Disposition of Cyromazine in Plants under Environmental Conditions†

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The disposition of cyromazine (Trigard) in plants under environmental conditions and the role of photodegradation in the formation of melamine were studied. Cyromazine was applied to three *Brassica sp.* vegetables, and residues were analyzed by high-pressure liquid chromatography. After multiple applications, both cyromazine and melamine were detected. Melamine residues constituted 14–36% of the total residues. After one application, the average half-life for cyromazine was 7 days. Melamine levels increased (3–5-fold) with time. Both compounds were readily removed by water. Percent residues removed decreased with increased elapsed time between application and washing. The role of photodegradation in the formation of melamine was studied by in vitro experiments. In glass dishes, cyromazine was dealkylated with the formation of melamine. Since the amount of melamine formed did not account for all of the lost cyromazine, volatilization of cyromazine and degradation to other metabolites is likely.

Cyromazine (*N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine, Trigard; Figure 1) is a systematic insecticide and an insect growth regulator effective against fly larvae and leaf miners. Its primary target for activity is the insect cuticle (Binnington et al., 1987, Friedel et al., 1988). Unlike other compounds in this triazine class, it lacks herbicidal activity.

The environmental fate of certain triazines, such as atrazine and simazine, has been reviewed. In general, they are metabolized by biological systems including bacteria, plants, and mammals (Fishbein, 1975) as well as degraded by photochemical reactions (Jordan et al., 1970; Marcheterre et al., 1988). The major degradative reactions are dealkylation and hydroxylation, and they are involved in the development of resistance to atrazines (Shimbabukuro, 1967; Shimbabukuro and Swanson, 1969). In contrast, there are limited data published on the environmental fate of cyromazine. Bacteria *Pseudomonas spp.* have been shown to utilize cyromazine as a nitrogen source (Cook et al., 1984; Cook and Hutter, 1981). Dealkylation of cyromazine may lead to the formation of melamine (1,3,5-triazine-2,4,6-triamine; Figure 1). Most of the published studies concerning melamine were initiated because of its use in melamine–formaldehyde amino resins and in fire-retardant finishes. Early studies in rats showed that melamine may be carcinogenic (Melnick et al., 1984); however, the toxicity may be due to a secondary effect from the development of renal bladder stones induced by melamine (Heck and Tyl, 1985). Melamine was not mutagenic in the Ames assay (Zeiger, 1987). Current Environmental Protection Agency guidelines require the analysis of both cyromazine and melamine for the establishment of tolerances in food commodities (*Fed. Regist.*, 1984).

The purpose of the present study was to investigate the disposition of cyromazine in crops under environmental conditions. In particular, we will study (1) the dealkylation of cyromazine after multiple and single applications, (2) the binding of residues to foliage, and (3) the role of photochemical reactions in the degradation of cyromazine. Three *Brassica spp.* vegetables, bok choy, napa cabbage, and Chinese mustard, were studied due to current interest in expanding the use of cyromazine on these minor crops.

EXPERIMENTAL SECTION

Materials. Analytical standards of cyromazine (purity 97.98%) and melamine (purity 99%) were obtained from the EPA Pesticides and Industrial Chemicals Repository (Research Triangle Park, NC). Stock solutions were made in methanol. Trigard 75W containing 75% cyromazine was obtained from Ciba-Geigy (Greensboro, NC). All other reagents were HPLC grade or analytical grade (Fisher Scientific Co., Springfield, NJ; Baxter Healthcard Corp., Muskegon, MI).

Multiple-Application Field Trials. Cyromazine (Trigard 75W) was applied to mature plants of bok choy, napa cabbage, and Chinese mustard in separate plots at a rate of 0.25 lb of active ingredient (AI)/acre with use of a CO_2 backpack sprayer. The trials were conducted at different times between 1987 and 1988 in Florida. Applications were made at weekly intervals for 7 weeks, and plants were harvested 7 and 14 days after the last application (preharvest interval, PHI). Each treatment condition was replicated in four plots. Four plants were harvested from each plot and frozen at −30 °C until analysis.

