Fate of Avermectin B_{1a} on Citrus Fruits. 1. Distribution and Magnitude of the Avermectin B_{1a} and ^{14}C Residue on Citrus Fruits from a Field Study

Michael S. Maynard,*[1] Yutaka Iwata,[2,3] Peter G. Wislocki, Chia C. Ku,[4] and Theodore A. Jacob

An 8 μg/mL solution of [^{14}C]avermectin B_{1a}, the approximate field application rate, was applied to oranges, lemons, and grapefruit; a 10-fold higher rate was also applied to oranges. Immediately postapplication, ^{14}C residues were 20–38 ng/g for the fruit treated at the field rate. Most of the residue was recovered in the surface solvent rinse at less than 2 weeks postapplication; however, after this time more of the residue was recovered from the rind fraction. The total recoveries of applied radioactivity were 61–90% and 33–50% at 1 and 12 weeks postapplication, respectively. The level of unextractable rind ^{14}C residue from oranges treated at the 10× rate and harvested at 12 weeks (a worse case) was 4.9% of the applied dose (<2 ppb at the field rate). The inner pulp samples for all treatments had ^{14}C residue levels below the detection limit of 0.4–0.8 ppb. The initial depletion half-life of avermectin B_{1a} was <1 week, with losses occurring within 30–40 min. For the 1–12-week postapplication period, the avermectin B_{1a} and ^{14}C residue depletion half-lives were 20–38 and 56–98 days, respectively. Differences in the rate of dissipation of avermectin B_{1a} due to fruit type and application rate were observed.

The avermectins, a newly discovered class of pesticidal agents, are macrocyclic lactones produced by the actinomycete *Streptomyces avermitilis* (Burg et al., 1979). The avermectin structures have been elucidated (Albers-Schonberg et al., 1981), and some of the biological activities have been reported (Campbell et al., 1983). Abamectin is the commercial product that is being developed as an acaricide/insecticide and contains avermectin B_{1a} as its major active ingredient. Abamectin (avermectin B_1 or MK-0936) consists of ≥80% avermectin B_{1a} and ≤20% avermectin B_{1b} (Figure 1). Abamectin has been shown to be very active against citrus rust mite (*Phyllocoptruta oleivora*) (McCoy et al., 1982). Abamectin has also demonstrated excellent control of other pests and is registered in the United States under the trademark AFFIRM for use against imported fire ants (Lofgren and Williams, 1982) and in the United States and several countries worldwide under the trademark AVID and VERTIMEC for use against dipterous leafminer (*Liriomyza trifolii*) and spider mites (*Tetranychus sp.*) on ornamentals.

This study investigated the fate of ^{14}C-labeled avermectin B_{1a} applied to citrus fruits in the field and was performed to support its registration for commercial use on citrus. The magnitude of residues of the parent compound was determined for the surface wash of the fruits and for the extract prepared from the rind. Total ^{14}C residue levels in these extracts, the exhaustively extracted rind matrix, and the pulps were also determined.

This study was conducted with [^{14}C]avermectin B_{1a} applied as an emulsifiable concentrate formulation containing 8 and 80 ppm of the active ingredient; oranges were treated at 8 and 80 ppm while lemons and grapefruit were treated only at 8 ppm. The use of the 8 ppm formulation simulated a field application rate of 0.025 lb of ai/500 gal per acre while the 80 ppm application was used as comparison to the field rate and for metabolite isolation. Treated and associated control fruit were sampled at 0, 1, 2, 4, 8, and 12 weeks postapplication. Field treatment, sample collection, and initial sample preparation were conducted at the University of California, Riverside. Sample combustion, liquid scintillation counting, HPLC analysis, and rind matrix extractions were performed at Merck.

Pesticide Metabolism and Environmental Safety Group, Department of Animal Drug Metabolism, Merck, Sharp and Dohme Research Laboratories, P.O. Box 450, Three Bridges, New Jersey 08887.

[1] Present address: Agricultural Division, Biochemistry Department, CIBA-GEIGY Corp., P.O. Box 18300, Greensboro, NC 27419.

[2] Former address: Department of Entomology, University of California, Riverside, CA 92521.

[3] Present address: Stauffer Chemical Co., 1200 South 47th St., Box No. 4023, Richmond, CA 94804.

[4] Deceased.

MATERIALS AND METHODS

One lemon, one grapefruit, and two navel orange trees were used for this study. All trees were fully mature and were located within the citrus groves on the University of California Citrus Research Center, Riverside, CA. Each tree had an open wooden frame with a fiberglass roof built around it to minimize exposure to precipitation. This roof structure did not block incident radiation. A polyethylene curtain, attached to the frame, was lowered to protect the treated side of the tree only during periods of rainfall and only for the first 6 weeks after application.

