ENVIRONMENTAL FATE OF AVERMECTIN [1/]

D. L. Bull

Cotton Insects Research Laboratory, ARS, USDA
College Station, TX 77841

ABSTRACT

In laboratory tests, ^{3}H-labeled avermectin B_1a (AVM) did not leach in representative agricultural soils (Lufkin fine sandy loam, Houston clay) and was degraded fairly rapidly (halflives of 3-4 wk at 0.1 ppm) in samples of these soils held under aerobic conditions. At least 13 radioactive transformation products were detected; the major product was identified as an equilibrium mixture of the 8α-hydroxy and corresponding ring-opened-aldehyde derivatives of AVM. AVM was not degraded under anaerobic conditions.

In field tests, radioactive residues were detected in all parts of cotton plants harvested 2 mon. after two spray applications of [^{14}C]AVM at a rate of 20 g AI/ha. The highest concentrations (1.045 ppm) were in leaves that had dropped and were collected from the ground; lowest concentrations were in lint (38 ppb) and seed (50 ppb). Weekly analyses of Bermuda grass grown in plots treated with granular formulations of [^{14}C]AVM at rates of 125, 375, and 1250 mg AI/ha indicated no radioactive material was taken up by the plants during an eight wk experimental period.

INTRODUCTION

As indicated in the other articles included in this publication, the avermectins appear to have excellent potential for use in controlling different arthropod pests of plants and animals. Since it is highly likely that these compounds will be used extensively in pest control, it is essential to obtain sufficient information on their posttreatment fate in order to facilitate assessment of the potential for environmental contamination or adverse effects on nontarget organisms.

Certain aspects of the fate of radiolabeled avermectin-B_1a (AVM) in different soils and plants were described in a recent report by Bull et al. (1984). This paper includes a review of some of the information those authors presented, as well as the results and discussion of subsequent work that has been done with ^{14}C-labeled AVM.

MATERIALS AND METHODS

Chemicals. Two different radiolabeled formulations of AVM were provided by Merck & Co., Inc., Rahway, NJ for the previous study reported by Bull et al.

[1/]This work was done in cooperation with Texas A&M University, Texas Agricultural Experiment Station, College Station, TX. This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation under FIFRA as amended. Also, mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the U. S. Department of Agriculture.

(1984) and the current studies discussed in this report. One preparation was labeled with ^{3}H at the 5-position of the molecule (specific activity 1.74 mCi/mg) and the other with ^{14}C at the 3-, 7-, 11-, 13-, and 23-positions (specific activity 16.4 μCi/mg). The carbon atoms in the AVM molecule illustrated in Fig. 1 are partially numbered to facilitate identification of the positions of these radiolabels.

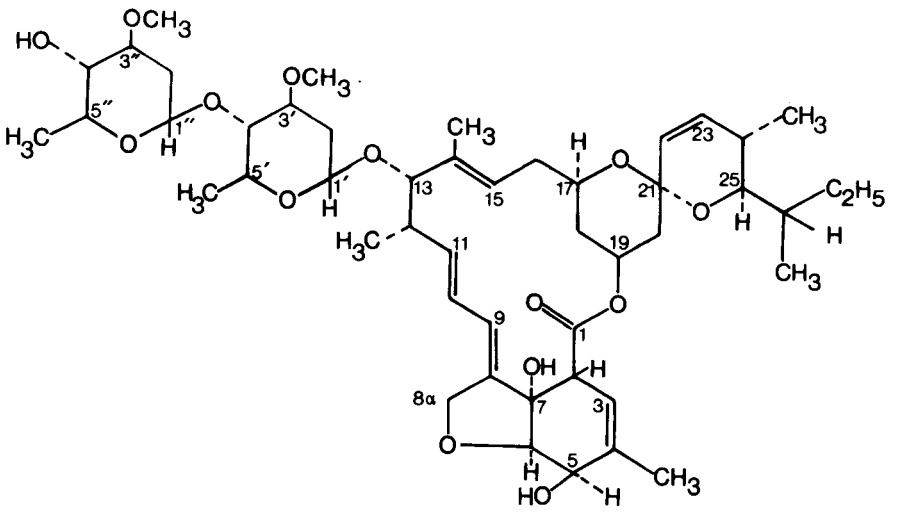

FIG. 1. Structure of avermectin-$B_{1}a$.

