Nontoxicity of Polar Abamectin Degradates from Citrus Fruit and Thin Film Photolysis

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After application to crops, abamectin (avermectin B_{1a} plus avermectin B_{1b}) degrades largely to a polar residue of unknown identity. Such polar degradates are also formed after photolysis of abamectin as a thin film on glass for approximately 1 day. To establish the safety of these residues, polar abamectin degradates were isolated from oranges treated with abamectin in the field and after photolysis of thin films on glass. These degradates were tested and found to have no adverse maternal or developmental effects in the CF_1 mouse, the laboratory species/strain most sensitive to abamectin toxicity. In addition, the degradates from photolysis of thin films were negative in the microbial mutagenesis assay.

INTRODUCTION

The avermectins are a class of macrocyclic lactones produced by the soil actinomycete *Streptomyces avermitilis*. They have been developed as potent, wide-spectrum agents against numerous endo- and ectoparasites in animals and man as well as against many destructive crop pests. The structural characterization (Davies and Green, 1986), mode of action, broad-spectrum efficacy in target species (Putter et al., 1981; Lasota and Dybas, 1991), and mammalian toxicity (Lankas and Gordon, 1989) of the avermectins have been reviewed.

Avermectin B_1 (B1) or abamectin (Figure 1), which consists of not less than 80% of the avermectin B_{1a} (B1A) homologue and not more than 20% of the avermectin B_{1b} (B1B) homologue, is currently experiencing rapid worldwide growth in usage as a miticide/insecticide for a number of crops. Abamectin is rapidly degraded, with partial loss of total residues, on crops such as cotton (Bull et al., 1984), citrus (Iwata et al., 1985; Maynard et al., 1989), and celery (Moye et al., 1990).

The identification of abamectin residue components in crops to date has been limited to B1A and its 8,9-*Z* photoisomer (Bull et al., 1984; Iwata et al., 1985; Maynard et al., 1989; Moye et al., 1990); the majority of abamectin residue components, however, have not been identified. Most of these unknown abamectin residues in crops are quite polar relative to B1A when assayed by reverse-phase HPLC (Moye et al., 1990; Crouch, unpublished results). In addition, avermectin B_{1a} is entirely degraded to polar residues after photolysis of thin films for 1 day or longer (Crouch et al., 1991). Since these unknown polar abamectin degradates in crops have not been identified, the present studies describe the isolation and toxicological testing of these residues en masse from citrus. In addition, polar abamectin degradates formed by photolysis of thin films on glass were isolated and subjected to toxicological investigations for comparison to field-generated material.

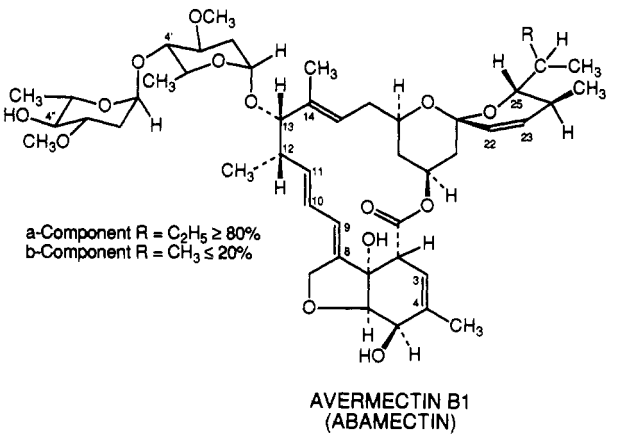

Figure 1. Structure of abamectin.

