could be due to worker contamination through contact with previously used equipment or to exposure to contaminated surfaces in the general work environment.

The developed procedure provides a rapid, reproducible, and sensitive method for monitoring occupational exposure to fenitrothion. The method should be useful for monitoring exposure to organophosphorus pesticides in general and is currently being employed in our laboratory for assessing exposure to azinphos-methyl.

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LITERATURE CITED

Bradway, D. E.; Moseman, R.; May, R. *Bull. Environ. Contam. Toxicol.* **1981**, *26*, 520.
Bradway, D. E.; Shafik, T. M.; Lores, E. M. *J. Agric. Food Chem.* **1977**, *25*, 1353.
Brokopp, C. D.; Wyatt, J. L.; Gabica, J. *Bull. Environ. Contam. Toxicol.* **1981**, *26*, 524.
Churchill, F. C.; Ku, D. N.; Miles, J. W. *J. Agric. Food Chem.* **1978**, *26*, 1108.
Dale, W. E.; Miles, J. W.; Churchill, F. C. *J. Assoc. Off. Anal. Chem.* **1976**, *59*, 1088.
Daughton, C. G.; Crosby, D. G.; Garnas, R. L.; Hsieh, D. P. H. *J. Agric. Food Chem.* **1976**, *24*, 236.
Eto, M. "Organophosphorus Pesticides: Organic and Biological Chemistry"; CRC Press, Inc.: Cleveland, OH, 1974; p 80–82.
Franklin, C. A. *Can. J. Physiol. Pharmacol.* **1984**, *62*, 1037.
Franklin, C. A.; Fenske, R. A.; Greenhalgh, R.; Mathieu, L.; Denley, H. V.; Leffingwell, J. T.; Spear, R. C. *J. Toxicol. Environ. Health* **1981**, *7*, 715.
Greenhalgh, R.; Blackwell, B. A.; Preston, C. A.; Murray, W. J. *J. Agric. Food Chem.* **1983**, *31*, 710.
Ives, F. N.; Guiffrida, L. *J. Assoc. Off. Anal. Chem.* **1970**, *53*, 973.
LeBel, G. L.; Williams, D. T. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 1353.
Lores, E. M.; Bradway, D. E. *J. Agric. Food Chem.* **1977**, *25*, 75.
Miles, J. W.; Dale, W. E. *J. Agric. Food Chem.* **1978**, *26*, 480.
Shafik, M. T.; Bradway, D.; Biros, F.; Enos, H. *J. Agric. Food Chem.* **1970**, *18*, 1174.
Shafik, M. T.; Bradway, D.; Enos, H. F. *J. Agric. Food Chem.* **1971**, *19*, 885.
Shafik, M. T.; Bradway, D.; Enos, H. F. *J. Agric. Food Chem.* **1973**, *21*, 625.
Shafik, M. T.; Enos, H. F. *J. Agric. Food Chem.* **1969**, *17*, 1186.

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Residues of Avermectin B_1a on and in Citrus Fruits and Foliage

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A 3 μg/mL solution of [^{3}H]-avermectin B_1a was applied to citrus fruits and leaves. Both fruits and leaves accepted 0.017 ± 0.004 μg/cm^2 of surface area treated. Postapplication (2~3 h) residues for orange rind, lemon rind, and lemon leaves were 0.066 ± 0.007, 0.038 ± 0.006, and 0.92 ± 0.23 μg/g, respectively, based on total tritiated water obtained by combustion. However, based on reversed isotope dilution analysis of acetone extracts, actual avermectin B_1a residues were 30% for orange rind and 16% for lemon rind of the corresponding combustion value. Residues were <0.001 μg/g in pulp of both treated mature fruits and lemons treated when ~2.5 cm in diameter and analyzed 60 days postapplication. Residues were <0.004 μg/g in new growth leaves sampled from tips of branches whose leaves had been treated 91 days earlier.