Single-Application Field Trials. Cyromazine (Trigard 75W) at a rate of 0.50 lb of active ingredient/acre were applied to the three vegetables planted in separate plots. The trials were conducted at the same time from April to May, 1989, at farms in the same vicinity as for the trials with multiple-application trials. Four plants were harvested at 2 h, 1 day, and 7 days after the application. Of the four plants collected per time period, two were subjected to washing by dipping ten times into a bucket containing 3–4 gal of fresh tap water. The napa and Chinese mustard were collected and washed as whole plants. After washing, the bok choy plants were separated into the top leafy portion (blade) and the bottom portion (petiole). The samples were frozen at −30 °C until analysis.

Residue Analysis. Each replicate was chopped in a food chopper, and 25.0-g subsamples were analyzed. The method

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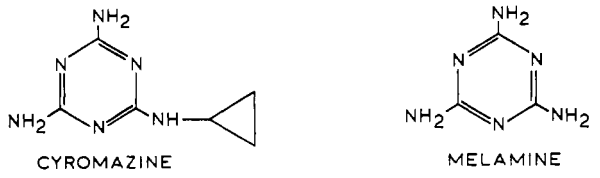

Figure 1. Chemical structures of cyromazine and melamine.

for the extraction of cyromazine and melamine was derived from the *Pesticide Analysis Manual* (1986) and Bardalaye et al. (1987). Briefly, the subsample was refluxed in 200 mL of methanol–water (95:5) for 1–2 h. After filtration, the sample volume was reduced and acidified with 0.2 N HCl. The aqueous solution was washed with dichloromethane once and then with hexane. The aqueous solution was adjusted to pH 3–3.5 with 1 N NaOH prior to column cleanup procedures.

An aliquot of the sample extract was loaded onto a cationic exchange column (Bond Elut; SCX, 500 mg/2.8 mL; Analytichem International, Harbor City, CA) and eluted with 5% NH_4OH in methanol. The sample was further cleaned up with an anionic exchange column (Bond Elut; SAX, 500 mg/2.8 mL) and eluted with water. The eluate was evaporated to dryness under a stream of nitrogen, and the final residue was dissolved in methanol. The samples were analyzed by high-pressure liquid chromatography (HPLC).

High-pressure liquid chromatography of Trigard in solution used for the single-application trials showed <0.4% contamination with melamine.

Storage stability and method recovery studies were performed by fortifying subsamples with analytical grade cyromazine and melamine of appropriate concentrations. For the recovery studies, the samples were processed 1 h after fortification. The average recovery of both compounds (0.1–5.0 ppm fortification) ranged from 91 to 121%. Storage of samples for up to 4 months at −30 °C did not result in any significant loss; the average recovery of cyromazine and melamine ranged from 74 to 97 and 69 to 88%, respectively, for all three crops.

Photodegradation Study. One milliliter of analytical cyromazine (1.5, 6, or 30 nmol), formulation cyromazine (6 or 30 nmol), or melamine (2, 8, or 40 nmol) dissolved in methanol was placed on Pyrex (9.0-cm diameter) glass Petri dishes. The methanol was allowed to evaporate in a fume hood, and the dishes were then placed in a greenhouse in direct sunlight. Additional dishes with 30 nmol of cyromazine were covered with aluminum foil. After specified time periods (2 h, 1 day, 1 week, 2 weeks, or 3 weeks), the dishes were removed and rinsed twice with 3 mL of methanol. The volume was reduced to 1 mL, and the sample was analyzed by high-pressure liquid chromatography. Four studies were conducted between December 1988 and May 1989.

High-Pressure Liquid Chromatography. Cyromazine and melamine residues were analyzed on a Perkin-Elmer liquid chromatograph equipped with a UV detector set at 215 nm and an amino column 25 cm × 4.6 mm (i.d.) (Alltech Associates Inc., Deerfield, IL). The mobile phase was 96% acetonitrile and 4% water, and the flow rate was 1 mL/min. The average retention times for cyromazine and melamine were 7 and 12 min, respectively.