MATERIALS AND METHODS

Chemicals. Technical grade abamectin (MK-936, approximately 82% B1A and 13% B1B, remainder minor avermectins and other impurities) was obtained at Merck Sharp & Dohme Research Laboratories (Rahway, NJ). Radiolabeled B1A ([^{14}C]-B1A, labeled at a single position per molecule at carbon 3, 7, 11, 13, or 23; specific activity, approximately 13 μCi/mg; radiochemical purity greater than 98%) was provided by the Labeled Compound Synthesis Group, Department of Animal and Exploratory Drug Metabolism, Merck Sharp & Dohme Research Laboratories. The [^{14}C]B1A was used undiluted or was diluted with B1A to a specific activity of 0.5 μCi/mg of B1A for the citrus study and was diluted with the technical grade abamectin to a specific activity of 3.6 nCi/mg of B1A for thin film photolysis. All solvents used were HPLC grade as supplied by EM Science (Cherry Hill, NJ).

Preparation of Polar Abamectin Degradates (PAD) from Citrus Fruit Residues. *Treatment of Trees and Fruit.* Orange trees bearing nearly mature Hamlin oranges were sprayed (Clermont, FL; November) with abamectin in a proprietary agricultural formulation diluted to 180 μg/g in water (30*X* usage rate) using an FMC Model 757 airblast sprayer as for normal agricultural practice. Sufficient trees to provide approximately 10 000 oranges were sprayed to the point of runoff with the diluted abamectin formulation; trees sufficient to provide a similar number of control oranges were treated with a blank formulation

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(containing no abamectin) diluted with water as for the abamectin treatment. Oranges were harvested 2 weeks postapplication and were stored in a refrigerated trailer (approximately 4 °C) for 7–10 days until processing. A companion study with [^{14}C]B1A was performed concurrent with the field study at a nearby location (Lake Alfred, FL) to estimate the PAD residue levels in the abamectin-treated fruit and for processing-methods development. Six trees bearing nearly mature Hamlin oranges were selected, and individual oranges were treated with [^{14}C]-B1A in a proprietary agricultural formulation by application of approximately 0.5 mL of an aqueous dilution containing 180 μg/g (30*X* usage rate) [^{14}C]B1A with a small brush to the point of runoff. The oranges were harvested at 2 weeks postapplication and were stored refrigerated for 2–14 days until processing.

Extraction of Avermectin Residues from Fruit. Residues were removed from the surface of oranges treated with abamectin using a specialized citrus rinsing apparatus (Omni Systems, Inc., Frenchtown, NJ). Briefly, the apparatus consisted of an inclined stainless steel trough fitted with an enclosed gutter and six spray nozzles through which methanol could be delivered at a controlled rate. Oranges were gravity-fed down the trough at a constant rate so that the volume of methanol per orange could be calculated. The methanol rinse was collected into glass bottles via a drain located at the bottom of the gutter and filtered through paper towels. The optimal conditions for removal of residues from the surface of oranges were previously determined using fruit treated with [^{14}C]B1A: approximately 27 mL of methanol per orange repeated twice for a total of three rinses per orange was required. Abamectin and vehicle-treated oranges were processed in groups of approximately 1000 fruit; the filtered methanol rinse was recycled two times before replacement with fresh solvent. The methanol rinses from abamectin- and vehicle-treated fruit (approximately 300 L total each from 10 000 fruit) were filtered again through filter paper and then concentrated to about 2 L under reduced pressure at 35 °C using a process-scale rotary evaporator (Buchi) fitted with a 20-L flask; the concentrated methanol rinses from the vehicle-treated fruit and abamectin-treated fruit were stored in a freezer (−20 °C) until further processing. Some of the oranges treated with [^{14}C]B1A were also processed by hand rinsing with methanol followed by extraction of the peel. These rinses were then stored in a freezer and were used for development of an isolation procedure for PAD (see below). Residues remaining in the peel of [^{14}C]B1A-treated oranges after the hand methanol surface rinsing were extracted with acetone in a blender after frozen peel fragments were powdered in a blender with dry ice. All ^{14}C residues were quantitated by liquid scintillation counting (LSC) with Packard Insta-Gel in a Packard 460 or 4530 counter using appropriate quench corrections.

