IV). Concentrations of aminocarb in soil, as observed previously (Sundaram et al., 1976), were much lower than those in the corresponding litter samples. The highest was 0.051 ppm (wet weight), detected in soil samples collected 2 h after the first application of 180 D (Table IV). The residues persisted in soil for about 5 days. Aminocarb was not detected in any of the soil samples collected 8 days after the second application. The difference in persistence of aminocarb in litter and in soil could be partly explained by the difference in acidity of the two matrices. The litter, which was composed of fallen needles, twigs, and organic detritus, was definitely acidic (pH 5.4), whereas the soil (sandy loam) had mild acidity with a pH of 6.3. It has been shown that the hydrolysis of aminocarb is pH dependent and the hydrolysis rate is much higher at elevated pH. According to the studies of Murphy et al. (1975) and Tessier et al. (1978), the estimated half-lives for aminocarb in aqueous buffers at 20 °C were 28.5 days at pH 7.0 and 90.2 days at pH 5.0. Since the pH of the soil was higher than that of the litter, hydrolytic degradation of aminocarb in soil may have been at a faster rate than that in litter.

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Registry No. Aminocarb, 2032-59-9.

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Bound Residues of Deltamethrin in Bean Plants

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Deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-*cis*-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropanecarboxylate] labeled with ^{14}C at the methyl or benzylic position formed unextractable (bound) ^{14}C residues in bean plant shoots. The amount of bound ^{14}C residues formed was higher in the benzylic label deltamethrin treated plants. Deltamethrin and a number of metabolites, present in the plant as bound ^{14}C residues, were released and identified with the high temperature distillation technique followed by thin-layer and gas chromatographic analysis, whereas a major portion of the unextractable products remaining was of unknown composition. A small proportion of the bound ^{14}C residues from plant tissue was released after incubation with enzymes.

Deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-*cis*-2,2-dimethyl-3-(2,2-dibromovinyl)-2,2-cyclopropanecarboxylate] is a new synthetic pyrethroid insecticide that is active against numerous species of insects when applied on field crops. Recent interest in deltamethrin metabolism in plants has centered primarily on the fate of this insecticide in cotton and bean plants (Ruzo and Casida, 1979; Cole et al., 1982). A large number of extractable metabolites have been successfully isolated and characterized after application of deltamethrin to cotton and bean leaves. However, a portion of the insecticide or its products were unextractable (bound), and greater quantities of these bound residues were present in outdoor samples (Ruzo and Casida, 1979). These bound and usually chemically unidentified residues may, however, be important. For example, they might become released on digestion of the contaminated food or their accumulation may be of significance to crops growing in the treated soils. The present study reports the formation of bound residues in bean plants when treated with radiolabeled deltamethrin and attempts to identify some of these residues. Crop material containing bound residues from the treated plants was also subjected to enzyme hydrolysis to determine the release of bound radioactivity.

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MATERIALS AND METHODS

Chemicals. Deltamethrin (^{14}C labeled and unlabeled) was a gift from Roussel-Uclaf-Procida through its subsidiary Hoescht of Canada, Ltd. The position of labeling,

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radiochemical purity (as determined by thin-layer chromatography), and specific activity were as follows: methyl labeled, 98%, and 56 mCi/mmol; benzylic labeled, 96%, 58 mCi/mmol. A portion of methyl- and benzylic-labeled material was individually mixed with purified deltamethrin and dissolved in acetone to give concentrations of 484 μg/mL (4.64×10^5 dpm) and 483 μg/mL (5.43×10^5 dpm), respectively. Base-catalyzed hydrolysis of deltamethrin followed by purification by thin-layer chromatography gave pure 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (Br_2CA). 3-Phenoxybenzaldehyde (PBald), 3-phenoxybenzoic acid (PBacid), and 3-phenoxybenzyl alcohol (PBalc) were purchased from Aldrich Chemical Co., Milwaukee. 3-(4-Hydroxyphenoxy benzoic acid (HO-PBacid) and 3-(4-hydroxyphenoxy)benzyl alcohol (HO-PBalc) were prepared following the procedure of Unai and Casida (1977).

