to Organic Solvent on the Recovery of Six OP Compounds from Fortified Soil Dust[a]

aqueous to organic solvent ratio[b]	recovery, %, of OP compound parathion	azinphosmethyl	malathion	parathion oxon	azinphosmethyl oxon	chlorpyrifos oxon
4:20	92	81	92	76	37	40
3:20	94	83	91	80	29	37
2:20	96	88	95	85	70	45
1:20	99	93	96	93	78	51
0.5:20	98	96	96	97	86	73
0.25:20	99	97	98	101	93	87
c	99	99	102	109	96	92

[a] Mean recovery (percent) over the fortification range of 10–350 ppm by using 1 or 3 g of sandy loam soil dust (<100 mesh). [b] The aqueous solvent was 20% (w/v) NaCl solution and the amount added varied as shown. The organic solvent was 15% acetone in hexane and the amount added was 20 mL. Aliquots of the upper phase were used for analysis. [c] No NaCl solution added.

is taken as 1.5 g for calculation purposes. To the dust is added 0.25 mL of 20% NaCl solution and 20 mL of 15% acetone in hexane solution. After the mixture was shaken, an aliquot of the organic phase is removed for analysis. Table IV gives recovery values for six OP compounds added to soil and extracted by using different amounts of added salt water. If no water is added, the extract is somewhat yellowish in color, and difficulties are encountered due to suspended soil particles in the extract. Increasing amounts of added water correspondingly eliminate these two problems, but the amount of water-soluble OP compounds recovered decreases with increased use of water.

Field Testing of the Method. The prototype field method was tested for on-site determination of the dissipation rates of three OP insecticides. Orange trees were sprayed separately with wettable powder formulations of parathion, malathion, and methidathion and an emulsifiable concentrate formulation of methidathion by using both low-volume and dilute sprays. At each of 11 dates spanning the period 3–62 days postapplication, four 40 leaf disk samples were collected from each treatment plot. Two samples were processed and analyzed immediately in the field for total organophosphorus residues, and two samples were returned to the laboratory, processed by using the method of Iwata et al. (1977), and analyzed by gas chromatography for both the parent insecticide and its oxygen analogue. Figures 1–4 show the correlation between the mean values obtained by the field method and the GLC method. The GLC values are the sum of the parent insecticide and its oxygen analogue.

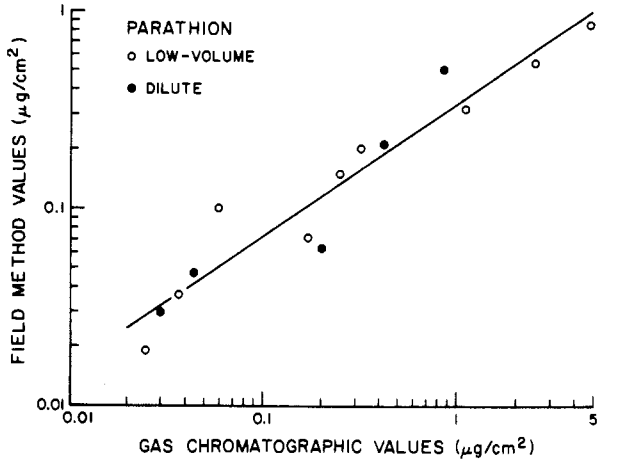

Figure 1. Correlation between total (thion and oxon) dislodgeable foliar OP residues obtained by the colorimetric field method and the GC laboratory method after spraying trees with low-volume and dilute sprays of a wettable powder formulation of parathion.