Isolation of Polar Abamectin Degradates from Methanol Rinse of Citrus Fruit. The optimal conditions for isolation of PAD from the total residues in the concentrated methanol rinse of abamectin-treated fruit by a liquid–liquid partitioning method described below were previously developed on a small scale using the methanol rinse from [^{14}C]B1A-treated fruit (180 μg/g, 14 days PHI) and the concentrated methanol rinse from the abamectin-treated oranges. The procedure was then scaled up for isolation of PAD residues from field-treated fruit as follows: a 30-L glass separator equipped with a Teflon propellor stirrer, a baffle, and a stopcock located at the bottom was thoroughly rinsed in order with hexane, hot tap water, deionized water, and methanol before use. The methanol rinse from oranges treated with water and agricultural formulation only (no abamectin) was processed first followed by that from abamectin-treated oranges. Two liters of the concentrated methanol rinse, 10 L of deionized water, and 12 L of hexane were placed in the separator. The stirrer was set so as to form a uniform mixture without splashing; after 10 min, the extraction was stopped, and the aqueous and organic layers were drawn off separately into clean glass flasks. The aqueous layer was then reextracted four times with 12 L of hexane. The resultant five hexane extracts were combined and concentrated via rotary evaporation under reduced pressure (35 °C) by continuously feeding the hexane extract into the same 20-L flask on a process-scale Buchi rotary evaporator. After all 60 L of the hexane extracts had been reduced to 200–300 mL, the concentrated hexane layer was removed and the evaporating flask rinsed with hexane and methanol. The rinses were combined with the concentrated hexane extract, dried, and reconstituted in methanol; this hexane fraction (CITHEX) from abamectin-treated fruit contained B1A, B1B, and moderately polar plus nonpolar residues as defined under HPLC Methods for ^{14}C Residues. The hexane-extracted aqueous layer was concentrated in the Buchi apparatus at 40 °C until a volume of about 200 mL remained. The flask was rinsed with methanol, the rinse was dried, and the concentrated aqueous layer was added to the dried rinse; this aqueous fraction (CITAQ) from abamectin-treated fruit contained PAD. The CITAQ fractions from both vehicle- and abamectin-treated oranges were an opaque brown suspension containing some black tarlike material and were used without further processing for mouse teratology studies.

Preparation of Polar Abamectin Degradates from Photolysis of Abamectin Thin Films. Technical grade abamectin (B1) was dissolved in methanol and applied to 60 95-mm glass Petri dishes (Fisher Scientific) to give thin films of approximately 16 μg/mm^2 after drying (6.89 g total material); an additional 2 Petri dishes contained [^{14}C]B1A and B1 at the same film thickness and were used to estimate residue components. The dishes were then placed on an aluminum foil surface under two racks of six General Electric 275-W Suntanner bulbs located 66 cm from the dish bottoms (contained in a fume hood) and photolyzed for 30 h (50 °C). The B1 thin film residues were recovered from the dishes by a methanol rinse, combined, and concentrated for preparative HPLC; the [^{14}C]B1A thin film residues were also recovered by a methanol rinse for HPLC radioassay. The PAD residues were isolated from the total B1 thin film photolysis residues by HPLC on a Whatman Magnum 20 ODS column eluted with 85% methanol/15% water at a flow rate of 22.5 mL/min. Approximately 0.4 g of B1 thin film residues was chromatographed at a time, the eluate was monitored at 245 nm, and those residues whose retention time (t_R) was between 0 and 0.6 that of B1A were designated PAD. The eluate containing PAD was dried to a constant weight by rotary evaporation (Buchi Model R-110) and placed in a vacuum desiccator containing $CaSO_4$ for several days until a constant weight was obtained; the dried PAD from photolysis of B1 thin films was a yellow powder.

Analytical High-Pressure Liquid Chromatography (HPLC) Analysis of Abamectin Residues. *HPLC Equipment.* The HPLC systems consisted of Du Pont Zorbax or IBM C_{18} columns (4.6 × 250 mm, 5–7-μm particle size), a Spectra-Physics SP8700 solvent delivery system, a Rheodyne 7125 injector, an LDC Spectromonitor III, Spectra-Physics SP8440, or Hewlett-Packard 1040A diode array detector, a Spectra-Physics SP4200M or Hewlett-Packard HP 85B data station, and a Pharmacia Frac-100 collector. The eluate was monitored at 245 nm with the variable-wavelength detectors or from 190 to 400 nm with the diode array detector. One-minute fractions of eluate were collected for additional HPLC or for LSC with Packard Insta-Gel in a Packard 460 or 4530 counter.