Treatment. Twenty-one fruits of each of the three citrus cultivars were treated with a solution containing 8 ppm of ^{14}C-labeled avermectin B_{1a}. The treatment solution contained 540 μg of [3,7,11,13,23-^{14}C]avermectin B_{1a} and 30 μL of a proprietary emulsifier in 67 mL of distilled water. The specific activity and radiochemical purity of the [^{14}C]avermectin B_{1a} were 16.4 μCi/mg and 99+%, respectively. The solution was mixed well and refrigerated overnight prior to its use. A total of 78 oranges were treated with a solution containing 80 ppm of ^{14}C-labeled avermectin B_{1a}. The solution contained 5.40 mg of [^{14}C]avermectin B_{1a} and 300 μL of proprietary emulsifier in 67 mL of distilled water. Its preparation was similar to that of the 8 ppm solution. To serve as control fruit, six fruits of each of the three citrus cultivars were treated with a solution containing only 30 μL of emulsifier in 67 mL of distilled water; six oranges were treated with a solution containing 300 μL of eulsifier in 67 mL of distilled water. A small artist's paintbrush was used to apply 0.5 mL of appropriate treatment solution to each fruit. All applications were made on Dec 7, 1982.

Sampling. At each sampling time, three oranges, lemons, and grapefruits treated with 8 ppm solution and three oranges treated with the 80 ppm solution were randomly picked and placed in separate plastic bags. Immediate

0021-8561/89/1437-0178$01.50/0 © 1989 American Chemical Society

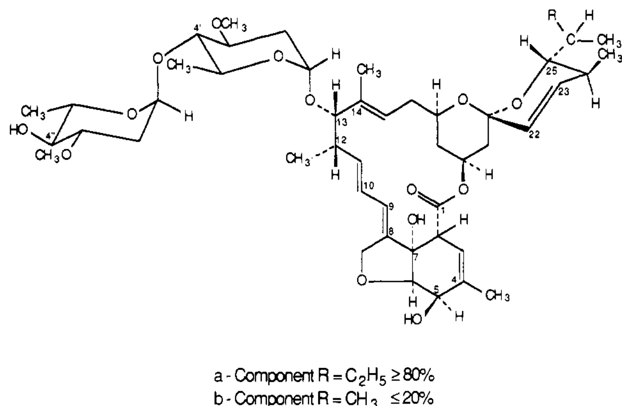

Figure 1. Structures of the two major components of abamectin.

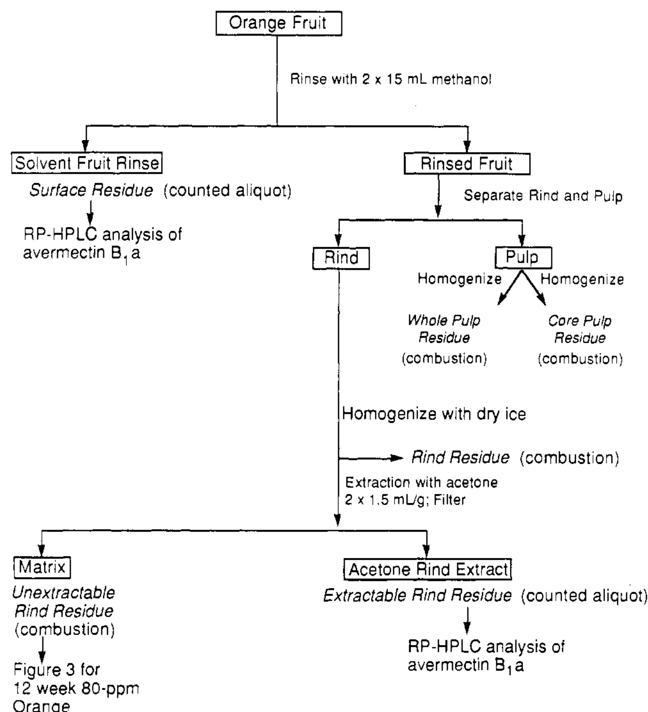

Figure 2. Orange fruit processing procedure.

postapplication samples (0 time) were collected 30–45 min after treatment and processed immediately. Additional samples were taken at 1, 2, 4, 8, and 12 weeks after application. For the 80 ppm treatment, an additional 15 oranges were sampled at 2, 4, 8, and 12 weeks and processed as a group separate from the other three 80 ppm treated oranges.

Processing. The sets of 3 and 15 fruits at each sampling time were processed as 3- and 15-fruit composite samples. This procedure is outlined in Figure 2. Briefly, each fruit of the set was rinsed twice with 15 mL of methanol, and aliquots of the two rinses were taken for scintillation counting. The methanol fruit rinses are considered to be the surface residues. The rind (peel) was separated from the pulp (edible portion), and the weight of each composite sample was determined. The rind was homogenized with dry ice, and 2-g subsamples were removed for combustion and scintillation counting. The remaining homogenized rind sample was extracted twice with acetone (1.5 mL/g of rind); each macerate was filtered to separate the spent rind (matrix) and the acetone extract. Samples of the matrix were taken for combustion and scintillation counting, and samples of the acetone extract were taken for scintillation counting. The pulp residue was determined as a whole-pulp and as an inner-pulp core