Mass Spectrometry. The presence of melamine in the samples was verified by gas chromatography/mass spectroscopy (GC/MS) of the HPLC fraction containing melamine as identified by retention time. The conditions were essentially the same as those reported earlier (Toth and Bardalaye, 1987). The sample was chromatographed on a 30 m × 0.25 mm DB-17 capillary column (J&W Scientific, Folsom, CA), interfaced directly to the ion source of a Finnigan Model 4500 quadrupole GC/MS system (San Jose, CA). The injection was splitless with injection port temperature of 250 °C, and the oven temperature program was 150 °C (0.5 min) to 280 °C at 10 °C/min with 1-min hold at 280 °C.

Electron impact mass spectra were obtained with a source temperature of 150 °C and electron energy of 70 eV. The scan limits were 40–250 Da, and the scan rate was 2 scans/s.

Table I. Residue Levels of Cyromazine and Melamine in Bok Choy and Napa and Chinese Mustard Sprayed with Trigard at 0.25 lb of Active Ingredient/Acre Weekly for 7 Weeks and Harvested 7 or 14 Days after the Last Application (PHI)

commodity	PHI, days	residue level,[a] nmol/g		% total residue[b]	
		cyromazine	melamine	cyromazine	melamine
bok choy	7	16.1 ± 4.4	6.1 ± 1.8	73	27
	14	8.9 ± 0.6	5.0 ± 1.1	64	36
napa cabbage[c]	14	8.1 ± 1.6	3.0 ± 0.5	73	27
Chinese mustard	7	23.0 ± 5.6	3.7 ± 1.1	86	14
	14	16.1 ± 4.3	3.2 ± 0.8	84	16

[a] Residue levels are expressed as nanomoles/gram wet weight of crop, and values are means ± standard deviations from four samples. [b] Total residue was calculated as the sum of cyromazine and melamine levels. [c] Samples for PHI at 7 days were not available.

RESULTS AND DISCUSSION

After multiple applications of Trigard, both cyromazine and its dealkylated product, melamine, were detected in the three crops studied (Table I). Since the trials were conducted at different times, differences in the residue levels among crops may be due to environmental conditions such as rainfall and sunlight intensity as well biological and morphological factors. Within the data for each crop, there was a time-related decrease in the cyromazine residue level depending on the preharvest interval. Fourteen days after the last application, cyromazine levels in bok choy and Chinese mustard were 55% and 70%, respectively, of those obtained after 7 days.

Melamine was detected in all crops, and its identification by HPLC was confirmed by GC/MS. The retention time and electron impact mass spectrum of the resulting GC peak from the treated sample matched those from analytical standard solution (data not shown). The residue levels ranged from 3.0 to 6.1 nmol/g and accounted for 14–36% of the total residues calculated as the sum of cyromazine and melamine. In contrast to the results for cyromazine, the amount of melamine did not change significantly with longer PHI. Other metabolic pathways may be involved in addition to degradation in the accumulation of melamine residues.

To further determine the fate of cyromazine in these crops, a second trial was conducted with one application to all three crops under similar environmental conditions. The application rate was increased to 0.5 lb of AI/acre to assure adequate levels of residues for the studies. At this rate, no phytotoxicity was observed. Cyromazine and melamine were detected in all three crops (Figures 2 and 3). Bok choy petiole and blade were analyzed separately to locate the primary site of deposit of cyromazine. Petioles, which are usually shielded by the blades, contained relatively low levels of cyromazine at less than 2.5 nmol/g compared to the blades at 37.3–90.9 nmol/g. Also, residue levels were influenced by the sample composition. The blade, on a per gram basis, contains more surface area than the petiole. The greater exposed surface area would account for the higher residue levels found in samples containing the blade than those with the petiole or the two parts.