Soils and Their Treatment. Lufkin fine sandy loam was used for the laboratory studies with [^{14}C]AVM. The properties of this soil, as well as the others used in the aforementioned studies with [^{3}H]AVM (Bull et al. 1984) were reported previously by Bull and Ivie (1982). Samples of loam were collected from adjacent fields, air-dried, and sieved to pass a 35-mesh screen. Ten-gram samples in 20-ml glass vials were treated at a rate of 1.0 ppm with [^{14}C]AVM added in a small volume of methanol. After the solvent evaporated, samples of treated soil were mixed thoroughly, moistened to field capacity with distilled water, then held at ambient temperature and high relative humidity (>90%) in ventilated glass containers located in a fume hood.

Another study was made to compare the fate of [^{14}C]AVM in soil held in aerobic or anaerobic conditions. Samples of loam were treated with [^{14}C]AVM as described. One group was held entirely in aerobic conditions, another group entirely in anaerobic conditions, and a third group was changed from aerobic to

anaerobic conditions at 4 wk posttreatment. Anaerobic conditions were established by purging vials containing treated loam with nitrogen. Sufficient distilled water was added to flood the soil and then the vials were sealed. In all laboratory studies, treated samples were protected from direct exposure to light. All tests were replicated three times.

At definite times posttreatment, samples of soil were extracted three times with acetonitrile. Combined solvent extracts were radioassayed and analyzed by thin layer chromatography, and air-dried extracted soil was analyzed via oxygen combustion, as described by Bull et al. (1984).

Plants and Their Treatment. Cotton plants used for tests of the fate of [^{14}C]AVM were of the Deltapine 213 variety grown in the field with standard production procedures.

All applications of [^{14}C]AVM to cotton were made with emulsifiable concentrate (EC) formulations. These spray solutions were prepared by dissolving appropriate amounts of the insecticide in a blank EC solvent mixture (provided by Merck & Co., Inc.) and then adding water to the desired volume. Individual leaves were treated *in situ* by spreading 100 µg of [^{14}C]AVM in 150 µl of aqueous emulsion uniformly over the upper surface of each leaf. Plants bearing treated leaves were covered with plastic-covered cages during occasional brief periods of inclement weather.

At definite times posttreatment, individual leaves were collected and then analyzed for unabsorbed and absorbed radioactive materials with the radiometric and TLC procedures described by Bull et al. (1984).

Bound radiocarbon in air-dried extracted leaf tissue was determined via standard oxygen combustion techniques using an R. J. Harvey (Patterson, NJ) biological oxidizer. Samples (100 mg/each) were burned for 2 min at a combustion temperature of 900°C. Combustion gases were passed through 10 ml of a trapping solution consisting of a 1:1 mixture of Carbosorb II (Packard Instr. Co., Inc., Downers Grove, IL) and liquid scintillation cocktail. After each combustion, the trapping solution was mixed with an additional 10 ml of cocktail and radioassayed with a liquid scintillation counter. Recovery of known amounts of [^{14}C]AVM applied to untreated samples was >98%. These tests with individual leaves were replicated from three to six times.

A small plot of cotton (366 row cm) was treated with [^{14}C]AVM by spraying the plants at a rate of 20 g AI in 100 L of aqueous emulsion/ha. This plot was treated first on August 15, 1983 and then again on August 22. At the time of treatment, these plants were at the peak of the fruiting cycle and there were no open bolls. After treatment, the plot was enclosed in a screened cage to exclude infestations by insect pests; the top of the cage was covered with plastic to minimize wash-off of AVM by occasional rainfall. It should be emphasized, however, that the plants were exposed to heavy wind and rains associated with hurricane Alicia on August 18. Damaged plants were removed prior to the second treatment and analyzed separately.

All plant materials in the treated plot of cotton, including leaves and other plant parts that had been shed during the 2-mon posttreatment period, were collected on October 18, 1983. Plants were divided into various parts and dried 48 h at 45°C. Foliage and woody parts of the plant were milled to a fine powder; lint and seeds were separated with a manual ginning apparatus and then seeds were acid-delinted, dried, and milled. Subsamples (250 mg/each) of the different plant parts were analyzed for radiocarbon content via oxygen-combustion as described above.