HPLC Methods for ^{14}C Residues. The methanol rinses of citrus fruit or Petri dishes were chromatographed using a Zorbax C_{18} column eluted with 1 mL/min methanol/water at the following compositions: 0–35 min, 85% methanol; 35–37 min, 85–100% methanol; 37–60 min, 100% methanol (C18HPLC). This C18HPLC analysis was used to broadly classify and quantitate [^{14}C]B1A residues as polar (t_R up to 0.6 that of B1A), moderately polar (t_R between 0.6 and 0.95 that of B1A), or nonpolar (t_R greater than that of B1A) similar to previous studies (Moye et al., 1990; Crouch et al., 1991). The PAD from photolysis of [^{14}C]-B1A thin films were also obtained by this method for further HPLC characterization. The PAD obtained by C18HPLC of the total residue from photolysis of [^{14}C]B1 thin films or by liquid–liquid partitioning of [^{14}C]B1A residues from citrus were chromatographed by a step gradient method (SGHPLC) using an IBM column eluted with 1 mL/min methanol/water at the following compositions: 0–8 min, 30% methanol; 8–10 min, 30–45% methanol; 10–20 min, 45% methanol; 20–22 min, 45–70% methanol; 22–45 min, 70% methanol; 45–70 min, 70–100% methanol; 70–90 min, 100% methanol.

HPLC Methods for Nonradiolabeled Avermectin B_{1a}. The concentrated aqueous fractions from liquid–liquid partitioning of the methanol rinse of vehicle and abamectin-treated oranges were assayed for B1A by a modification (J. Cobin, personal communication) of the fluorescence derivatization HPLC method of Tway et al. (1981). The CITHEX fraction from abamectin-

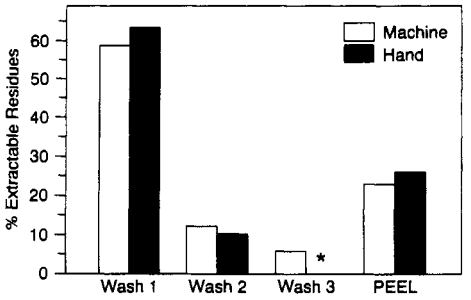

Figure 2. Extractable abamectin residues in orange peel. Washes are total residues removed by methanol rinses of whole fruit treated with [^{14}C]B1A; peel was extracted with acetone after two or three methanol rinses. The asterisk indicates that the methanol rinse was not performed.

treated oranges was assayed for B1A by HPLC (conditions as for C18HPLC above) following cleanup of the sample by solid-phase extraction; a standard curve of peak area vs micrograms of purified B1A was constructed, and [^{14}C]B1A was added to the sample prior to cleanup to monitor recovery.

Miscellaneous Analytical Procedures. The CITAQ fractions from abamectin- and vehicle-treated oranges were assayed for total solids by evaporation of an aliquot under vacuum in a desiccator at room temperature until constant weight was achieved.

Toxicological Procedures. *Microbial Mutagenesis Assay.* Strains of *Salmonella typhimurium* (TA1535, TA97a, TA98, TA100) and *Escherichia coli* (WP2, WP2 uvrA, WP2 uvrA pKM101) were used to detect the mutagenicity of PAD isolated from thin film photolysis (in DMSO) at 100–10 000 μg/plate in the presence and absence of rat liver S9 in a standard plate incorporation assay (Maron and Ames, 1983). The tests were also run with appropriate strain-specific diagnostic mutagens (2-aminoanthracene, methyl methanesulfonate, daunomycin, hydrazine sulfate, sodium azide, and ICR-191) and solvent controls (DMSO). The PAD from citrus were not tested for mutagenicity.