The avermectins are a family of new pesticidal agents that are extracted from the mycelia of the actinomycete, *Streptomyces avermitilis*, which was first isolated from a soil sample collected in Japan (Burg et al., 1979; Miller et al., 1979). The chemical structures of the avermectins were elucidated by Albers-Schönberg et al. (1981). Some of the extraordinary biological activities of the avermectins have recently been reviewed (Campbell et al., 1983). MK-936, consisting of at least 80% avermectin B_1a and not more than 20% avermectin B_1b (see Figure 1), has shown good efficacy in laboratory and field tests as an acaricide for the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), and appears promising for the control of the citrus red mite, *Panonychus citri* (McGregor) (McCoy et al., 1982). The latter is one of the three most serious pests attacking California citrus. It also shows great promise for the control of many other economically important arthropods including imported fire ants (Schuster and Everett, 1983; Putter et al., 1981; Lofgren and Williams, 1982).

To obtain preliminary information on residue levels, dissipation rates, and translocation resulting from a field treatment, tritium-labeled avermectin B_1a was applied at an anticipated use level to orange and lemon fruits and lemon leaves, and samples were collected and analyzed. Field treatment, sample collection, and sample preparation were conducted at the University of California, Riverside (UCR), and sample combustion, liquid scintillation counting, and reversed isotope dilution analyses (RIDA's) were performed at Merck.

EXPERIMENTAL SECTION

Formulation. To 88 mL of 1,2-propanediol in a 500-mL round-bottom flask was added 6.15 mL of a [5-^{3}H]-avermectin B_1a solution (2.88 mg of avermectin B_1a/mL, 3.9 mCi/mL in ethanol). After removing the ethanol under reduced pressure, 174 mg of an avermectin B_1a preparation (L-676,895-00P32), containing 162.3 mg of unlabeled av-

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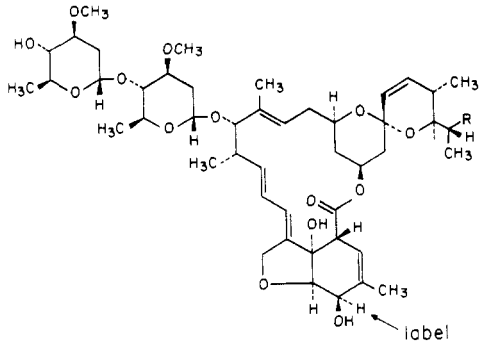

Figure 1. Structure of avermectin B_1a (Albers-Schönberg et al., 1981). The position of the tritium label is indicated by the arrow. MK-936 consists of at least 80% avermectin B_1a (R = C_2H_5) and not more than 20% avermectin B_1b (R = CH_3).

ermectin B_1a, and 12 mL of Triton X-100 surfactant were added. The solution was allowed to equilibrate overnight (1.8 mg of avermectin B_1a/mL, 0.24 mCi/mL, specific activity = 296 dpm/ng). An aliquot of this solution was sent to UCR where it was diluted 600-fold with water and used for the field treatment of citrus fruits and foliage.

Treatment. One mature lemon tree and one mature orange tree, both located within a citrus grove on the University of California Citrus Research Center, Riverside, CA, were used. Fifteen nearly mature lemons, 15 nearly mature oranges, 12 small lemons (each ~10 g and 2.5 cm in diameter), and mature lemon leaves on four separate branches were treated by brief immersion into a 3 ppm avermectin B_1a solution on July 21, 1980.

Sampling. At each sampling, three lemon and three orange fruits and six leaves were removed. Immediate postapplication samples (0 day) were collected 2~3 h after treatment. Additional samples were collected after 15, 30, 60, and 91 days had elapsed. Treated small lemons were collected 60 and 91 days postapplication, and new growth leaves were collected 91 days postapplication from the tips of branches from which treated leaves had not previously been removed.

Processing. For each fruit, three samples of rind and two samples of pulp (edible portion of the fruit) were obtained. Each rind sample consisted of two 1.0-cm diameter cores excised with a cork borer and obtained from opposite sides of the equatorial circumference. Each pulp sample consisted of a portion of a core sample obtained with a 1.0-cm (lemon) or 1.6-cm (orange) diameter cork borer. Each leaf sample consisted of two 2.5-cm diameter disks excised from a single leaf.