RESULTS AND DISCUSSION

Soil Studies. The results of studies of the fate of [^{14}C]AVM after treatment of Lufkin fine sandy loam at a rate of 1.0 ppm (Table 1) were somewhat similar to those reported previously for [^{3}H]AVM (Bull et al. 1984). The apparent halflife of the parent compound in loam in this study was ca. 14 days, compared with halflives for [^{3}H]AVM of ca. 21 days at the 0.1 and 1.0 ppm treatment levels and ca. 40 days at 50 ppm (Fig. 2). In tests conducted with

similar procedures, Bull et al. (1984) reported that the halflives for [^{3}H]AVM in Houston clay were ca. 28 days at 0.1 ppm and 50 days at 1 ppm (Fig. 3) and ca. 8 wk at 1.0 ppm in a coarse sand.

TABLE 1. Degradation of [^{14}C]AVM in Lufkin Fine Sandy Loam Treated at a Rate of 1.0 ppm and Held in Aerobic Conditions.

Days post-treatment	Distribution of radioactivity, % of dose in--								
	Compounds[a/]								
	4	5-6	7	8-10	11	12	13-14	Unextractable	Lost
0	0.0	0.0	0.0	0.0	99.0	0.0	0.0	1.0	0.0
14	2.1	2.5	12.0	0.0	50.3	12.0	3.3	6.9	10.9
28	4.4	3.4	16.1	0.0	25.2	15.1	3.2	10.9	21.7
56	5.4	5.8	8.9	5.2	11.0	10.9	2.1	15.8	34.9
84	6.2	6.3	8.4	4.2	8.1	8.0	1.7	18.8	38.3

[a/]See Bull et al. (1984) for TLC R_f values.

On the basis of TLC behavior, the array of radioactive products of the degradation of [^{14}C]AVM in Lufkin fine sandy loam was similar to that reported for [^{3}H]AVM treatments. These products and their relative concentrations over time are shown in Table 1. [^{14}C]AVM and at least 10 radioactive products were detected in solvent extracts of treated soil. Minor compounds (<5% of dose) are combined in the table for convenience and the numerical designations are the same as those used in previous studies with [^{3}H]AVM. Compound 11 is AVM and compound 7 coincides chromatographically with the major metabolite of [^{3}H]AVM (Fig. 4) which was identified as an equilibrium mixture of the 8α-hydroxy derivative and corresponding ring-opened aldehyde derivative of AVM in an approximate 1:2.5 ratio (Bull et al. 1984). Similarly, compounds 4 and 12 were also found to be major products of the degradation of [^{14}C]AVM in loam.

There were progressive increases in the proportions of the applied [^{14}C]AVM that could not be accounted for in the different analyses (Table 1). The nature of this "lost" radioactive material is unknown. Bull et al. (1984) detected evolution of $^{14}CO_2$ in biometer flasks containing Lufkin fine sandy loam treated with [^{14}C]AVM at a rate of 10 ppm; however, the accumulated amount of $^{14}CO_2$ trapped through 27 wk posttreatment was only 4.1% of the dose. It should be noted that the degradation of [^{14}C]AVM in the 100-g samples of treated loam in the biometer flasks was somewhat slower than that observed in other conditions with 10-g samples; the parent compound still accounted for ca. 49% of the dose at 28 wk posttreatment. Bull et al. (1984) concluded that under the conditions of the biometer tests, which were also conducted in a way to minimize exposure of treated samples to light, that there was very little oxidative cleavage of carbon to carbon bonds at the labeled positions.

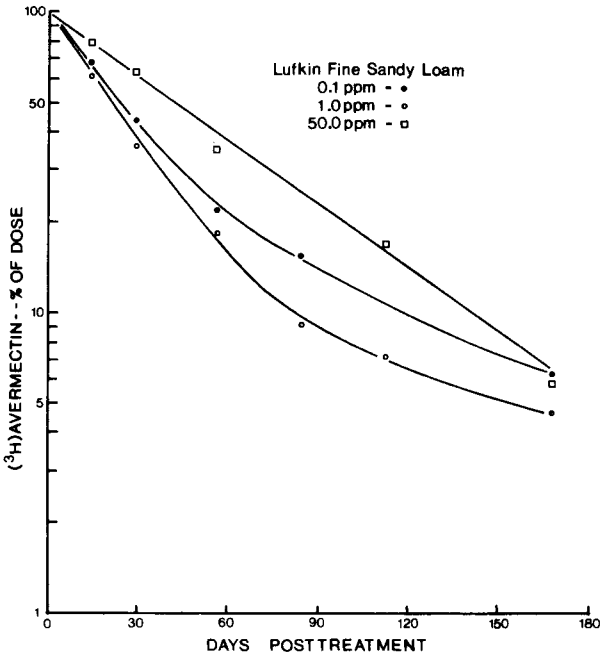

FIG. 2. Rate of aerobic degradation of [^{3}H]AVM after application to Lufkin fine sandy loam at rates of 0.1, 1.0, and 50 ppm. (Adapted from Bull et al. 1984 with permission.)