β-Glucosidase was purchased from Nutritional Biochemicals Corp., Cleveland, OH, cellulase from All Japan Biochemicals Corp., and protease (*Streptomyas grieseus*) from Sigma Chemical Co., St. Louis, MO.

Plant Treatment. Seafarer bean plants were grown in Hoagland nutrient solution (500 mL/jar) for 30 days in a growth chamber at 21 °C. A day length of 12 h was maintained with artifical light (1.8×10^4 lx). The plants were then injected into the stem with methyl-labeled (20 μg, 0.05 μCi) or benzylic-labeled (22 μg, 0.04 μCi) deltamethrin in acetone (20 μL) per plant. The control plants were injected with acetone (20 μL) only. Ten days after treatment the plants were harvested and sectioned into shoots and roots. The latter was washed with cold water, and the shoots and roots were stored at −20 °C.

Generation and Determination of Bound ^{14}C Residues in Plant Tissues. The plant tissues were thawed at room temperature, cut into small pieces, and extracted twice by homogenation (polytron) in ice-cold ether (1:10 w/v) for 1 h. The homogenate was filtered under suction and the sample residue washed with ether (×3). The insoluble material from shoot or root tissue was then soaked and occasionally shaken in 150 mL of acetonitrile–chloroform (2:1) for 16 h. The mixture was filtered under suction and washed with acetonitrile–chloroform (2:1) mixture (×3). This procedure removed all the extractable ^{14}C as further extraction of the insoluble material with other solvents such as acetone, methanol, or aqueous acidic methanol did not result in any further release of ^{14}C. The filtrates and washes were combined, concentrated to a small volume, and analyzed for total extractable ^{14}C.

The extracted shoot or root samples containing only bound ^{14}C residues were dried at room temperature for 24 h and divided into three parts. One part of the tissue was combusted to $^{14}CO_2$ to determine the total bound ^{14}C. The other part was used to release the bound ^{14}C residues by the high-temperature distillation (HTD) technique (Khan and Hamilton, 1980). The released material was collected in four traps, the first two contained acetone, the third contained methanol, and the last trap contained oxisorb to trap any released $^{14}CO_2$. The distillates were concentrated to a small volume and subjected to thin-layer chromatographic (TLC) separation and finally analyzed by TLC, gas chromatography (GC), and liquid scintillation counting (LSC). The third portion of the extracted tissue was used for enzyme incubation study as described below.

In Vitro Incubation with Enzyme. Ground plant tissue (200 mg) containing bound ^{14}C residues was suspended in 10 mL of appropriate buffer containing β-glucosidase (80 mg), cellulase (100 mg), or protease (80 mg) in glass-stoppered Erlenmeyer flasks (50 mL) and incubated at 37 °C for 48 h. Control flasks contained only appropriate buffer and plant tissues but no enzyme. At the end of the incubation the mixture in the flask was acidified with concentrated HCl (pH 2) and extracted with ether (4 × 5 mL), and then the released ^{14}C was determined.

Determination of Radioactivity. Combustion of dried plant tissues was done in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. The $^{14}CO_2$ was absorbed in and admixed with an appropriate volume of oxisorb and oxiprep (Packard Instrument Canada, Ltd.). Aliquots of various extracts and $^{14}CO_2$ released by combustion were analyzed in a Beckman Series 8000 liquid scintillation spectrometer, using an external standard and correcting the data for quenching. In some instances the dark color in solutions from the HTD distillate caused interferences in the LSC determination. An aliquot of these solutions (0.5 mL) was absorbed on ground filter paper, combusted to yield $^{14}CO_2$, and analyzed as described before.