All samples represented ~0.5 g of fresh weight of the substrate. After recording the exact fresh weights, the samples were each placed in a combustion cup and lyophilized. Samples were sent to Merck where they were combusted, and the water produced was trapped to determine by liquid scintillation counting the amount of tritium present. Samples fortified with radiolabeled avermectin B_1a at levels comparable to those present in actual samples were also prepared and analyzed by combustion along with the field samples.

Extracts. Acetone extracts of rind and pulp were prepared by macerating 20 g of substrate twice, each time with 100 mL of acetone and a 2 min blending time, and by using a 50-mL acetone rinse. The acetone was removed from the combined extracts, and the aqueous residue was sent to Merck for RIDA. The solid extraction residue recovered by filtration was analyzed by combustion.

Sample Combustion. A Packard Tri-Carb B306 Sample Oxidizer was used. To minimize carry over contamination and background contributions, replicate samples were grouped together and oxidized in order of increasing expected tritium levels (successively pulp, rind, and leaves). Each group of samples was separated from others by water blanks (combustion cups containing 300~500 μL of water) and dry blanks (empty combustion cups); the radioactivity level of the dry blank was subtracted from that of each of the following samples. Generally, dry blank values varied in the range 20–100 dpm but were quite consistent within a day's run. In addition, lyophilized untreated citrus tissues were oxidized prior to each set of corresponding treated samples.

Liquid Scintillation Counting. A Packard Tri-Carb 460CD Liquid Scintillation system was used. This instrument was equipped with a "dpm option" which allowed the automatic calculation of dpm values based on an efficiency curve generated from quenched standards. Samples from the Sample Oxidizer were counted in 20-mL glass vials containing 15 mL of Monophase 40 (Packard Instruments) scintillation cocktail. The inclusion of a vial containing 15 mL of Monophase 40 as the first sample allowed automatic correction for the instrument background. Samples from the high-performance liquid chromatographic (HPLC) system (the last purification step in the RIDA procedure) were counted in 7-mL polyethylene Minivials containing 5 mL of Aquasol 2 (New England Nuclear) scintillation cocktail. Final counts were taken after allowing 6~12 h of equilibration for Sample Oxidizer samples; equilibration for 24~36 h was required for many of the HPLC samples.

Reversed Isotope Dilution Analysis. For all but the very earliest samples taken, an accurately known amount of unlabeled avermectin B_1a was added as the tissues were being extracted at UCR. For the others, it was added to the extracts upon arrival at Merck. The extract was rinsed with CH_2Cl_2 from the shipping vial into a separatory funnel. The water–acetone mixture was extracted three times with CH_2Cl_2. An aliquot of the aqueous residue was counted. The combined CH_2Cl_2 extracts were reduced to 2~5 mL. An aliquot of this concentrate was oxidized and counted as the crude extract was intensely colored. An aliquot of the remainder was applied to a small column containing a bottom layer of 1.0 g of acid-washed alumina (Baker) and a top layer of 1.0 g of Pitt-type CAL carbon packed in CH_2Cl_2. A "spent and wash" fraction consisting of 2–5 mL of CH_2Cl_2 was collected and consistently contained negligible or no tritium. Avermectin B_1a was eluted with 15 mL of 15% (v/v) 2-propanol in CH_2Cl_2. An aliquot of the eluate (usually 100 μL) was taken for sample oxidation; the remainder was taken to dryness and streaked on a prewashed 20 × 20 cm silica gel 60 (F-254, E. Merck) plate in methanol. On each edge, a marker spot of avermectin B_1a was placed to aid in the location of the desired zone. The avermectin B_1a region, as visualized under 254-nm ultraviolet (UV) light, was scraped from the plate; methanol eluted the avermectin B_1a from the adsorbent. In preparation for HPLC, the methanol was evaporated to dryness, and the residue was redissolved in 1.00 mL of HPLC mobile phase (acetonitrile + methanol + water, 62 + 18 + 20, v/v/v). After filtration through a 0.5 μm Millipore filter to remove particulate matter, 100 μL was removed for counting, and 500 μL was injected into the HPLC system.