Since the bok choy was analyzed as separate parts, the residue levels on the petiole and blade could not be compared to those for the whole plants of napa cabbage and Chinese mustard. Examination of the residue data for napa cabbage and Chinese mustard showed that the cyromazine and melamine levels in these plants were similar. The difference in the configuration between the tight-

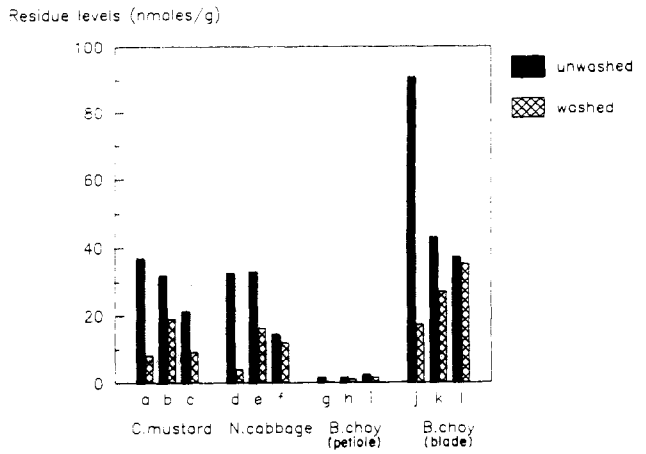

Figure 2. Disappearance of cyromazine in Chinese mustard, napa cabbage, and bok choy after a single application of Trigard at 0.5 lb of AI/acre. Plants were harvested 2 h (a, d, g, j), 1 day (b, e, h, k), or 7 days (c, f, i, l) after application. They were either washed or unwashed before processing for analysis by HPLC. Residues levels are expressed as nanomoles/gram wet weight (100 nmol/g = 16.7 ppm), and values are means of duplicate determinations.

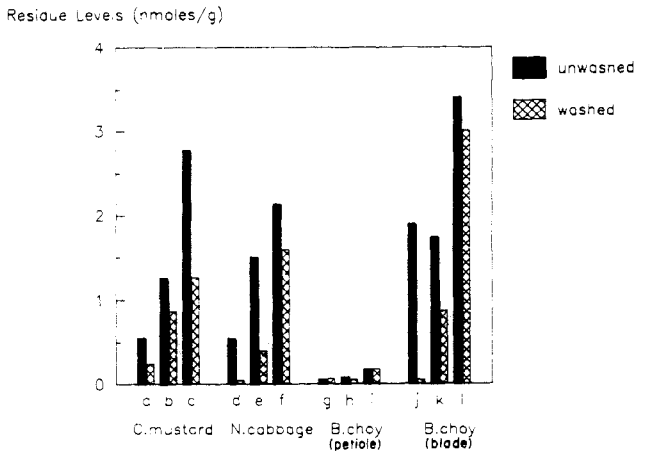

Figure 3. Formation of melamine in Chinese mustard, napa cabbage, and bok choy after a single application of Trigard at 0.5 lb of AI/acre. Plants were harvested 2 h (a, d, g, j), 1 day (b, e, h, k), or 7 days (c, f, i, l) after the application. They were either washed or unwashed before processing for analysis by HPLC. Residues levels are expressed as nanomoles/gram wet weight, and values are means of duplicate determinations.

headed napa cabbage and open-headed Chinese mustard apparently did not have any effect on the residue levels.

In all crops, cyromazine remaining in the plant declined with time (Figure 2), with an average half-life of about 7 days. The decrease in concentration may be due to growth dilution, rainfall, volatilization, and degradation. Melamine residues were detected in all samples. In contrast to the decline of cyromazine with time, melamine levels increased with time (Figure 3). Within 7 days, there was a 3–5-fold increase in melamine residues. However, in all cases, melamine was less than 11% of the total residues.

Further studies were conducted to determine whether any of the residues were removable by ordinary washing procedures. In general, washing resulted in a decrease of both cyromazine and melamine in the plants (Figures 2 and 3). The extent of loss was dependent on the PHI as a lower percentage of residues was removed by the washing procedure at the longer PHI. Two hours after application, approximately 80% of the residues was removed from the crop matrix. At later time periods,