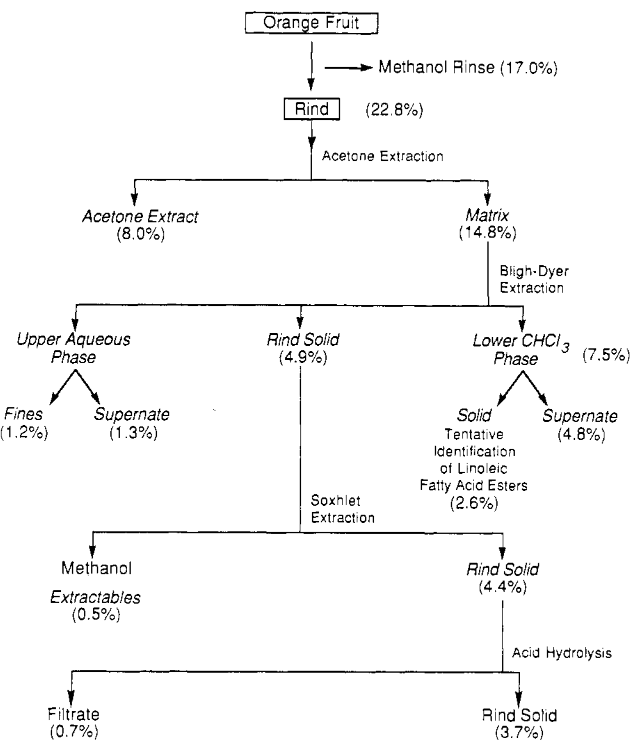

Figure 3. Processing procedure and distribution of the total ^{14}C residue in the 12-week 80 ppm treated oranges. Values in parentheses are the total ^{14}C residues recovered in the fraction as a percentage of the total ^{14}C residue for the zero time fruit.

sample. The inner-pulp sample was obtained by excising a transverse core section from each whole pulp by using a 23-mm-i.d. cork borer; a 1–3-mm portion was removed from each end of the core with a razor blade. The composite core sample was homogenized, and subsamples were taken for combustion and scintillation counting. The whole-pulp sample consisted of the remainder of the fruit after removal of the core samples. The whole-pulp sample was homogenized, and subsamples were taken for combustion and scintillation counting.

A more extensive processing of the rind fraction was conducted with the 12-week, 80 ppm treated oranges. The rind matrix after acetone extraction was processed further as outlined in Figure 3. A 40-g portion of the matrix was extracted by using the Bligh–Dyer extraction method (Bligh and Dyer, 1959). Five successive extractions, each consisting of 200 mL of CH_3OH, 180 mL of H_2O, and 200 mL of $CHCl_3$, were conducted and the layers separated. Two additional 300-mL CH_3OH extractions were performed on the spent solid and combined with the upper aqueous layer. Aliquots from each aqueous and $CHCl_3$ layer and the exhaustively extracted matrix were counted for radioactivity. The aqueous layer contained fine particulate material that was removed by filtration through a 0.2-μm Nalgene filter. Aliquots of the filtrate were taken for scintillation counting.

The $CHCl_3$ layer from the Bligh–Dyer extraction was processed by taking a sample of the lower layer from the first extraction and removing the solvent under a stream of nitrogen at ambient temperature. The residue was washed with methanol/tetrahydrofuran (THF) (75/25), and the remaining residue was solubilized in $CHCl_3$. Upon addition of CH_3OH, a white precipitate formed that was separated and washed twice with CH_3OH. The solid was then subjected to NMR and mass spectrometric analyses.

The spent solid from the Bligh–Dyer extraction was further investigated for unextractable residues. A 1.5-g subsample of the Bligh–Dyer spent solid was extracted

with methanol by Soxhlet extraction for 74 cycles. The radioactivity in the methanol extract was counted. The extracted solid was subjected to hydrochloric acid hydrolysis (50 mL, pH 1.3, stirring for 25 h at ambient temperature). The solid was removed by filtration, and the radioactivity in the filtrate was counted.

Avermectin B_{1a} Determination. To determine the level of avermectin B_{1a}, the methanol fruit rinses and the acetone rind extracts were analyzed by RP-HPLC. The amount of radioactivity eluting as a definitive peak at the retention time of an avermectin B_{1a} standard was determined and expressed as a percent of the total radioactivity eluting from the LC column. This value represents the percent of avermectin B_{1a} in the surface rinse or the rind extract. The fraction of avermectin B_{1a} in the extract multiplied times the concentration of the ^{14}C residue in the extract, expressed as nanogram equivalents per gram of whole fruit, gave the nanograms of avermectin B_{1a} per gram of whole fruit (ppb). The levels of avermectin B_{1a} from the methanol fruit rinse and the corresponding acetone rind extract were summed to yield a value for the total extractable avermectin B_{1a}. The radioactivity eluting through the LC column was 85–97% of that present in the various extracts analyzed. Duplicate injections of a sample gave reproducible percent values for avermectin B_{1a}. To confirm that the radioactivity eluting at the retention time of avermectin B_{1a} was indeed avermectin B_{1a}, the avermectin B_{1a} peak from one sample was collected and injected on a different RP-HPLC system. The radioactivity attributed to avermectin B_{1a} from the first LC system eluted at the retention time of an avermectin B_{1a} standard on the second system. The RP-HPLC conditions used for the determination of the level of avermectin B_{1a} in the fruit extracts are described below. A 100% CH_3OH column wash was collected after the isocratic run for accurate recovery determination.

Instrumentation. *High-Performance Liquid Chromatography* (*HPLC*). A laboratory data control system was used with a 4.6 mm × 250 mm DuPont Zorbax ODS column and a precolumn containing Whatman Pell ODS packing. The mobile phase was 85/15 (v/v) CH_3OH/H_2O, and the flow rate was 1.0 mL/min. The column effluent was monitored at 245 nm, and 1-min fractions were collected and counted for radioactivity.