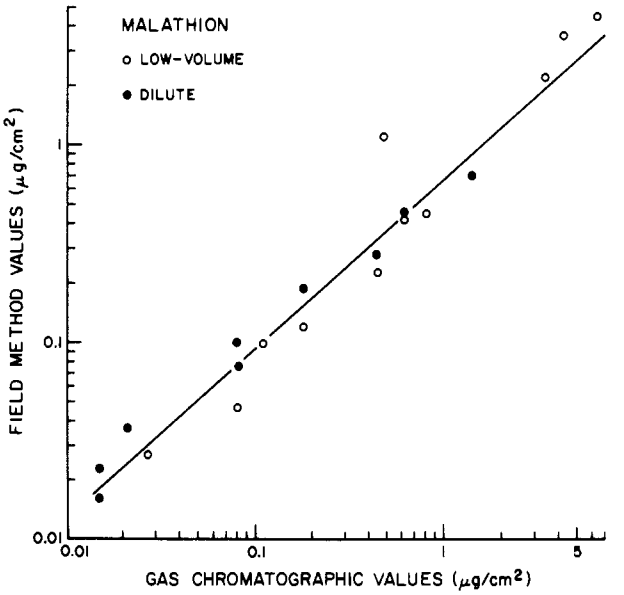

Figure 2. Correlation between total (thion and oxon) dislodgeable foliar OP residues obtained by the colorimetric field method and the GC laboratory method after spraying trees with low-volume and dilute sprays of a wettable powder formulation of malathion.

The correlation lines were described by the following equations: $\ln y = 0.67 \ln x - 1.09$ with the correlation coefficient (r) of 0.96 for parathion; $\ln y = 0.87 \ln x - 0.380$ with $r = 0.99$ (one datum point omitted for the calculation) for malathion; $\ln y = \ln x - 0.313$ with $r = 0.93$ for the methidathion emulsifiable concentrate formulation; $\ln y = \ln x - 0.133$ with $r = 0.99$ for the methidathion wettable powder formulation. The correlation between the laboratory method and the field method is quite good for total OP insecticide residues. The scatter of points is due in part to the use of only two samples for each method at each sampling date. The slope of the correlation equation would be 1.00 if a one-to-one correspondence for the two methods was achieved. Values below 1.00 indicate that lower res-

Table V. Distribution of Dislodgeable Foliar Residues at Various Locations of Sprayed Lemon Trees after Application as Dilute and Low-Volume Sprays

elapsed days	sampling position, height, ft	mean residue, $\mu g/cm^2$,[a] for direction				overall mean, $\mu g/cm^2$
		east	west	north	south	
		Dilute Application				
7	6	0.11 ± 0.01	0.11 ± 0.02	0.07 ± 0.007	0.07 ± 0.01	0.09
	4	0.17 ± 0.02	0.11 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.12
	1.5	0.19 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.15
14	6	0.04 ± 0.006	0.04 ± 0.006	0.02 ± 0.005	0.03 ± 0.003	0.03
	4	0.08 ± 0.01	0.05 ± 0.01	0.04 ± 0.006	0.05 ± 0.01	0.05
	1.5	0.06 ± 0.02	0.05 ± 0.02	0.04 ± 0.004	0.04 ± 0.006	0.05
		Low-Volume Application				
11	6	0.18 ± 0.06	0.12 ± 0.02	0.11 ± 0.03	0.11 ± 0.01	0.13
	4	0.12 ± 0.01	0.15 ± 0.03	0.12 ± 0.01	0.23 ± 0.04	0.16
	1.5	0.14 ± 0.04	0.08 ± 0.01	0.24 ± 0.02	0.26 ± 0.02	0.18
17	6	0.05 ± 0.01	0.05 ± 0.008	0.05 ± 0.004	0.04 ± 0.02	0.05
	4	0.05 ± 0.03	0.04 ± 0.003	0.07 ± 0.02	0.09 ± 0.03	0.06
	1.5	0.05 ± 0.02	0.03 ± 0.003	0.10 ± 0.03	0.03 ± 0.03	0.05

[a] Three replicate samples.