Mouse Teratology Studies. Mated CF_1 female mice were dosed once daily by oral gavage with PAD from citrus or thin film photolysis in 0.5% aqueous methylcellulose at 0.25, 0.5, or 1.0 mg of abamectin residues/kg from days 6 through 15 of gestation. Control groups were treated with 0.5% aqueous methylcellulose or with CITAQ from vehicle-treated oranges at 50, 100, and 200 mg of total solids kg^{-1} day^{-1}. A total of 25 females were used for each treatment group. The mice were sacrificed on day 17 of gestation and the numbers of live, dead, and resorbed fetuses were noted as well as fetal weights. All fetuses were given external and skeletal examinations, and every third fetus and all externally malformed or dead fetuses from each litter were given a visceral examination. Statistical analyses were based on an analysis of variance or covariance using a least significant difference procedure after normalizing for nonparametric data by a normalized rank transformation (Tukey, 1962). Results were considered statistically significant at $p \leq 0.05$.

RESULTS

Assays of Abamectin Residues from Citrus Fruit and Photolysis of Thin Films. The proportions of total ^{14}C residues in orange peel removed by methanol rinses of whole fruit by hand ($n = 5$) or by the citrus-rinsing apparatus ($n = 65$) followed by acetone extraction of the peel are summarized in Figure 2; the two procedures were essentially equivalent with about three-fourths of the extractable residues present in the methanol rinses. The acetone-extracted peel was not assayed for radioactivity; previous studies with oranges treated with 80 μg/g [^{14}C]-abamectin indicated that about 95% of the peel residues were extractable at 14 days PHI using similar methods (Maynard et al., 1989).

The C18HPLC radioprofile of residues in the combined methanol rinses of hand-processed fruit is shown in Figure 3; the B1A residues are designated polar, moderately polar,

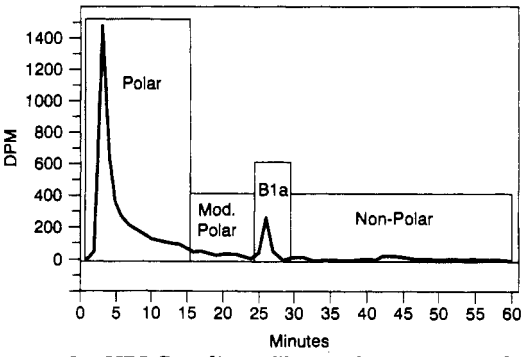

Figure 3. C18HPLC radioprofile of abamectin residues in methanol rinse of whole fruit. Fruits were treated with [^{14}C]-B1A and rinsed three times with methanol by hand.

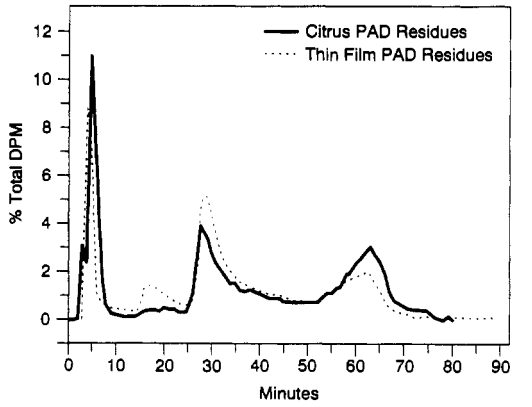

Figure 4. SGHPLC radioprofile of PAD from orange fruit and thin film photolysis. The PAD were obtained by C18HPLC of residues in the methanol rinse of orange fruit treated with [^{14}C]-B1A (Figure 3) or from photolysis of abamectin thin films containing [^{14}C]B1A on glass.