Liquid Scintillation Counting (*LSC*). A Tri-Carb 460 or 4530 (Packard, United Technologies, Inc.) with a dpm converter was used. Samples were counted in Insta-gel (Packard, United Technologies, Inc.). Appropriate quench curves were determined with standards.

Sample Combustion. A Tri-Carb B-306 sample oxidizer (Packard, United Technologies, Inc.) was used for sample combustion. Carbon-14 was collected as $^{14}CO_2$ in 8 mL of Carbosorb and 13 mL of Permafluoro V (Packard, United Technologies, Inc.). Combustion efficiency was determined each time and was greater than 96; dpm values were determined by liquid scintillation counting by using appropriate quench curves.

Materials. The ^{14}C labeled avermectin B_{1a} was prepared as previously described (Ku et al., 1985). The emulsifier was supplied internally by the Agricultural Formulations Group. All other chemicals used were reagent grade or better, and solvents were HPLC grade or better. All materials were purchased from commercial suppliers.

RESULTS

^{14}C Residue Levels. The total ^{14}C residues in the various fruit fractions were expressed as nanograms of avermectin B_{1a} equivalents per gram of whole fruit (ppb) (Table I). The residue levels were presented in these units

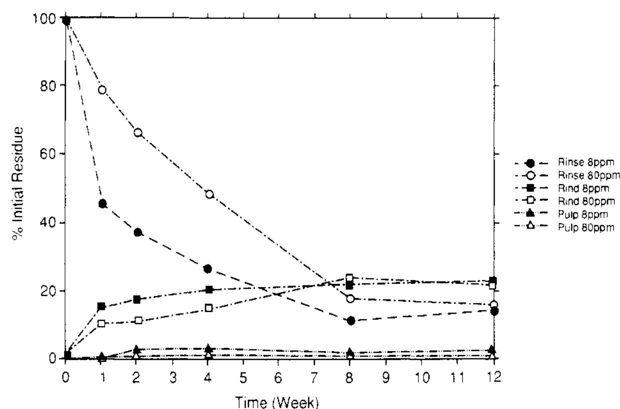

Figure 4. Distribution of the total residue in the fruit fractions for oranges.

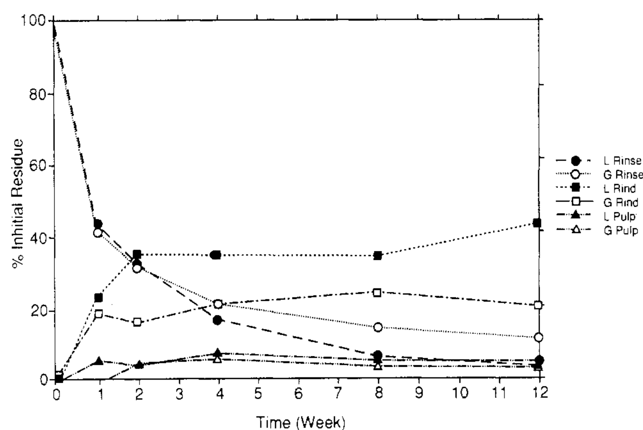

Figure 5. Distribution of the total residue in the fruit fractions for lemons and grapefruit.

for comparison purposes. Actual rind and pulp residue levels (ppb) are higher based on the weights of actual fraction. The initial residues (zero time) were 20–38 and 203 ppb for the 1×- and 10×-treated fruits, respectively. A decrease in the total ^{14}C residues with time was observed; the largest decrease (10–40%) occurred during the first week postapplication for all fruits. Excluding the first week and assuming first-order kinetics, the dissipation half-lives for the total ^{14}C residue were between 56–98 days (Table IV). At the field rate, the residue dissipation half-lives were observed to follow the order lemon (56 days) < grapefruit (84 days) < orange (98 days). The half-life of total ^{14}C residues from 1 to 12 weeks postapplication on oranges treated at the 10× rate was less than at the 1× rate (59 vs 98 days).

^{14}C Residue Distribution. The distributions of the ^{14}C residues, expressed as a percentage of the total initial ^{14}C residue, as a function of time postapplication are shown in Figures 4 and 5. The fruit surface ^{14}C residue, as determined from the analyses of surface rinses, decreased rapidly within the first week postapplication and more slowly thereafter. Concurrently, the ^{14}C rind residue, as determined from the combustion of the rinds, increased rapidly within the first week and more slowly thereafter. The rind residues attained near maximal levels after 2 weeks, while the fruit surface residues did not attain near minimal levels until 8 weeks (Figures 4 and 5).

During the initial 8 weeks, the decrease in the fruit surface residues and the increase in the rind residues were observed to be slower for the 10× than for the 1× application rate (Figure 4). After 8 weeks, both the fruit surface and rind residues were similar for the 1× and 10× application rates when expressed as a percent of the corresponding total initial ^{14}C residue.