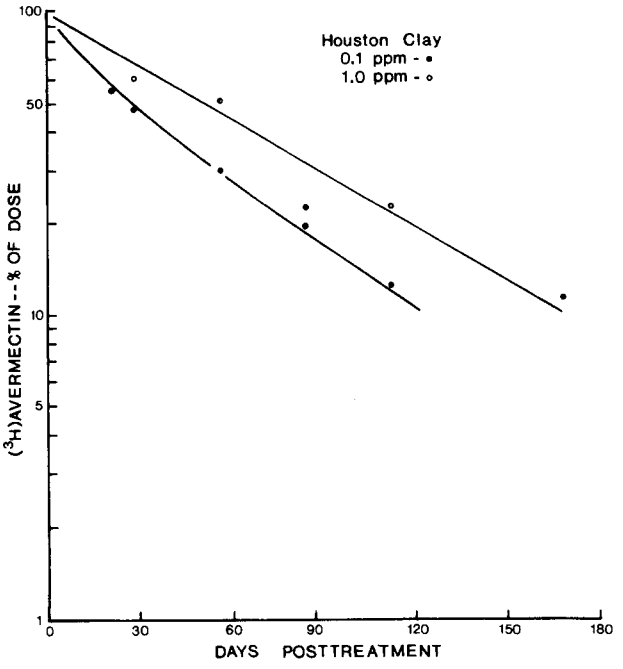

FIG. 3. Rate of aerobic degradation of [^{3}H]AVM after application to Houston clay at rates of 0.1 and 1.0 ppm. (Adapted from Bull et al. 1984 with permission.)

In their studies with [^{3}H]AVM, Bull et al. (1984) reported that a volatile radioactive material, accumulating to as much as 30% of the dose of [^{3}H]AVM applied to different soils, ultimately condensed in the water used to maintain high levels of humidity in the sample holding containers. This condensed radioactivity was probably tritiated water. Since special studies demonstrated that the tritium label of AVM is stable and apparently does not exchange with unlabeled hydrogen (as is sometimes the case with tritium), the authors speculated that the apparent release of tritium resulted from metabolic oxidation at the 5-carbon position of either the parent molecule or its degradation products.

The results of studies comparing the fate of [^{14}C]AVM in Lufkin fine sandy loam held in aerobic or anaerobic conditions indicated there was no degradation in samples held entirely in anaerobic conditions (Fig. 5). Recovery of the applied [^{14}C]AVM was quantitative at all times posttreatment. Degradation of [^{14}C]AVM in samples held in totally aerobic conditions was comparable to that discussed above, but when some of the samples were changed after 4 wk from aerobic to anaerobic conditions, the subsequent rate at which [^{14}C]AVM degraded decreased considerably.

Bull et al. (1984) studied the possible leaching of [^{3}H]AVM in Lufkin fine sandy loam, Houston clay, and sand with a soil/TLC method and found that there was none, a finding that would be expected in view of the fact that the water solubility of AVM is only ca. 6 ppb.

Plant Studies. The results of studies of the fate of [^{14}C]AVM after application to individual cotton leaves were very similar (Table 2) to those reported in comparable studies with [^{3}H]AVM (Bull et al. 1984). Although the total concentrations of surface radioactive materials were fairly persistent, the parent compound was somewhat unstable, with an apparent halflife of less than 24 h. As was the case in similar studies with [^{3}H]AVM (Bull et al. 1984), TLC analyses of the external rinses indicated that the radioactive material moved with heavy streaking from the origin to the solvent front. A large number of poorly resolved radioactive zones were observed, including large amounts of a polar material that remained at the origin. Based upon the TLC behavior of the unabsorbed radioactive materials and upon available information on the instability of AVM to ultraviolet light (Bull et al. 1984), the rapid dissipation of surface residues of the parent compound can most likely be attributed to photodegradation induced by sunlight. Our experiences during these studies indicated that AVM is readily washed off treated foliage by rainfall. In this study plants were protected during occasional periods of inclement weather; however, some of the losses of applied radiocarbon shown in Table 2 may have resulted from heavy dews that sometimes occurred.