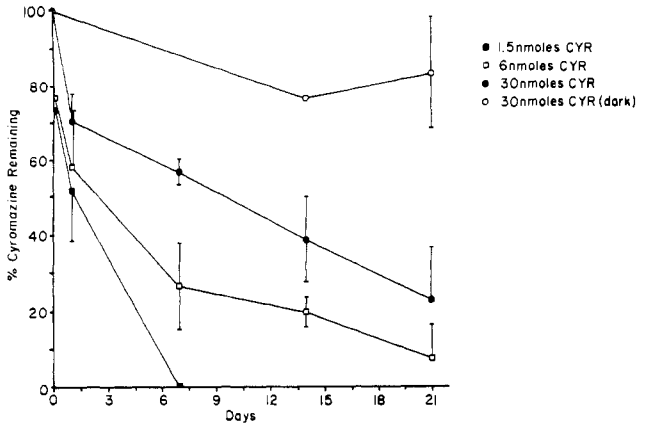

Figure 4. Time course of cyromazine disappearance from glass dishes. Cyromazine (CYR) was added to Petri dishes at 1.5, 6, or 30 nmol and exposed to sunlight or at 30 nmol and covered with foil. Amount of cyromazine remaining is expressed as a percent of the initial amount. Values presented are means ± SD of two to six determinations.

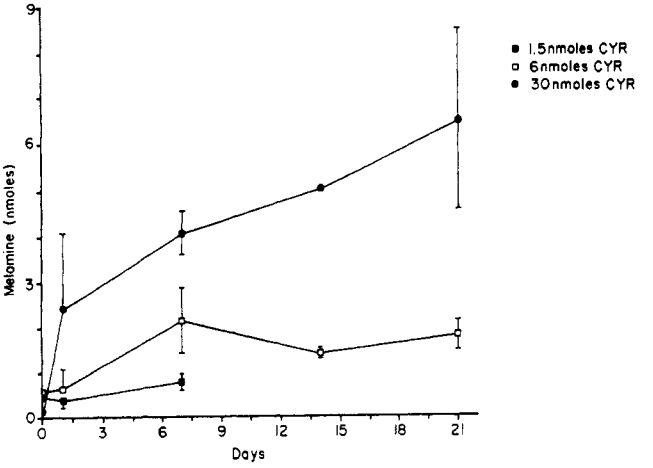

Figure 5. Time course of melamine formation from cyromazine. Cyromazine (CYR) was added to Petri dishes at 1.5, 6, or 30 nmol and exposed to sunlight. Amount of melamine formed is expressed as nanomoles. Values presented are means ± SD of two to six determinations.

the incorporation of cyromazine into molecules on the plant surface and/or into the plant may have protected cyromazine from removal by the washing procedure. Kerler and Schonherr (1988a,b) have shown that the permeance of several triazines into the leaf cuticular membrane is dependent on the chemical lipophilicity.

The formation of melamine in these vegetables treated with Trigard may be mediated by plant enzymatic reactions or photochemical processes. Since it is known that triazines undergo dealkylation by photodegradation, experiments using glass Petri dishes to simulate leaf surfaces were set up to examine this reaction. Results showed that the degradation of cyromazine was both time- and concentration-dependent (Figure 4). At the lowest concentration (1.5 nmol) studied, all the added substrate was degraded in 7 days. In contrast, at 30 nmol, 22% of added cyromazine remained after 3 weeks. This inverse concentration effect is probably due to a physical phenomenon where molecules below the top layer are shielded (Jordan et al., 1965). There was no difference in the extent of degradation of cyromazine in the form of analytical standard or as formulation in Trigard, indicating that the inert ingredients in the formulation (25% by weight) did not have any influence on the reaction (data not shown).