Chromatography and Analysis. The concentrated solution from the HTD distillate was directly applied at the bottom of the central linear region of the TLC plate (20 × 20 cm precoated silica gel GF chromatoplates with 1.0-mm gel thickness, Analtech, Inc., Newark, DE) while the two side linear regions were used for reference authentic compounds. The plate was developed with a hexane–benzene (2:8) solvent system 3 times. The developed TLC plate was divided into three fractions: (i) R_f 0.85, deltamethrin; (ii) R_f 0.71, PBald; (iii) R_f origin 0.33 for Br_2CA, PBacid, PBalc, HO-PBacid, and HO-PBalc. Fractions i and ii were scraped from the glass support and extracted with ether and then with methanol. The combined extracts were concentrated and aliquots were analyzed by LSC while the remaining portions were subjected to TLC using toluene–hexane–acetic acid (15:3:2) and formic acid saturated toluene–ether (10:3) developing solvent systems for further identification. The TLC plate with fraction iii was further developed by using the toluene–hexane–acetic acid (15:3:2) solvent system. The gel region (R_f 0.06–0.29 for [*benzyl*-^{14}C]deltamethrin; R_f 0.39–0.51 for [*methyl*-^{14}C]deltamethrin) was extracted with methanol, and the extract was concentrated to a small volume and analyzed by TLC for identification using toluene–hexane–acetic acid (15:3:2) and formic acid saturated toluene–ether (10:3) solvent systems. The acid fractions (R_f 0.68, Br_2CA; R_f 0.51, PBacid; R_f 0.31, HO-PBacid) from the TLC plates of the latter solvent system were extracted with methanol and derivatized to form pentafluorobenzyl esters (Ehrsson, 1971; Saleh et al., 1980). The derivatized material was subjected to TLC separation using a hexane–benzene (1:1) developing solvent system (R_f 0.53, Br_2CA ester; R_f 0.32, PBacid ester; R_f 0.27, HO-PBacid ester), the fractions were extracted with ether, and ether extract was evaporated just to dryness, and the residue was dissolved in hexane and analyzed by LSC and GC.

The gas chromatograph was a Varian Model 3700 equipped with a ^{63}Ni detector. The column was 2.5 m × 0.25 cm i.d. glass tube packed with 3% SE-30 on Chromosorb WHP. The operating conditions were as follows: column temperature 200 °C for HO-PBacid and PBacid esters and 180 °C for Br_2CA ester; detector and injector port temperatures 340 and 240 °C, respectively. The carrier gas nitrogen flow rate was 30 mL/min.

RESULTS AND DISCUSSION

Table I shows the distribution of ^{14}C residues in bean plants. The bean plants contained about 35% and 85% of the ^{14}C applied in the form of methyl- and benzylic-

Table I. Extractable and Bound ^{14}C Residues in Bean Plants Treated with [^{14}C]Deltamethrin

	extractable ^{14}C, %		bound ^{14}C, %	
plant tissue	methyl label	benzylic label	methyl label	benzylic label
shoot	31.4	73.5	3.0	9.7
root	0.6	1.5	0.1	0.3

Table II. Bound ^{14}C Residues in Shoots of Bean Plants Treated with [^{14}C]Deltamethrin

	% of the released ^{14}C	
compound	methyl label	benzylic label
deltamethrin	14	11
Br_2CA	13	
PBald		6
PBacid		9
PBalc		6
HO-PBacid		4
polar products[a]	11	12

[a] Very polar metabolites or conjugates remained at the origin of the TLC plates.

labeled deltamethrin, respectively. The remaining radioactivity was lost from the plants by evaporation and/or transpiration. There was very little translocation of ^{14}C residues in the root tissues, and the levels were too low to permit any further analysis of these samples. Bound ^{14}C remaining in the solid material from shoots after solvent extraction amounted to 3% (methyl label) and 10% (benzylic label) of the ^{14}C applied. In other studies, formation of bound ^{14}C residues resulting from topical application of ^{14}C deltamethrin to cotton leaves has been also reported, but the amounts were nearly similar for the two radiolabeling positions (Ruzo and Casida, 1979).