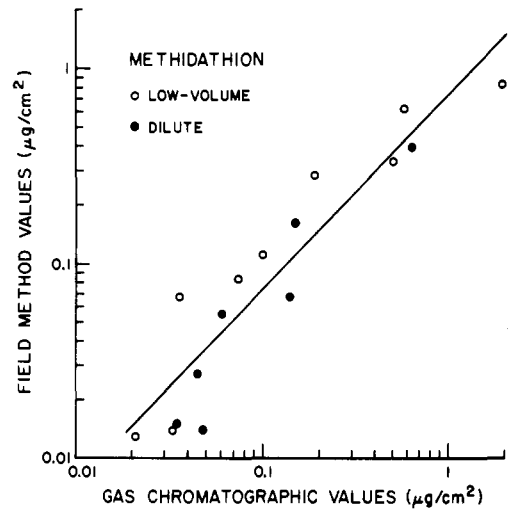

Figure 3. Correlation between total (thion and oxon) dislodgeable foliar OP residues obtained by the colorimetric field method and the GC laboratory method after spraying trees with low-volume and dilute sprays of an emulsifiable concentrate formulation of methidathion.

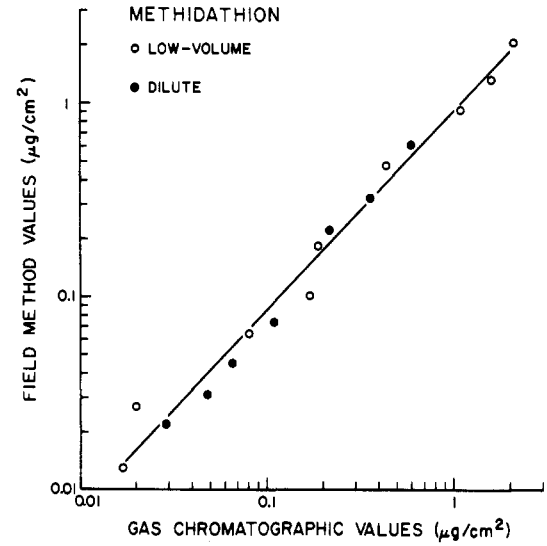

Figure 4. Correlation between total (thion and oxon) dislodgeable foliar OP residues obtained by the colorimetric field method and the GC laboratory method after spraying trees with low-volume and dilute sprays of a wettable powder formulation of methidathion.

idues are found by the field method than the laboratory method. For both EC and WP methidathion formulations the slope was 1.0. The 0.87 value for malathion is also quite good. The acceptable but lower than desirable value of 0.67 for parathion is attributed to the lipophilicity of parathion. The parathion, freed from the soil dust by desorption and solubilized in water, partitions from the aqueous wash into the citrus leaf wax. Thus, recovery of dislodgeable residues must be determined by adding known amounts of OP compound to the 20 mL of NaCl solution and adding it to actual leaf samples.

It should be mentioned that to generate the numerical data plotted in Figures 1–4 for the field method, it was necessary to take aliquots of the hexane extract smaller than the 10 mL specified under Materials and Methods. The aliquot size varied with the nature of the insecticide used and the days of postapplication, and appropriate dilutions were made, if necessary, to keep the maximum absorbance readings under 1.0 absorbance unit.

Residue Distribution on Citrus Trees. Residues on a tree are expected to vary somewhat due to nonuniform insecticide application and different rates of dissipation from different portions of the tree due to microclimates. Trees were sprayed with Supracide 40WP by using 3.5 lb of AI $(100\ gal)^{-1}\ acre^{-1}$ and 3.5 lb of AI $(1400\ gal)^{-1}\ acre^{-1}$, and separate samples were taken from the 1.5-, 4-, and 6-ft heights of the trees and from the north, south, east, and west sides of the trees. The spray rigs traveled in an east–west direction. Accordingly, the north and south sides of the tree which face the source of the spray would be expected to receive higher initial deposits of insecticides and subsequently retain relatively higher postapplication residues. Data obtained by using the field method are given in Table V. Only the low-volume treatment indicates that significantly higher residues are present on the north and south sides of the tree. This is expected as the oscillating boom gives generally quite uniform spray coverage as a result of the use of a large volume of spray solution. For both treatments, the trend of the data is that residues are higher at the 1.5- and 4-ft levels than at the 6-ft level. Samples should be collected at the 4-ft level on the between-row sides of the trees to obtain samples containing maximum residues.