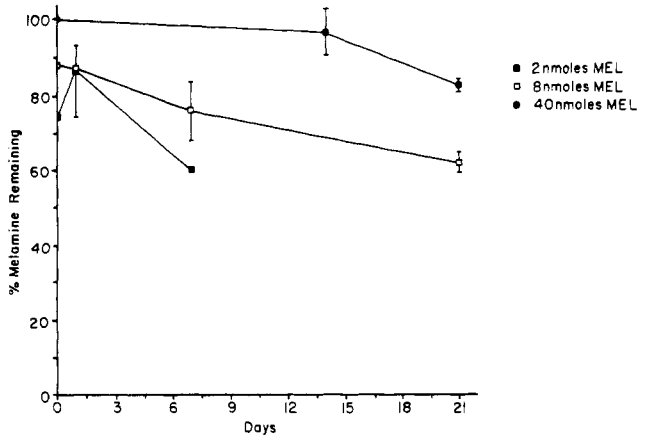

Figure 6. Time course of melamine disappearance from glass dishes. Melamine (MEL) was added to Petri dishes at 2, 8, or 40 nmol and exposed to sunlight. Amount of melamine remaining is expressed as a percent of the initial amount. Values presented are means ± SD from two experiments.

The loss of cyromazine may be attributable to photochemical reactions since there was little loss of cyromazine in dishes protected from sunlight. Formation of melamine from the dealkylation of cyromazine was detected. The appearance of melamine was both time- and concentration-dependent (Figure 5). Two hours after exposure to sunlight, 0.14 nmol of melamine was detected in the dishes fortified with 1.5 nmol of cyromazine. By 7 days, only melamine (0.80 nmol) was recovered while cyromazine was not detectable. Increasing the cyromazine from 1.5 to 30 nmol resulted in higher levels of melamine. The amounts of melamine recovered after 7 and 21 days were 4.0 and 6.5 nmol, respectively. On the basis of the extent of cyromazine degradation (Figure 4), the amount of melamine recovered did not stoichiometrically account for the cyromazine loss. The possibilities are that cyromazine may be converted to other products in addition to melamine and/or that melamine once formed may be further degraded.

Volatilization of cyromazine may be an additional factor under the photodegradation study conditions since the vapor pressure of cyromazine, at less than 1.6×10^{-6} mmHg (20 °C), is similar to those reported for *s*-triazines (Jordan et al., 1970). Jordan et al. (1970) have shown that several triazines were volatile under environmental conditions. At 35 °C, approximately 15% of the atrazine (vapor pressure 3.0×10^{-7} mmHg) applied to the soil was lost after 1 day.

The possibility that melamine may be degraded into other metabolites was examined by plating melamine on the Petri dishes and exposing them to sunlight. There was a time- and concentration-dependent disappearance of melamine (Figure 6). Similar to the findings for cyromazine, loss of melamine was greater at the lower concentrations (2 and 8 nmol) than at the higher concentration (40 nmol). After 3 weeks, 60% of the added amount (8 nmol) remained on the dish. Although Jutzi et al. (1982) observed that the bacteria *Pseudomonas spp.* can utilize melamine, resulting in the formation of ammelide, ammeline, and cyanuric acid, bacterial biotransformation was unlikely in the glass dish system. Photodegradation of melamine has not been reported. The procedures used in this study would preclude the detection of melamine metabolites (ammelide, ammeline, cyanuric acid) since they are relatively insoluble in organic solvents (Beilstein et al., 1981).

Under field conditions, foliar application of Trigard on *Brassica* vegetables resulted in both cyromazine and melamine residues. Some of the residues were readily removed by washing with water, indicating that at least in the early time period, e.g., 2 h after application, most of the residues were not absorbed into the plant. The residues remaining in the crop matrix after washing may be due to both the incorporation into plant components of cyromazine and melamine produced by photochemical reactions and/or by plant biotransformation systems. While bacterial and plant degradation processes cannot be ruled out in the field-treated samples, we have demonstrated that photochemical reactions (dealkylation) and volatilization may play a role in the disposition of cyromazine on leaf surfaces.