Table I. Polar ^{14}C Degradates and [^{14}C]B1A Content of Abamectin Residues from Thin Films on Glass and Citrus Fruit As Assayed by C18HPLC

residue source	% PAD	% B1A
thin film[a]	81.0	7.3
citrus fruit rinse[b] (before partitioning)	82.3	6.8
citrus fruit rinse[c] (hexane fraction)	2.7	5.9
citrus fruit rinse[d] (aqueous fraction)	74.7	0.0

[a] Total residues from photolysis of abamectin thin films on glass. [b] Total residues from methanol rinses of orange surface. [c] Residues in CITHEX fraction after partitioning. [d] Residues in CITAQ fraction after partitioning.

or nonpolar relative to t_R of B1A as described under Materials and Methods and as in previous studies (Moye et al., 1990; Crouch et al., 1991). The PAD obtained by C18HPLC of total residues from photolysis of abamectin thin films or from the methanol rinse of citrus fruit (residues eluting before 0.6 that of B1A t_R) are compared by their SGHPLC radioprofiles in Figure 4 and appear qualitatively similar.

The PAD and B1A contents of ^{14}C residues in the methanol rinses of hand-processed oranges (before and after hexane partitioning) and of a photolyzed abamectin thin film are summarized in Table I. Essentially all citrus PAD are in the aqueous fraction (CITAQ), and all B1A is in the hexane fraction (CITHEX); also, a similar proportion of B1A and PAD is seen in the methanol rinses of thin films and oranges (Table I).

The total natural products and B1A per vehicle- and abamectin-treated orange and the PAD and B1A per abamectin-treated orange estimated from [^{14}C]B1A-treated

Table II. Natural Product and B1A Content of Field-Treated Oranges

	vehicle[a]-treated	abamectin-treated
natural products,[b] mg of solids/orange	1.68	1.67
estimated PAD residues,[c] (μg/orange)		10.6
estimated B1A,[d] μg/orange		0.96
assayed B1A,[e] μg/orange	0.0	1.14

[a] Vehicle is agricultural formulation diluted in water. [b] Total solids in CITAQ fraction per orange. [c] Estimated by C18HPLC of CITAQ fraction of [^{14}C]B1A-treated fruit. [d] Estimated from B1A content of methanol rinse of [^{14}C]B1A-treated fruit determined by C18HPLC. [e] From B1A content of CITHEX fraction assayed by HPLC.

Table III. Teratogenicity Testing of PAD from Citrus Fruit in CF_1 Mice (23–25 Litters per Group)

observation	vehicle[a]	CITAQ control,[b] 200 mg kg^{-1} day^{-1}	CITAQ abamectin,[c] 1.0 mg kg^{-1} day^{-1}
no. of implants per pregnant female	13.1	13.4[d]	12.2[d,e]
no. of live fetuses per pregnant female	11.5	12.3[d]	11.6[d,e]
fetal wt, g	0.94	0.95[d]	0.95[d,e]
fetuses (*n*) with			
external malformations[f]	2/277	3/282	2/289
visceral malformations[f]	0/84	0/89	1/91
skeletal malformations[f]	5/277	14/282	12/289

[a] Vehicle is 0.5% aqueous methylcellulose. [b] Dose is milligrams of total solids from partitioned methanol rinse of oranges treated with blank agricultural formulation in water. [c] Dose is milligrams of PAD from field-treated fruit estimated from [^{14}C]B1A-treated oranges. [d] Any difference from vehicle controls not significant ($p > 0.05$). [e] Any difference from CITAQ control not significant ($p > 0.05$). [f] Statistical analysis not performed.

Table IV. Teratogenicity Testing of PAD from Photolysis of Abamectin Thin Films in CF_1 Mice (21–24 Litters per Group)

observation	vehicle[a]	thin film PAD, 1.0 mg kg^{-1} day^{-1}
no. of implants per pregnant female	13.2	13.4[b]
no. of live fetuses per pregnant female	11.3	12.4[b]
fetal wt, g	0.92	0.95[b,c]
fetuses (*n*) with		
external malformations	3/237	4/298[b]
visceral malformations	0/73	2/93[b]
skeletal malformations	12/237	13/298[b]

[a] Vehicle is 0.5% aqueous methylcellulose. [b] Difference from vehicle-treated group not significant ($p > 0.05$). [c] Statistical analysis performed with adjustment for time of sacrifice.

fruit are summarized in Table II. The yield of natural products (expressed as milligrams of total solids/orange) was essentially the same for vehicle- and abamectin-treated oranges (Table II). A yield of approximately 10–11 μg of PAD residue per field-treated orange was estimated from the [^{14}C]B1A-treated oranges, and the micrograms of B1A per orange estimated from the same data was in close agreement with the actual B1A content of the field-treated oranges (Table II).