Results of combustion analyses of plant materials collected from the small plot of cotton that was sprayed twice with [^{14}C]AVM indicated that all parts of the plants contained some radioactive material (Table 3). The highest concentrations of radiocarbon (1.045 ppm) were found in leaves that had dropped and were collected from the ground. Residues were higher in these leaves because many of them were on the plants when treatments were applied. Relatively low levels of radiocarbon, only 50 ppb of [^{14}C]AVM equivalents, were detected in seeds. The unidentified radioactive material was about equally divided between the hull and seed meat. Lint also contained ca. 38 ppb of radiocarbon. In view of the very poor water solubility of AVM and its instability in sunlight, it is possible that the radioactive materials distributed within the plants resulted from the incorporation of smaller radioactive fragments released upon decomposition of the parent compound. Radioactive residues in core samples of soil (1 g subsamples analyzed by oxygen combustion) taken from the plot at the same time plants were harvested were insignificant--only 2 ppb near the soil surface and none at depths below 7.5 cm. Levels of radiocarbon in the damaged plants removed from the plot before the second treatment also contained appreciable levels of radiocarbon (Table 3) but they were somewhat less than in those treated twice.

TABLE 2. Fate of [^{14}C]AVM after Foliar Application to Individual Cotton Leaves in the Field (ca. 100 µg/leaf).[a/]

Days post-treatment	Distribution of radiocarbon, % of dose (±SE)					
	External rinse		Internal extract		Unextr. residue	Lost
	Total	AVM	Total	AVM		
0	100.0 ± 0.0	98.9 ± 0.1	0.0	0.0	0.0	0.0
1/4	84.4 ± 2.5	56.8 ± 0.1	3.7 ± 0.4	2.6 ± 0.1	2.8 ± 0.1	9.0
1	82.7 ± 11.2	45.1 ± 6.0	8.6 ± 1.4	5.7 ± 1.0	6.3 ± 1.0	2.4
2	60.1 ± 11.2	13.9 ± 2.7	8.2 ± 0.6	4.4 ± 0.3	12.6 ± 1.5	19.1
4	43.7 ± 6.6	4.7 ± 1.1	9.5 ± 1.8	3.2 ± 1.2	26.1 ± 4.7	20.7
8	19.3 ± 11.0	1.7 ± 0.3	15.9 ± 3.3	3.6 ± 0.3	23.1 ± 4.1	41.7

[a/]Treated leaves rinsed with methanol and then homogenized three times with a 9:1 mixture of actetone and water; samples analyzed via TLC using solvent mixture of 10:3:1 ethyl acetate, benzene, and 2-propanol. Extracted tissues were dried and analyzed (100 mg samples) for residual radiocarbon by oxygen combustion.

TABLE 3. Combustion Analyses of Samples from Cotton Plot Treated With [^{14}C]AVM.

Sample[a/]	Dry wt (g)	ppm[b/] (±SD)
main stem/branches	510.0	0.071 ± .005
roots	155.7	0.025 ± .003
leaves on plant	223.7	0.396 ± .027
leaves on ground	311.5	1.045 ± .018
bract/calyx	229.4	0.229 ± .015
whole seed	357.3	0.050 ± .003
seed hull		0.046 ± .002
seed meat		0.044 ± .004
lint	271.0	0.038 ± .003
soil 0.0- 7.5cm	--	0.002 ± .001
7.5-15.0cm	--	0.000
15.0-25.0cm	--	0.000

[a/]These samples were from plants sprayed twice with [^{14}C]AVM. Subsamples of plants collected and analyzed after one treatment contained the following levels (ppm) of radiocarbon: main stem/branches - 0.052 ± 0.006, roots - 0.022 ± 0.005, dried leaves - 0.245 ± 0.005.
[b/]Ppm of [^{14}C]AVM equivalents.

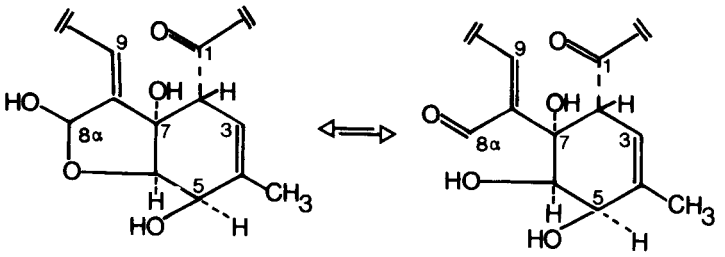

FIG. 4. Subsection of the molecule of [^{3}H]AVM degradation product 7, showing the 8α-hydroxy and furan ring-opened aldehyde derivatives of AVM in equilibrium mixture.