The identity of the bound ^{14}C residues in the shoot tissues was determined by the HTD technique (Khan and Hamilton, 1980). In preliminary experiments it was observed that HTD of the reference standard of [^{14}C]deltamethrin resulted in a recovery of 87–91% radioactivity in the distillates. Furthermore, HTD of the air-dried extracted control plants shoot samples to which [^{14}C]deltamethrin (10 ppm) was added resulted in 63% recovery of the radioactivity in distillates while 12% remained in the residual dark colored plant material after HTD and about 25% was lost during the process.

HTD of the solvent extracted treated shoot samples containing bound ^{14}C residues showed that the amount of radioactivity in the combined solutions from the three traps and the quartz tube was 47% (methyl label) and 35% (benzylic label) of total bound ^{14}C residues. The residual dark colored polymerized plant material left in the porcelain boat after HTD still contained 30% (methyl label) and 22% (benzylic label) radioactivity. It was also observed that during HTD of samples 3–7% bound ^{14}C residues were thermally decomposed to $^{14}CO2$. Furthermore, 20% (methyl label) and 36% (benzylic label) ^{14}C remained unaccounted for at the end of experiment. The latter may be partly attributed to the surface adsorption to glass vessels during the working procedure (Helmuth et al., 1983; Akhtar, 1982).

The unextractable or bound residues of a few synthetic pyrethroids in plants have been reported in earlier studies (Ruzo and Casida, 1979; Ohkawa et al., 1980; Roberts, 1981; Roberts and Standen, 1981; Cole et al., 1982; Gaughan and Casida, 1978). However, the identity of these residues is not known. We report now the identity of some of the bound residues in bean plants treated with [^{14}C]deltamethrin. TLC, GC, and LSC analyses of the HTD distillates indicated the presence of a number of bound ^{14}C products in the shoot samples (Table I1). We were able to identify only about one-third of the bound ^{14}C residues due to the possible loss of the material in extensive TLC procedures and poor recoveries resulting by adsorption to glass surfaces (Akhtar, 1982; Helmuth et al., 1983). However, it should be realized that these type of residues would not be detected by conventional analytical procedures, thereby resulting in an underestimation of plant burden of total pesticide residues.

Table III. Release of Bound ^{14}C Residues from Bean Plant Shoots after Incubation with Enzyme

	^{14}C released, % of the bound ^{14}C	
incubation	methyl label	benzylic label
buffer I[a]	45	15
buffer I + β-glucosidase	45	23
buffer I + cellulase	55	27
buffer II[b]	56	30
buffer II + protease	54	35

[a] 0.2 M acetate buffer, pH 4.75. [b] McIvaine's standard buffer, pH 7.55.

Enzyme hydrolysis studies were undertaken to determine the release of bound ^{14}C residues from bean plant tissues. It was observed that incubation of plant tissue containing bound ^{14}C residues with buffer alone resulted in a substantial release of radioactivity. The release was more pronounced in the case of methyl-labeled [^{14}C]deltamethrin treated plant tissues. A small increase in the release of bound ^{14}C residues was observed due to cellulase from methyl labeling position treated samples (Table III). However, both the enzymes β-glucosidase and cellulase released bound ^{14}C residues from benzylic labeled [^{14}C]-deltamethrin treated material (Table III). The release was slightly more in the case of cellulase. It is likely that more bound ^{14}C residues were associated with the cellulose fraction of the plant material. Okhawa et al. (1980) also observed a small release of bound residues from bean plants on hydrolysis with a mixture of β-glucosidase and cellulase. However, they did not present any data on the release of bound ^{14}C residues with buffer alone. Therefore, caution should be exercised in the interpretation of results on enzyme hydrolysis of bound pesticide residues.