The HPLC system consisted of an Altex Model 110A pump, a Waters Associates Model U6K injector, an ES Industries 50 cm × 9.6 mm i.d. Chromegaprep 10 μm C_{18} reversed-phase column maintained at 50 °C, and a LDC Spectromonitor II UV detector set at 245 nm (λ_{max} for avermectin B_1a). Mobile-phase flow rate was 5.0 mL/min.

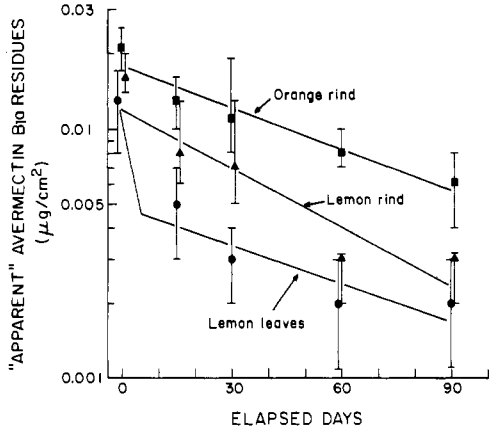

Figure 2. Disappearance of radioactivity from orange (■) and lemon (▲) rind and lemon leaves (●) after field treatment with ^3H-labeled avermectin B_1a. Radioactivity was determined by sample combustion and is therefore expressed in terms of μg of "apparent" avermectin B_1a residues per cm^2 of treated surface represented by the sample. Each datum point represents the mean value of nine sample analyses for rind and six sample analyses for leaves; vertical lines give the ranges of residue values found. In the cases of lemon rind at 60 and 91 days, all values were either 0.002 or 0.003 μg/cm^2. The mean in both cases was between 0.002_5 and 0.003 μg/cm^2. Since only one significant figure is justified, the means and the upper range values shown coincide.

Eluate fractions of 1.5 mL each were collected every 0.3 min by using an Isco Golden Retriever fraction collector. Fractions containing avermectin B_1a (as determined from the strip chart recording of UV absorbance vs. time) were diluted to an exact volume with mobile phase, and aliquots were removed for counting and UV determination of avermectin B_1a content by using a Beckman Model UV 5260 spectrophotometer.

RESULTS AND DISCUSSION

When tritium-labeled avermectin B_1a (see Figure 1) was used, orange and lemon fruits and lemon leaves were field-treated with a solution containing 3 μg of avermectin B_1a per mL (3 ppm). Data and discussion on the behavior of avermectin B_1a under field conditions are given below.

Rind and Leaves. Figures 2 and 3 show the recovered radioactive residues expressed in terms of "apparent" avermectin B_1a obtained for samples collected 2–3 h and 15, 30, 60, and 91 days postapplication. For these graphs, all the radioactivity recovered by sample combustion to tritiated water was taken to be due to undegraded avermectin B_1a. Figure 2 shows the residue levels expressed in terms of μg per cm^2 of treated surface area represented by the sample. Both orange and lemon rind and lemon leaves accepted the same amount of active ingredient per unit surface area treated (0.017 ± 0.004 μg/cm^2, mean ± standard deviation). Figure 3 shows the residue levels expressed in terms of μg per gram (ppm) of sample. On a weight basis, the residue levels are quite dissimilar for rind and leaves due to the difference in the ratio of weight of substrate to the treated surface area of the substrate. This also accounts for the different ppm levels for orange vs. lemon rind. The immediate postapplication residues were quite low for citrus rind and were 0.066 ± 0.007 and 0.038 ± 0.006 ppm for orange and lemon rind, respectively. Since no determinable residues were found in the edible portion of the fruit, the 0-day residue on a whole fruit basis would already be only about 0.01 ppm for both citrus fruits as oranges are about 20% and lemons about 30% rind by weight (Gunther, 1969). For regulatory purposes, fruit tolerances are based upon whole fruit.