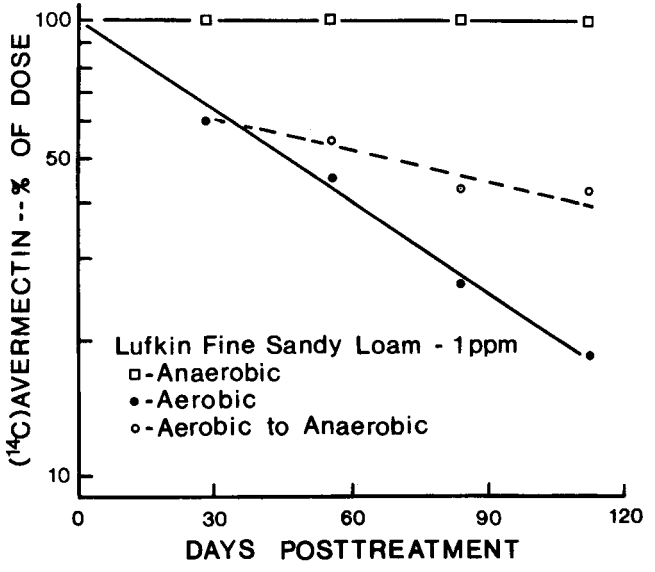

FIG. 5. Rate of aerobic and anaerobic degradation of [^{14}C]AVM after application to Lufkin fine sandy loam at a rate of 1.0 ppm.

Bull et al. (1984) conducted studies to assess the possible uptake by plants of residues in soil treated with AVM. For this, cotton seeds were grown either in samples of loam that had been freshly treated with [^{3}H]AVM at a rate of 10 ppm or in samples of solvent-extracted or unextracted treated loam that had been aged for 3 months in a greenhouse. Seedlings were harvested when they began to form the first true leaves and then dried and analyzed by oxygen combustion. Results indicated very little radioactivity (ca. 0.1 ppm) was taken up and translocated into the stems and leaves of the seedlings. Higher concentrations were measured in samples of roots (ca. 3 ppm) but it is not known if this radioactivity was inside root tissue or simply adsorbed on the surface. As expected, the lowest levels of radioactivity were in seedlings (0.02 ppm in stems and leaves, 0.15 ppm in roots) grown in soil that had been extracted with solvents prior to planting.

In another field test, Bull et al. (1984) treated small plots of vigorously-growing Bermuda grass with a formulation of AVM that is being evaluated for fire ant control. This was a granular formulation consisting of pregel defatted corn grits (69.989%), soybean oil (30%), and [^{14}C]AVM (0.011%). These granules were applied manually to the soil surface in the plots at three different rates--125, 375, and 1250 mg AI/ha. At weekly intervals through 8 wk posttreatment, all the grass in each plot was harvested. The samples were dried and milled, and then subsamples (250 mg/each) were analyzed for radiocarbon by oxygen combustion. No radioactive residues were detected in any grass samples, nor was any radiocarbon detected in soil samples collected and analyzed at the end of the 8-wk experimental period. It is, however, important to mention that 16 days of measurable rainfall totalling 26 cm occurred during the test.

CONCLUSIONS

These studies and those reported by Bull et al. (1984) have shown that under aerobic conditions, AVM degrades progressively at fairly rapid rates in representative agricultural soils. Circumstantial evidence suggests that rates of AVM degradation in the field would be more rapid than those seen under laboratory conditions. This apparent instability in soil, coupled with the poor leaching potential of AVM, suggests that possible contamination of soil surfaces with AVM during field application would not lead to serious problems with persistent residues or with contamination of surface or subterranean water. These studies further suggest that spray applications of AVM on cotton plants or broadcast applications of granular formulations of the compound on the soil at the recommended rates probably will not result in the development of intolerable residues in plant materials that might eventually be consumed by livestock or humans.

LITERATURE CITED

Bull, D. L., and G. W. Ivie. 1982. Fate of O-[4-[(4-chlorophenyl)thio]phenyl] O-ethyl S-propyl phosphorothioate (RH-0994) in soil. J. Agric. Food Chem. 30:150-5.

Bull, D. L., G. W. Ivie, J. G. MacConnell, V. F. Gruber, C. C. Ku, B. H. Arison, J. M. Stevenson, and W. J. A. VandenHeuvel. 1984. Fate of avermectin B_{1a} in soil and plants. J. Agric. Food Chem. 32:94-102.