Toxicity Testing of Polar Abamectin Degradates. The results of teratology studies of PAD in the CF_1 mouse are summarized in Tables III and IV for the highest dose groups only. For PAD residues from citrus fruit (CITAQ abamectin) or the CITAQ fraction from control oranges (CITAQ control) no effects were observed relative to vehicle controls (Table III). Also, for PAD from photolysis of abamectin thin films there appeared to be no differences from vehicle controls (Table IV). No evidence of maternotoxicity was seen with PAD residues from citrus fruit or thin films (data not shown).

Table V. Microbial Mutagenesis Assay (−S9 Activation) of PAD from Photolysis of Abamectin Thin Films on Glass

	E. coli revertants per plate[a]		
	WP2	WP2 uvrA	WP2 uvra PKM101
μg/plate			
0	41.4	16.1	89.9
100	41.3	11.3	81.7
300	36.3	13.7	81.3
1000	33.3	18.0	96.0
3000	35.0	16.3	110.0
10000[b]	39.7	14.7	140.0

	S. typhimurium revertants per plate[a]			
	TA1535	TA97a	TA98	TA100
μg/plate				
0	17.8	148.5	25.3	191.7
100	16.7	156.3	27.3	198.3
300	16.3	157.0	24.0	207.7
1000	16.0	157.3	21.7	195.0
3000	18.7	185.0	24.0	194.3
10000[b]	24.0	213.7	23.3	241.7

[a] An assay is considered positive if the number of revertant colonies is at least 2-fold of solvent control with an evident dose-related increase. [b] Precipitate was observed but colonies were discernible.

Table VI. Microbial Mutagenesis Assay (+S9 Activation) of PAD from Photolysis of Abamectin Thin Films on Glass

	E. coli revertants per plate[a]		
	WP2	WP2 uvrA	WP2 uvra PKM101
μg/plate			
0	50.0	10.7	55.7
100	43.7	13.7	55.7
300	53.3	8.3	59.7
1000	51.7	12.7	63.7
3000	49.0	12.0	69.0
10000[b]	63.7	15.7	96.3

	S. typhimurium revertants per plate[a]			
	TA1535	TA97a	TA98	TA100
μg/plate				
0	19.3	194.8	27.4	191.8
100	17.3	186.0	24.0	199.3
300	18.3	195.3	26.7	205.3
1000	18.0	206.0	24.7	197.7
3000	22.7	201.0	25.3	198.0
10000[b]	30.7	199.7	25.7	250.3

[a] An assay is considered positive if the number of revertant colonies is at least 2-fold of solvent control with an evident dose-related increase. [b] Precipitate was observed but colonies were discernible.

The PAD from photolysis of abamectin thin films was tested for mutagenicity in three strains of *E. coli* and four strains of *S. typhimurium* at 0.1–10 mg/plate in the absence and presence of metabolic activation. No 2-fold or greater increases in revertants were observed at the highest testable dose (limited by solubility of the test material), and therefore PAD are considered negative in the microbial mutagenesis assay (Tables V and VI).

DISCUSSION

The presence of abamectin and its degradates on crops intended for human consumption necessitates identification of those residues of possible toxicological concern. Since abamectin degrades on plants (Moye et al., 1990) or after photolysis of thin films (Crouch et al., 1991) to multiple polar compounds that have proved intractable to HPLC resolution and purification to date, structural determinations of these compounds have not been possible. Therefore, the toxicity of the entire fraction of these polar degradates from abamectin-treated fruit and from photolysis of thin films was studied.