Figure 3 shows that a large loss of "apparent" avermectin B_1a residues occurred from the lemon leaves prior to the second sampling at 15 days postapplication. The immediate postapplication residue was 0.92 ± 0.23 ppm. Due to the limited amount of data represented in Figure 3 and the probable complex nature of the degradation, we can only assume that the avermectin B_1a residue dissipations are following first-order kinetics. Figure 3, however, is similar to other residue dissipation curves for citrus (Gunther, 1969). The residue dissipation from leaves is described by

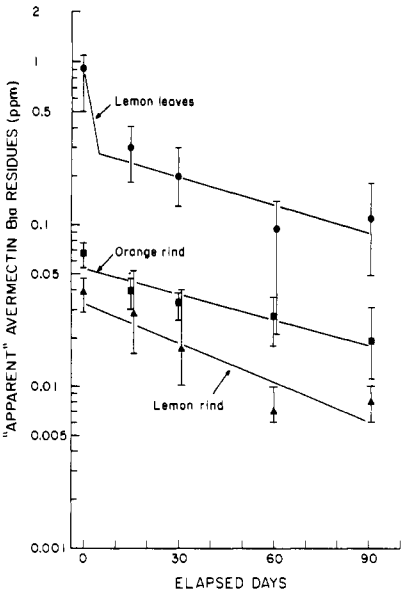

Figure 3. Disappearance of radioactivity from orange (■) and lemon (▲) rind and lemon leaves (●) after field treatment with ^3H-labeled avermectin B_1a. See Figure 2 for additional details.

$$\text{residue, ppm} = -0.014 \ln t + \ln 0.31$$

where t is time in days; because of the rapid initial decline, the 0-day point was omitted. The correlation coefficient (r) is −0.87 and the half-life ($t_{1/2}$) is 50 days. The residue dissipation from orange rind is described by

$$\text{residue, ppm} = -0.012 \ln t + \ln 0.054$$

$$r = -0.95,\ t_{1/2} = 58 \text{ days}$$

The residue dissipation from lemon rind is described by

$$\text{residue, ppm} = -0.019 \ln t + \ln 0.033$$

$$r = -0.93,\ t_{1/2} = 36 \text{ days}$$

Fresh untreated rind and leaf disk samples were fortified with radiolabeled avermectin B_1a and subjected to the same treatment as the samples as a check on the analytical procedure. Samples were fortified at the levels anticipated in the field samples. One fortified sample each of orange rind, lemon rind, and lemon foliage was prepared for the 0-, 15-, 30-, 60-, and 91-day samplings. Fortified samples were oxidized and counted as were the field samples. Recoveries for orange rind fortified at 0.15, 0.14, 0.023, 0.0077, and 0.0077 ppm, respectively for the five sampling dates, were 99, 97, 95, 100, and 101%, respectively. Recoveries for lemon foliage fortified at 2.5, 2.4, 0.45, 0.011 (inadvertent fortification error), and 0.11 ppm, respectively for the five sampling dates, were 96, 94, 96, 100, and 104%, respectively. No "corrections" were applied to the data based on these high recovery values.

The presence of avermectin B_1a residues is based on the radioactivity recovered by combustion of the lyophilized substrate. Thus, no matter how extensive the degradation of the parent compound, no decline in "apparent" residues will be noted unless the tritium label is lost from the

substrate. Due to the size and complexity of the avermectin molecule, it is hypothesized that no radiolabel will be lost unless a chemical reaction occurs at the 5-position (See Figure 1). This can be loss of label through either oxidation of the 5-OH group or through proton exchange. The first mechanism leads equally to loss of radioactivity and of parent compound; the second leads to loss of label only. Proton exchange has not been detected in numerous studies of [5-^{3}H]-avermectin B_1a in various substrates including soil (Bull et al., 1984) and plants (Gruber and Jacob, 1983). In addition, Maynard et al. (1983) reported that ^{14}C-labeled avermectin B_1a applied to citrus fruits showed behavior very similar to that of the tritiated avermectin B_1a used in this study. This indicates that proton exchange at the 5-position is not an important process. Because of the low vapor pressure of avermectin B_1a ($\leq 1.5 \times 10^{-9}$ mmHg), volatilization of the parent molecule is not expected to be important, but volatilization of degradative fragments could be.