Method Application. The variable reactivities of OP compounds with NBP and variable recoveries from the aqueous leaf washes put constraints on the utility of the method. Thus, if several insecticides have been applied either sequentially or in admixture, results must be interpreted in terms of the most toxic insecticide used if the

spray history is known and in terms of the most toxic insecticide registered if the spray history is unavailable.

The method reported herein is a prototype and has been subjected to further refinement (Gunther et al., 1980). For example, a battery-operated, factory-thermostated heating unit has supplanted the camp stove used herein.

California Assembly Bill 1090 (AB 1090) allows growers more flexibility by making provisions for testing groves for safe conditions rather than having to automatically wait until the expiration of a preset reentry interval. Knaak et al. (1980) have suggested toxicological safe levels for azinphosmethyl, methidathion, and parathion as being 3.1, 0.6, and 0.09 $\mu g/cm^2$ for citrus. The field method can readily determine these levels. However, each insecticide will require specific instructions as to the volumes of salt water and hexane to be added and removed for use. If the final measurement obtained by the analyst is below the absorbance value provided with the kit and the procedure, then the field can be deemed safe for worker entry. The absorbance value must be determined by using the actual reagents and equipment used in the final field kit. Prototype kits will be field tested more extensively.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of J. Virzi, J. Barkley, G. E. Carman, J. Pappas, T. Dinoff, D. Aitken, and M. Wells.

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Received for review April 24, 1980. Accepted October 27, 1980. This work was supported by the U.S. Environmental Protection Agency under Contract R805 642-01 entitled "Rapid Field Measurements of Organophosphorus Pesticide Residues".

Effects of Atrazine Treatment of a Corn Field Using Different Application Methods, Times, and Additives on the Persistence of Residues in Soil and Their Uptake by Oat Plants

Shahamat U. Khan,* Paul B. Marriage, and Allan S. Hamill

Treatment of corn fields with the herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] resulted in the formation of its 2-hydroxy analogue as the only measurable metabolite in a clay loam soil. The time and method of atrazine application (preplant incorporated, preemergence, and postemergence) and the presence of oil/surfactant additives in the herbicide formulation had no long-term effect on its persistence. However, postemergence application and the presence of additives resulted in a slightly greater initial degradation rate of atrazine in soil. Both atrazine, in less than phytotoxic amounts, and hydroxyatrazine persisted into the following growing season from all treatments. The residues from the field-treated soil were taken up, metabolized, and conjugated by oats seeded in the spring.

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] is a widely used selective herbicide for weed control in corn. Applications may be made prior to planting with soil incorporation, immediately after planting before crop and weed emergence, and after crop and weed emergence. In the latter instance the addition of oils, surfactants, and their mixtures is recommended to enhance atrazine penetration into the leaf surface of weeds. In addition, atrazine for application at planting is now available in wettable powder and flowable formulations with the latter containing various additives.

It has been suggested that atrazine application in oil–water emulsions could affect and possibly decrease the persistence of atrazine (Sylwester, 1966; Sweet et al., 1979). Time and/or method of application may also effect atrazine persistence and degradation. It has been observed that treatments applied after corn emergence generally result in greater atrazine persistence or carry over than earlier treatments (Peters and Keeley, 1964; Frank, 1966; Burnside, 1976; Burnside and Schultz, 1978), and this may be related to soil moisture conditions.

In a previous study it was suggested that atrazine degradation products could persist beyond the growing season following a single atrazine application (Khan and Marriage,

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0021-8561/81/1429-0216$01.25/0