Table I. Distribution of the Total ^{14}C Residue in the Various Fruit Fractions Expressed as Nanograms of Avermectin B_{1a} Equivalents per Gram of Whole Fruit[a]

fruit/treatment	time, weeks	fruit surface	rind[b]	whole pulp[c]	total
orange/8 ppm	0	37.4	0.5	<DL	37.9
	1	17.4	5.8	<DL	23.2
	2	14.3	6.7	1.3	22.2
	4	10.2	8.0	1.4	19.6
	8	4.7	8.7	1.1	14.6
	12	6.0	9.2	1.4	16.6
lemon/8 ppm	0	28.9	0.6	<DL	29.5
	1	12.8	6.8	1.8	21.4
	2	9.6	10.4	1.3	21.3
	4	5.0	10.1	2.3	17.5
	8	1.8	9.9	1.6	13.3
	12	1.0	12.4	1.4	14.7
grapefruit/8 ppm	0	20.0	0.3	<DL	20.3
	1	8.4	3.9	<DL	12.3
	2	6.4	3.4	1.0	10.7
	4	4.3	4.3	1.2	9.8
	8	2.9	4.8	0.7	8.4
	12	2.2	4.0	0.6	6.8
orange/80 ppm	0	200.1	2.8	<DL	202.9
	1	159.3	21.5	1.6	182.6
	2	134.9	23.5	2.0	160.3
	4	99.4	31.0	4.1	134.5
	8	38.1	48.7	4.7	91.5
	12	34.7	46.3	3.7	84.4

[a] Total radioactivity in each fraction was measured. Values are the mean of triplicate measurements. [b] Actual rind levels are shown in Table II. [c] The core pulp sample (i.e., pulp with ring-pulp interface carefully removed) for all fruits and application rates were below the detection limit (0.4–0.8 ppb). Actual pulp levels are approximately 1.2 times higher based on pulp weight. DL = detection limit.

Rind. After the whole fruit was washed with methanol to remove surface residues, the rind was removed and extracted with acetone. The percentages of extractable and unextractable ^{14}C residues were determined. The results for this extraction as well as the actual residue concentration in the rind and matrix are given in Table II. For all fruit, 63–72% and 32–39% of the rind residue were extractable for the 1- and 12-week samples, respectively. The unextractable rind residues were 28–37% and 61–68% of the total rind residue for 1- and 12-week samples, respectively (Table II). At the field application rate, the amount of rind residue that was unextractable by this procedure was between 35–38 and 70–92 ppb for 1 and 12 weeks postapplication, respectively. Expressed as nanograms of avermectin B_{1a} equivalents per gram of whole fruit, the unextractable rind residue was between 1.4–2.4 and 2.7–7.9 ppb for 1- and 12-weeks postapplication, respectively.

The rind remaining after acetone extraction (matrix) from the 10×-treated oranges harvested at 12 weeks postapplication was processed as shown in Figure 3. The values in parentheses are the total ^{14}C residues in that fraction as a percent of the total initial ^{14}C residue. After the Bligh–Dyer and Soxhlet extractions and the acid hydrolysis of the rind matrix, only 3.7% of the total initial ^{14}C residue remained associated with the rind solid. This procedure removed 75% of the residue found in the rind matrix. This value does not include the fines found in the upper aqueous layer containing 1.2% of the initial residue. Including the fines (1.2%) with the solid (3.7%), this procedure then removes 67% of the residue found in the rind matrix. With the value of 4.9% of the total initial ^{14}C residue, the amount of unextractable rind residue for the 12-week, 10×-treated oranges was 9.9 ppb whole fruit concentration. With the 4.9% value for the 1×-treated fruit, the amounts of unextractable rind residue at all time periods postapplication would be less than 2 ppb whole fruit concentration or less than 31 ppb actual concentration.

Table II. Acetone Extraction of the Rind ^{14}C Residue from Citrus Fruit[a]

fruit (rate)	time, weeks	rind, ppb	matrix, ppb	acetone extractable
orange (8 ppm)	0	2.2	1.9 (18)	(82)
	1	20.3	34.5 (34)	(66)
	2	28.0	52.7 (49)	(51)
	4	32.4	82.4 (48)	(52)
	8	35.6	81.5 (48)	(52)
	12	28.5	92.4 (61)	(39)
lemon (8 ppm)	0	<1.5	1.1 (13)	(87)
	1	16.8	38.3 (36)	(64)
	2	31.7	74.0 (47)	(53)
	4	21.2	65.1 (57)	(43)
	8	23.1	89.1 (60)	(40)
	12	14.5	73.9 (63)	(37)
grapefruit (8 ppm)	0	1.6	<1.2 (0)	(100)
	1	17.2	37.8 (37)	(63)
	2	15.3	36.8 (47)	(53)
	4	23.4	59.6 (49)	(51)
	8	20.8	79.3 (61)	(39)
	12	18.4	69.5 (68)	(32)
orange (80 ppm)	0	10.7	11.0 (18)	(82)
	1	85.9	122.2 (28)	(72)
	2	100.0	140.7 (41)	(59)
	4	137.7	284.3 (42)	(58)
	8	207.0	505.3 (47)	(53)
	12	171.9	650.3 (65)	(35)

[a] Values reported are the actual nanograms of avermectin B_{1a} equivalents per gram of rind or matrix (ppb). Values in parentheses are the percents of radiolabeled ^{14}C residue in the rind that was extracted by acetone or remained with the matrix fraction. Values are the means of triplicate measurement.