Rind samples were extracted with acetone to determine how much of the radiolabel was not extractable. The solid residues recovered after filtration of the acetone macerates were combusted to determine how much radiolabel remained with the solid residue after two acetone extractions of the rind sample. The amount of radioactivity recovered was compared to the corresponding unextracted lyophilized combustion samples to obtain an estimate of unextractable radioactivity. For 0-, 15-, 60-, and 91-day orange rind samples, unextractable radioactivity was 12, 32, 47, and 46%, respectively; for lemon rind it was 23, 37, 48 and 52%, respectively. For 60- and 91-day lemon leaf samples, unextractable radioactivity was 69 ± 7 and 72 ± 5%, respectively. These values indicate that the parent compound undergoes rapid and extensive binding and/or chemical transformation. Degradative fragments may even be reutilized by the plant.

Pulp Residues. Three orange and three lemon fruits were collected at each sampling interval, and two subsamples of each fruit were analyzed for radioactivity by sample combustion of the lyophilized substrate. Samples were collected at 0 (2–3 h postapplication), 15, 30, 60, and 91 days postapplication. For orange pulp (edible portion of the fruit), three 0-day subsamples contained radioactivity equivalent to 0.002, 0.002, and 0.001 ppm and one 15-day subsample contained radioactivity equivalent to 0.001 ppm. All other subsamples contained residues of less than 0.001 ppm. The minute residues found are attributed to unavoidable contamination at these levels from the residue-bearing rind during sample preparation. Penetration of the acaricide through the rind within 2–3 h to the pulp would be unlikely. Also, radiolabel that penetrates into the pulp would likely remain inside the fruit and be found in subsequent samplings; this was not the case. All lemon pulp residues were less than 0.001 ppm.

The 0-, 15-, 30-, 60- and 91-day pulp samples also included untreated control samples and one fortified control for both orange and lemon pulp. Orange pulp samples were fortified at 0.0009, 0.0006, 0.0008, 0.0008, and 0.0008 ppm, respectively for the five sampling dates, and recoveries as determined from combustion results were 104, 85, 95, 94, and 105%, respectively. Lemon pulp samples were fortified at 0.0011, 0.001, 0.001, 0.001, and 0.001 ppm, respectively for the five sampling dates, and recoveries were 95, 97, 118, 99, and 95%, respectively. No "corrections" were applied to the data based on these recoveries.

Small Fruit. Field treatment of small green lemons ~2.5 cm in diameter with the 3 ppm avermectin B_1a solution resulted in 0-day residues of 0.020 ± 0.004 ppm on a whole fruit basis. The residue based on the treated surface area was 0.021 ± 0.003 $\mu g/cm^2$ and was about 30% higher than the 0.016 ± 0.002 $\mu g/cm^2$ obtained for the 0-day samples of the treated nearly mature lemons.

The small lemons were sampled 60 and 91 days postapplication. Pulp residues were less than 0.001 ppm and indicated that no translocation from the rind into the edible portion had occurred. "Apparent" avermectin B_1a rind residues for the 60-day samples of 0.007 ± 0.002 ppm or 0.002 ± 0.001 $\mu g/cm^2$ were identical with those for the 60-day samples of the treated nearly mature fruit. Rind residues for the 91-day samples of 0.004 ± 0.001 ppm were less than those for the 91-day samples (0.008 ± 0.001 ppm) of the treated nearly mature fruit. The overall data indicate that growth dilution of the initial residues is operative for the treated small lemon fruit.

The 0-, 60-, and 91-day samples also included untreated control and fortified control samples. Recovery for the 0-day sample fortified at 0.089 ppm was 94% based on sample combustion. Recoveries for the 60- and 91-day pulp samples fortified at 0.0009 ppm were 91 and 95%, respectively, and the corresponding rind samples fortified at 0.0075 ppm were 91 and 103%, respectively. No "corrections" have been applied to the data based on these recovery values.