The PAD from photolysis of abamectin thin films were easily obtainable in subgram to gram amounts by preparative scale C18HPLC and could be quantitated directly by dry weight or indirectly by assay of small amounts of radiolabeled material photolyzed under the same conditions. The PAD from field-treated citrus fruit, however, could only be assayed indirectly using fruit treated with [^{14}C]B1A under similar conditions as there are no spectroscopic or chemical analytical methods available for PAD at present. The B1A contents of oranges treated with 180 μg/g [^{14}C]B1A or the field-treated oranges (180 ppm abamectin) were remarkably similar (Table II), suggesting that comparable degradation to PAD had occurred. Rinsings of [^{14}C]B1A-treated fruit by hand or in the citrus-rinsing apparatus used to extract PAD residues for teratology studies appeared to be of similar efficiency in extracting total residue; the ^{14}C residues from hand-rinsed fruit were used both to estimate total PAD residues content and to develop a procedure to remove B1 from the PAD residues of field-treated fruit. The partitioning procedure devised for isolation of PAD residues from field-treated oranges completely removed the toxic parent compound from the CITAQ fraction used in the teratology studies and left essentially all of the PAD (Table I).

The ^{14}C-labeled PAD from citrus fruit or from thin films appeared qualitatively similar when assayed by SGHPLC (Figure 4), and the degrees of degradation of B1A were also similar (Table I). The isolation of PAD residues from abamectin-treated crops in amounts sufficient for teratology studies, though, required an heroic effort; for example, only an estimated 100 mg of PAD was extracted from approximately 10 000 oranges treated with abamectin, and the CITAQ fraction tested contained about 6 mg of PAD/g of orange fruit natural products (Table II). The natural products from 10 000 fruits treated with blank agricultural formulation served as a control in the teratology study as no attempt to purify PAD in the CITAQ fraction was made; the yield of natural products per orange was essentially identical for vehicle- and abamectin-treated trees (Table II).

Teratology studies of PAD from thin films or orange fruit indicated no toxicity relative to 0.5% methylcellulose at 1.0 mg kg^{-1} day^{-1} (Tables IV and V). These results are consistent with the qualitative similarities of PAD from thin films and citrus (Figure 4). Similar teratology studies of abamectin in mice, the most sensitive species, indicate a NOEL for fetal and developmental toxicity at 0.2 mg kg^{-1} day^{-1}; however, in contrast to PAD, abamectin caused maternotoxicity at 0.075 mg kg^{-1} day^{-1} with a NOEL of 0.05 mg kg^{-1} day^{-1} (Lankas and Gordon, 1989). The PAD from thin films were nonmutagenic relative to solvent controls in the presence or absence of metabolic activation in *S. typhimurium* and *E. coli* mutagenicity assays (Tables V and VI) as is abamectin itself (Lankas and Gordon, 1989). In regard to acute toxicity, abamectin was at least 500 times as toxic in mice as PAD from photolysis of abamectin thin films (Wislocki et al., 1989).

The loss of acute toxicity in mammals when abamectin is degraded to PAD, which are likely to be greatly altered from abamectin in structure, is not surprising as in vitro assays indicate that loss of the disaccharide moiety or reduction of double bonds of avermectin B_{1a} greatly decreases binding to dog brain synaptosomes (Pong and Wang, 1980). The present studies demonstrate that in addition to a loss of acute toxicity when abamectin is extensively degraded to polar residues, there is no evidence for genotoxicity of these polar residues.

ABBREVIATIONS USED

B1, avermectin B_1; B1A, avermectin B_{1a}; B1B, avermectin B_{1b}; C18HPLC, high-pressure liquid chromatography with octadecyl silane bonded phase column; CITAQ, aqueous fraction after partitioning of fruit rinse; CITHEX, organic fraction after partitioning of fruit rinse; DMSO, dimethyl sulfoxide; HPLC, high-pressure liquid chromatography; LSC, liquid scintillation counting; PAD, polar abamectin degradates; t_R, retention time; SGHPLC, step gradient HPLC.

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