Table III. Levels of Avermectin B_{1a} in the Total Extractable Residue[a]

time, weeks	8 ppm treated orange, ppb	8 ppm treated lemon, ppb	8 ppm treated grapefruit, ppb
0	29.35 (99.7)	23.45 (100.0)	16.69 (100.1)
1	3.26 (91.3)	0.80 (80.5)	0.50 (88.4)
2	1.67 (79.6)	0.60 (71.2)	0.24 (76.0)
4	1.47 (73.3)	0.23 (53.7)	0.13 (66.0)
8	1.08 (63.2)	0.09 (43.2)	0.08 (56.8)
12	0.59 (57.7)	0.06 (37.5)	0.04 (51.6)

[a] Values reported are the total amount of avermectin B_{1a} determined by HPLC analysis of the methanol fruit rinses and acetone rind extracts and expressed as nanograms of avermectin B_{1a} per gram of whole fruit. Values are a single measurement of the composite sample representing three fruits. Values in parentheses are the sum of ^{14}C residues in the methanol rinse and acetone rind extract as a percentage of the total ^{14}C residue.

While the Bligh–Dyer $CHCl_3$ lower phase was being processed (Figure 3), a white solid was formed that contained radioactivity. This solid was partially purified by washing with methanol and reprecipitation. The NMR and mass spectrometric data tentatively identified this material as a mixture of linoleic fatty acid esters. Further characterization was not possible due to the limited amount of material.

Extractable Residue. The total extractable ^{14}C residue from these fruits is defined as the sum of the ^{14}C residue recovered in the surface rinse and the acetone rind extract. The levels of the extractable residue as a percent of the total radiocarbon residue present for the 1×-treated fruit are shown in Table III. At 1 week, between 81 and 91% of the total residue was extractable. These values decreased with time postapplication to 38–58% at 12 weeks. Most of the residue was extractable except for the 8- and 12-week lemon samples.

Table IV. Depletion Half-Life (Days) of Total Residue and Avermectin B_{1a}

fruit/treatment	total residue	avermectin B_{1a} surface residue	avermectin B_{1a} total extractable
orange/8 ppm, 1–12 weeks	98 (0.901)[a]	31 (0.930)	38 (0.945)
lemon/8 ppm, 1–12 weeks	56 (0.973)	15 (0.980)	20 (0.968)
grapefruit/8 ppm, 1–12 weeks	84 (0.964)	23 (0.948)	20 (0.944)
orange/80 ppm, 1–12 weeks	59 (0.956)	20 (0.964)	nd

[a] Correlation coefficient.

The amount of avermectin B_{1a} in the total extractable residue is the sum of the avermectin B_{1a} measured in the surface rinse and in the acetone rind extract for each sample (Table III). At zero time (30–45 min), the levels of avermectin B_{1a} were 17–29 ng/g (Table III) while the total residue was 20–38 ng/g (Table I) for the 1×-treated fruit. This indicates a rapid breakdown of avermectin B_{1a} during this dry down period. Within the first week after application, the amount of avermectin B_{1a} was shown to decrease by 97, 97, and 89% on lemons, grapefruits, and oranges, respectively. After 1 week, a more gradual decline in the avermectin B_{1a} levels was observed. The half-lives of avermectin B_{1a} in the total extractable ^{14}C residues after 1 week were between 20 and 38 days (Table IV). The levels of avermectin B_{1a} after 1 week, as measured by its level in the total extractable residue, were <1 ppb on lemons and grapefruits and <1–3 ppb whole fruit concentration on oranges. The half-lives of avermectin B_{1a} in the total extractable ^{14}C residues were the same or greater than in the corresponding fruit surface ^{14}C residues (20–38 vs 15–31 days). Also, the half-life of avermectin B_{1a} in the fruit surface was shorter for the 10×- than for the 1×-treated oranges (20 vs 31 days).

Pulp. The ^{14}C residues in the pulp (edible portion) were investigated by sampling an inner-pulp fraction from which the rind/pulp interface was removed and a whole-pulp fraction lacking the inner pulp plug (see Materials and Methods and Table I). The effect of removal of the inner-pulp plug on the results for the whole-pulp fraction should be minimal. The ^{14}C residues in the inner-pulp fractions were less than the detection limit (DL) of <0.4–0.8 ppb for all samples including the oranges treated at the 10× rate. At all time points the whole-pulp fractions contained ^{14}C residues between <0.6–1.5 (DL) to 2.3 ppb and <1.5 (DL) to 4.7 ppb whole fruit concentration for the 1× and 10× application rates, respectively (Table I). Actual whole pulp residue levels for the 1×-treated fruit are between 0.7–1.8 (DL) and 2.8 ppb.

DISCUSSION

Total ^{14}C Residue. The immediate postapplication radiocarbon residue levels for oranges, grapefruit, and lemons were 20–38 ppb at the field application rate. These values were very similar to values from a previous field study (Iwata et al., 1985) and picked fruit studies (Maynard et al., 1989) at the approximate field application rate.

The dissipation profile of the total radiocarbon residue was biphasic with a rapid loss within the first week, which was the first time point after the zero time sampling, and slower thereafter. The estimated dissipation half-life of the first rapid phase was less than 1 day. The dissipation half-life of the second slower phase was determined to be 56–98 days. The persistance of the ^{14}C residue on the fruit followed the order lemon < grapefruit < orange. A biphasic dissipation of the total residue was previously reported on citrus fruits for avermectin B_{1a} (Iwata et al., 1985) and other pesticides. Because the fruits were protected from rain and the vapor pressure of avermectin B_{1a} is <10^{-8} Torr, rainfall and volatilization of avermectin B_{1a} were not factors in the ^{14}C residue dissipation.