"New Growth" Leaves. When the leaves on a selected lemon branch were treated by immersion of the individual leaves on the branch, care was taken to prevent contamination of the new growth leaves on the tip of the branch. To determine if any translocation had occurred, the new growth leaves were sampled 91 days postapplication. Residues found were less than 0.004 ppm (limit of detection).

Reversed Isotope Dilution Analysis. Rind. Orange and lemon rinds sampled 0, 15, 30 and 60 days postapplication were extracted with acetone and the extracts subjected to RIDA. In spite of determined efforts to avoid contamination (especially at the HPLC stage), some did occur with a few samples due to the extremely minute residue levels involved. Thus, only upper limits of actual avermectin B_1a content can be given and these are based on pre-HPLC measurements. At a specific activity of 296 000 dpm/μg of avermectin B_1a, 0.001 ppm of avermectin B_1a in 20 g of rind represents 5920 dpm at 100% recovery over the cleanup procedure. At a representative value of 20% recovery, ~1200 dpm would be expected. On this basis, values below 0.0001–0.0002 ppm cannot be quantitated reliably. Limit of detection estimates are based primarily on sample size.

On the basis of analyses of single 20-g orange rind samples, the actual levels of avermectin B_1a at 0, 15, 30, and 60 days were 0.020, 0.003, 0.0006, and 0.0004 ppm, respectively, and 0.006, 0.0004, 0.0002, and <0.0001 ppm, respectively, for lemon rind.

Pulp. Orange and lemon pulp samples collected 30 and 60 days postapplication were subjected to RIDA. None of the four samples contained detectable levels (>0.0002 ppm) of avermectin B_1a.

Leaves. Dual 2.0–3.3-g samples of lemon leaves collected 30 and 60 days postapplication were subjected to RIDA. Values obtained were 0.0012 and 0.0014 ppm for the two 30-day samples and <0.0002 and <0.0004 ppm for the two 60-day samples.

The mean 0-day (2~3 h postapplication) avermectin B_1a residues based upon total radioactivity determined by sample combustion were previously given as 0.066 ppm for orange rind and 0.038 ppm for lemon rind. Minimum

degradation or metabolism would have been expected for 0-day samples. However, the corresponding RIDA values for extractable avermectin B_1a residues were considerably lower indicating extensive and rapid metabolism and/or degradation. The RIDA values for orange and lemon rind were 0.020 and 0.006 ppm, respectively, or 30 and 16%, respectively, of the sample oxidizer values. Subsequent samples show continuing degradation of the actual avermectin B_1a present.

CONCLUSIONS

It should be noted that application was by dip and not by spray and that this was an experimental formulation not contemplated for commercial development. Thus, both the initial residues and their rates of dissipation may be different from those of other formulations delivered under normal treatment conditions. Assuming for illustration purposes an application of 1,000 gal per acre (93.5 hL/ha), the amount of avermectin B_1a applied would be 11.4 g (0.025 lb) per acre. According to these results, whole fruit residues of avermectin B_1a at harvest would be less than 0.001 ppm. In addition, translocation of avermectin B_1a from the treatment sites would be so low as to be undetectable. Because of its low application rate and short persistence, avermectin B_1a should cause minimal environmental and food contamination.

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Registry No. Avermectin B_1a, 65195-55-3.

LITERATURE CITED

Albers-Schönberg, G.; Arison, B. H.; Chabala, J. C.; Douglas, A. W.; Eskola, P.; Fisher, M. H.; Lusi, A.; Mrozik, H.; Smith, J. L.; Tolman, R. L. *J. Am. Chem. Soc.* **1981**, *103*, 4216.

Bull, D. L.; Ivie, G. W.; MacConnell, J. G.; Gruber, V. F.; Ku, C. C.; Arison, B. H.; Stevenson, J. M.; VandenHeuvel, W. J. A. *J. Agric. Food Chem.* **1984**, *32*, 94.