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Fate of Avermectin B_{1a} in Rats

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Male and female rats were administered [^{3}H]avermectin B_{1a} or a mixture of [^{3}H]- and [^{14}C]avermectin B_{1a} as a single oral dosage at 1.4 or 0.14 mg/kg. Most of the dose (69-82%) was recovered in the feces, with 1% or less found in the urine. The total residue levels in liver, kidney, muscle, and fat tissues were <5.3 ppm at 1 day after dosing and essentially depleted within 7 days after dosing. For all tissues analyzed, the depletion half-life of the total radioactive residue was approximately 1.2 days, while the half-life of avermectin B_{1a} was between 0.6 and 1.0 day. The tissue residue was shown to be qualitatively similar between the tissue type, dose, sex, pretreatment with or without unlabeled avermectin B_{1a}, and label (^{3}H or ^{14}C). A major metabolite (3″-desmethyl) and a minor metabolite (24-hydroxymethyl) isolated and identified from rat liver microsomal incubations of avermectin B_{1a} were identified in the rat tissues. These two metabolites and avermectin B_{1a} accounted for >85% of the tissue residue. The fate of [^{3}H]avermectin B_{1a} was the same as the fate of [^{14}C]avermectin B_{1a}, demonstrating the stability of the ^{3}H label on avermectin B_{1a} and the validity of its use in animal metabolism studies.

Avermectins, a new class of biological agents that contain a macrocyclic lactone, are produced by *Streptomyces avermitilis* (Burg et al., 1979). Their structures have been elucidated (Albers-Schönberg et al., 1981), and some of the biological activities have been reported (Campbell et al., 1983). Ivermectin (22,23-dihydroavermectin B_1) is a member of the avermectin class that is registered as an anthelmintic for use as an animal health drug. Abamectin (avermectin B_1) is the commercial product that is being developed by Merck & Co. Inc. as an acaricide/insecticide. The major active ingredient in abamectin is avermectin B_{1a} (Figure 1). This report describes the findings from rat metabolism studies with avermectin B_{1a}. Some of these data have been presented previously (Maynard et al., 1985a).

The major objective of this study was to describe the fate of avermectin B_{1a} in rats. The total residue levels in tissues were determined to evaluate the distribution and elimination of the radiolabeled avermectin B_{1a} residues. The amount of avermectin B_{1a} and the identity of the metabolites found in the liver, kidney, muscle, and fat tissues were determined. The effect of dose level, pretreatment with unlabeled avermectin B_{1a}, sex of the rats, and ^{3}H vs ^{14}C labeling of avermectin B_{1a} on the metabolic fate of avermectin B_{1a} were investigated.

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Male and female rats were administered a single oral dose at 1.4 and 0.14 mg/kg of ^{3}H-labeled avermectin B_{1a} or a mixture of ^{3}H- and ^{14}C-labeled avermectin B_{1a}. One group of animals received 14 single daily oral doses of unlabeled avermectin B_{1a} at 0.14 mg/kg before receiving a single dose of [^{3}H]avermectin B_{1a} at 0.14 mg/kg. Animals were sacrificed at 1, 2, 4, and 7 days after dosing; 15 tissue and fluid samples were taken, which accounted for the entire animal less skin and tail. Tissue extracts were analyzed for avermectin B_{1a}, and two metabolites were identified by cochromatography with standards.

MATERIALS AND METHODS

Animal Treatment. Male (ca. 295 g) and female (ca. 217 g; nulliparous and nonpregnant) CRCD rats were obtained from Charles River, MA. Animals were indiscriminately selected for grouping and administered a single oral (gavage) dose of [^{3}H]avermectin B_{1a} (labeled at carbon 5) or a mixture of [^{3}H]- and [^{14}C]avermectin B_{1a} (labeled at carbons 3, 7, 11, 13, and 23). Two dosage levels, 1.4 and 0.14 mg/kg, were employed. At the 1.4 mg/kg dosage level, one group of rats received [^{3}H]avermectin B_{1a} (group 1) and another group received a mixture of [^{3}H]- and [^{14}C]avermectin B_{1a} (group 4). At the 0.14 mg/kg dosage level, the rats received [^{3}H]avermectin B_{1a} either alone (group 2) or preceded by (group 3) 14 single daily oral doses of unlabeled avermectin B_{1a} at 0.14 mg/kg. The final specific activities of the [^{3}H]avermectin B_{1a} alone and mixed with [^{14}C]avermectin B_{1a} were 350 and 33 μCi/mg, respectively. The final specific activity of the [^{14}C]avermectin B_{1a} was 16.4 μCi/mg. The labeled avermectin B_{1a} and unlabeled

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