Surface ^{14}C Residue. The initial residue was observed to rapidly migrate from the fruit surface into the rind for all fruits. The surface rinse (surface residue) depletion pattern was similar to the total radiocarbon residue depletion pattern; i.e., both exhibited a rapid decrease within the first week and decreased slower thereafter. The loss in the total radiocarbon residue was proportional to the loss of the fruit surface residues, which was only partially attributed to the residue partitioning into the rind. Therefore, the dissipation of the total radiocarbon residues was occurring from the fruit surface. In general for the three fruit cultivars treated at the field application rate, the distribution patterns of the ^{14}C residue between the surface and the rind were similar; differences were observed in the rate of distribution. The rate of distribution followed the order orange < grapefruit < lemon.

Rind ^{14}C Residue. The ^{14}C rind residues attained near maximal levels within the first 1–2 weeks postapplication. Extraction of the methanol-rinsed rind with acetone removed most of the rind residue for fruits harvested at the early sampling periods. The extractability of the residues in the rind was shown to decrease with time after application. The ^{14}C residue remaining in the rind after acetone extraction was more extensively removed by the Bligh–Dyer and Soxhlet extraction and the acid hydrolysis procedure. The 10×-treated orange samples harvested 12 weeks postapplication were used for this procedure and represent a worse-case situation. After exhaustive extraction, at least 67% of the ^{14}C residue in the rind matrix was removed. At the field application rate, 67% extraction of the rind matrix residue would translate to unextractable rind residues of less than 2 ppb whole-fruit concentration (Table I) or 11–31 ppb actual concentration (Table II). Furthermore from these extractions, it was indicated that incorporation of radioactivity into linoleic fatty acid esters may have occurred. Moreover, incorporation of radioactivity into linoleic and palmitic fatty acids was shown in cotton seeds from plants treated with [^{14}C]avermectn B_{1a} (Ku, C. C., unpublished data). Therefore, in addition to low remaining levels of unextractable rind residues, the ^{14}C residue present may represent ^{14}C-labeled fragments recycled into natural constituents in the rind.

Pulp Residue. Two types of pulp samples were analyzed. One sample was essentially peeled edible fruit, and the other was a pulp sample with the rind–pulp interface carefully removed. The innerface-free pulp fraction did not contain detectable residues (<0.40–<0.8 ppb) even at the 10× application rate. Because the whole edible pulp fraction contained residues (1–2 ppb whole fruit concentration), it is concluded that the trace residues were located at the pulp to rind interface. The origin of these trace residues may be due to unavoidable contamination that occurred during the fruit peeling process or movement of the residue from the rind into the rind/pulp interface.

Avermectin B_{1a}. The total extractable ^{14}C residue was analyzed by HPLC to determine the level of avermectin B_{1a}. Most of the total radiocarbon residue was solvent extractable for samples collected within the first 4 weeks postapplication. Within the first 30–45 min (zero time), a rapid degradation of avermectin B_{1a} had occurred since only 77–82% of the total residue was measured as avermectin B_{1a}. This rapid degradation continued since at 1 week postapplication avermectin B_{1a} was only 3–11% of the total residue or 0.5–3.0 ppb. Therefore, the initial degradation half-life of avermectin B_{1a} was less than 1 week

on the basis of data presented. The real initial half-life is probably on the order of days since on cotton leaves the initial half-life was less than 1 day (Bull et al., 1984). The levels of avermectin B_{1a} remained higher on oranges than on lemons and grapefruit. The levels of avermectin B_{1a} found in this study at 1 and 2 weeks postapplication were very similar to levels reported previously in a field study (Iwata et al., 1985) and in a picked-fruit study (Maynard et al., 1989). After 1 week, the dissipation of avermectin B_{1a} was slower than during the first week. This biphasic dissipation phenomenon was previously observed for avermectin B_{1a} on citrus fruits (Iwata et al., 1985; Jenkins, J. J., personal communication; Maynard et al., 1989).

The persistence half-lives of avermectin B_{1a} on citrus fruits from 1 to 12 weeks postapplication were 20–38 days and observed in the following order: lemon < grapefruit < orange. The relationship between fruit types or application rate on the persistence half-life of avermectin B_{1a} was the same as on the persistence half-life of the total radiocarbon residue. In all cases, the half-life for avermectin B_{1a} was shorter than the corresponding half-life for the total radiocarbon residue. Therefore, it appeared that the degradation products(s) of avermectin B_{1a} was responsible for the loss in the total radiocarbon residue. As mentioned above, other factors such as removal by rain and volatilization of avermectin B_{1a} were not responsible for these losses. It therefore appears that the degradation of avermectin B_{1a} resulted in the eventual formation of volatile degradents.

Avermectin B_{1a} was shown to degrade in the presence of sunlight in an aqueous solution and on soil with a half-life of approximately 18 h (Ku et al., 1983). On picked orange fruit, the degradation of avermectin B_{1a} was more extensive in 1 week on sunlight-exposed than sunlight-protected fruit (Maynard et al., 1983). Avermectin B_{1a} when applied as a film to glass Petri dishes was shown to degrade faster in the presence of a sunlamp than in the dark ($t_{1/2}$ = 5 h vs 2 days) (MacConnell and Demchak, personal communication). Furthermore from another experiment, [^{14}C]avermectin B_{1a} applied as a thin film on glass Petri dishes and exposed to a sunlamp resulted in a large loss (55%) of the applied radioactivity in 6 days. This loss occurred mostly after the levels of avermectin B_{1a} were less than 2% of the total residue achieved in less than 1 day (Maynard and Gruber, unpublished data). Avermectin B_{1a} has been observed to degrade and form volatile components in the absence of light; however, this degradation is much less (days vs hours) than in the presence of sunlight or a sunlamp.