Burg, R. W.; Miller, B. M.; Baker, E. E.; Birnbaum, J.; Currie, S. A.; Hartman, R.; Kong, Y.-L.; Monaghan, R. L.; Olson, G.; Putter, I.; Tunac, J. B.; Wallick, H.; Stapley, E. O.; Oiwa, R.; Omura, S. *Antimicrob. Agents Chemother.* **1979**, *15*, 361.

Campbell, W. C.; Fisher, M. H.; Stapley, E. O.; Albers-Schönberg, G.; Jacob, T. A. *Science (Washington, D.C.)* **1983**, *221*, 823.

Gruber, V. F.; Jacob, T. A., unpublished data.

Gunther, F. A. *Residue Rev.* **1969**, *28*, 1.

Lofgren, C. S.; Williams, D. F. *J. Econ. Entomol.* **1982**, *75*, 798.

Maynard, M. S.; Gruber, V. F.; Ku, C. C. "Abstracts of Papers", 186th National Meeting of the American Chemical Society, Washington, D. C., Aug, 1983; American Chemical Society: Washington, D. C.; PEST 100.

McCoy, C. W.; Bullock, R. C.; Dybas, R. A. *Proc. Fla. State Hortic. Soc.* **1982**, *95*, 51.

Miller, T. W.; Chaiet, L.; Cole, D. J.; Cole, L. J.; Flor, J. E.; Goegelman, R. T.; Gullo, V. P.; Joshua, H.; Kempf, A. J.; Krellwitz, W. R.; Monaghan, R. L.; Ormond, R. E.; Wilson, K. E.; Albers-Schönberg, G.; Putter, I. *Antimicrob. Agents Chemother.* **1979**, *15*, 368.

Putter, I.; MacConnell, J. G.; Preiser, F. A.; Haidri, A. A.; Ristich, S. S.; Dybas, R. A. *Experientia* **1981**, *37*, 963.

Schuster, D. J.; Everett, P. H. *J. Econ. Entomol.* **1983**, *76*, 1170.

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Magnitude of Involvement of the Mammalian Flavin-Containing Monooxygenase in the Microsomal Oxidation of Pesticides

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The oxidation of sulfide-containing organophosphate and carbamate pesticides by the flavin-containing monooxygenase has been measured in mammalian microsomes made devoid of cytochrome P-450-dependent activity, primarily through the use of inhibitory antibodies against NADPH-cytochrome P-450 reductase. Rates of metabolism were determined for mouse, rabbit, and rat liver, lung, and kidney microsomes and for pig liver microsomes. Substrate specificity of the enzyme in different species and tissues is similar. Lung and kidney microsomes have high flavin-containing monooxygenase levels, and this enzyme is important relative to cytochrome P-450 in these tissues. Thioether-containing organophosphates are effective substrates for the flavin-containing monooxygenase in mouse liver microsomes, with K_m values between 3.5 and 36 μM. Thioether-containing carbamates are less effective substrates, having K_m values near 280 μM. Other substances oxidized include (methylthio)phenyl-containing organophosphates, certain phosphonodithioate pesticides, certain dithiocarbamate soil fumigants, ethylenethiourea, nicotine, selenourea, and diethylphenylphosphine.

INTRODUCTION

The flavin-containing monooxygenase (flavin monooxygenase), formerly known as *N,N*-dimethylaniline *N*-oxidase, EC 1.14.13.8, is an enzyme located in the endoplasmic reticulum. This enzyme and the cytochrome P-450-dependent monooxygenase system, also located in microsomes, are the major enzymes catalyzing the oxidation of lipophilic foreign compounds. Hydroxylated products produced by these enzymes have increased water solubility and serve as substrates for conjugative reactions forming glucuronide, glycoside, and sulfate derivatives which are readily excretable.

Techniques for the purification of pig liver flavin monooxygenase were developed some time ago and the capability of this solubilized enzyme to catalyze the oxidation of a wide variety of nucleophilic nitrogen- and sulfur-

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