Therefore, the degradation of avermectin B_{1a} appears to be enhanced by sunlight and the degradates eventually form products of greater volatility than the parent compound. Given no other mechanism of loss of the applied radiocarbon, the loss of radiocarbon resulting from the formation of volatile degradates by photolysis is the only explanation consistent with the results obtained in this study. Furthermore, because the half-life of avermectin B_{1a} was shorter on the fruit surface than in the total extractable ^{14}C residue, which includes the extractable rind residue, the migration into the rind provides protection from degradation of avermectin B_{1a}. This phenomenon may provide an explanation for the long residual acaricidal activity observed on citrus fruits in the field (McCoy et al., 1982).

In conclusion, the total ^{14}C residue levels are initially very low (20–37 ppb) and decrease to 33–50% after 12 weeks postapplication. Avermectin B_{1a} degrades very rapidly under normal environmental conditions following application and decreases to 3–11% of the total applied ^{14}C residue after 1 week. The pulp and unextractable rind ^{14}C residues are very low (2 ppb or less) at all time periods postapplication. These results indicate that residue exposure from abamectin applied to citrus fruits should be minimal for agricultural workers and consumers.

Registry No. Avermectin B_{1a}, 65195-55-3.

LITERATURE CITED

Albers-Schonberg, 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. Avermectins: Structure Determination. *J. Am. Chem. Soc.* **1981**, *103*, 4216.

Bull, D. L.; Gruber, V. F.; Ivie, G. W.; Ku, C. C.; MacConnell, J. G.; Stevenson, J. M.; VandenHeuval, W. J. A. Fate of Avermectin B_{1a} in Soil and Plants. *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. Avermectin, a New Family of Potent Anthelmintic Agents; Producing Organism and Fermentation. *Antimicrob. Agents Chemother.* **1979**, *15*, 361.

Campbell, W. C.; Fisher, M. H.; Stapley, E. O.; Albers-Schonberg, G.; Jacob, T. A. Ivermectin: a Potent new Antiparasitic Agent. *Science (Washington, D.C.)* **1983**, *221*, 823.

Iwata, Y.; MacConnell, J. G.; Flor, J. E.; Putter, I.; Dinoff, T. M. Residues of Avermectin B_{1a} on and in Citrus Fruits and Foliage. *J. Agric. Food Chem.* **1985**, *33*, 467.

Ku, C. C.; Jacob, T. A.; Maynard, M. S.; Scimone, A. Photodegradation of Avermectin B_{1a}. *Abstracts of Papers*, 185th National Meeting of the American Chemical Society, St. Louis, MO; American Chemical Society: Washington, DC, 1983; paper PEST 79.

Ku, C. C.; Hwang, S. C.; Kaplan, L.; Nallin, M. K.; Jacob, T. A. The Preparation of Carbon-14 Labeled Avermectin B_{1a}. *J. Label Compound Radiopharm.* **1985**, *22*, 451.

Lofgren, C. S.; Williams, D. F. Avermectin B_{1a}: Highly Potent Inhibitor of Reproduction by Queens of the Red Imported Fire Ant (Hymenoptera: formicidae). *J. Econ. Entomol.* **1982**, *75*, 798.

Maynard, M. S.; Gruber, V. F.; Ku, C. C. Distribution and Degradation of Avermectin B_{1a} on Citrus Fruits. *Abstracts of Papers*, 186th National Meeting of the American Chemical Society, Washington, DC; American Chemical Society: Washington, DC, 1983; paper PEST 100.

Maynard, M. S.; Halley, B.; Green, M.; Hwang, S. C.; Alvaro, R.; Bennett, B. Disposition and Metabolism of Avermectin B_{1a} in Rats. *Abstracts of Papers*, 190th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, DC, 1985a; paper AGRO 59.

Maynard, M. S.; Gruber, V. G.; Ku, C. C.; Wislocki, P. G.; Jacob, T. A. Disposition and Metabolism of Avermectin B_{1a} in Lactating Goats. *Abstracts of Papers*, 190th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, DC, 1985b; paper AGRO 60.

Maynard, M. S.; Ku, C. C.; Jacob, T. A. Fate of Avermectin B_{1a} on Citrus Fruits. 2. Distribution and Magnitude of the Avermectin B_{1a} and ^{14}C Residue on Fruits from a Picked Fruit Study. *J. Agric. Food Chem.* **1989**, following paper in this issue.

McCoy, C. W.; Bullock, R. C.; Dybas, R. A. Avermectin B1: a Novel Miticide against Citrus Mites in Florida. *Proc. Fla. State Hortic. Soc.* **1982**, *95*, 51.

Putter, I.; MacConnell, J. G.; Preiser, F. A.; Haidri, A. A.; Ristich, S. S.; Dybas, R. A. Avermectins: Novel Insecticides, Acaricides, and Nematicides from a Soil Microorganism, Streptomyces avermitilis. *Experientia* **1981**, *37*, 963.

Received for review December 24, 1987. Accepted May 12, 1988.