The results presented in this study show that treatment of bean plants with deltamethrin resulted in the formation of a small amount of bound residues. The latter were present in the form of the parent compound and its acid and hydroxy metabolites associated with plant constituents. In addition, a considerable amount of unidentified polar metabolites was also present in the form of bound residues. Although these bound residues were present in small amounts that may be neither detected nor determined by routine analysis involving solvent extraction, they can be of toxicological concern. Alternatively, being undetected and unidentified they may be assigned to some other disappearance factor when trying to account for the total pesticide residues in plants.

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Fate of Glyphosate in an Oregon Forest Ecosystem

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Glyphosate herbicide residues and metabolites were evaluated in forest brush field ecosystems in the Oregon Coast Range aerially treated with 3.3 kg/ha glyphosate. Deposits were recorded at various canopy depths to determine interception and residues in foliage, litter, soil, streamwater, sediments, and wildlife for the following 55 days. The half-life of glyphosate ranged from 10.4 to 26.6 days in foliage and litter and twice as long in soil. The treated stream peaked at 0.27 mg/L and decreased rapidly; concentrations were higher in sediment than in water and persisted longer. Coho salmon fingerlings did not accumulate detectable amounts. Exposure of mammalian herbivores, carnivores, and omnivores and retention of herbicide seemed to vary with food preference; however, all species had visceral and body contents at or below observed levels in ground cover and litter, indicating that glyphosate will not accumulate in higher trophic levels. (Aminomethyl)phosphonic acid was found at low concentrations but degraded rapidly. *N*-Nitrosoglyphosate was nondetectable.

Glyphosate herbicide is used in two predominant ways for managing forest vegetation (Newton and Knight, 1981). It is aerially applied (1) at rates of 1.7–3.3 kg/ha to remove herbs, shrubs, and hardwoods to prepare sites for planting conifers and (2) at rates of 0.82–1.24 kg/ha to selectively control competing vegetation once conifers are established. Environmental behavior of the chemical is presumed to be the same for both uses.

This paper reports the findings of an investigation on the distribution and fate of glyphosate aerially applied at the maximum registered-use rate to two hardwood communities in the Oregon Coast Range. Specific study objectives were to determine (1) glyphosate deposits in various strata of forest vegetation after aerial application and its persistence in these strata, in litter, and in soil, (2) glyphosate concentrations in streamwater, sediments, and fish after direct application to open streams, (3) glyphosate exposure and retention levels in various forest mammals, and (4) occurrence and persistence of (aminomethyl)-phosphonic acid, the major identifiable metabolite of glyphosate, and of *N*-nitrosoglyphosate, a trace impurity in glyphosate.

EXPERIMENTAL SECTION

Primary Study Site. The first site selected (primary site) was an 8-ha unit, roughly rectangular (about 200 × 400 m), about 12 km west of Summit, OR, in the Oregon Coast Range (Figure 1a). A small perennial stream flows through the site lengthwise. At the time of spraying, the flow rate was estimated at about 50 L/min, probably the lowest flow rate for the year.

Within the site are two large, shallow, beaver ponds. The terrain is moderately dissected, varying in elevation about 50 m. Bedrock is deeply weathered, horizontally stratified, Tyee sandstone. Soils are mostly of the Slickrock series, a very deep, loamy, forest soil of residual and colluvial origin that is common to the area. Soil pH is 4.0–4.7 and organic matter content approximately 3.8–5.2%. Soils of this type are highly productive for Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco], and the site was a typical one for conversion to conifers.

The climate in the area is characterized by mild summers and winters. Rainfall is estimated at about 230 cm annually, 87% of which occurs between Oct 1 and April 30 (Johnsgard, 1963).

Originally covered by Douglas fir, the site was burned by wildfire in the 1850s and later occupied by Douglas fir intermingled with red alder (*Alnus rubra* Bong.) and numerous small deciduous hardwood and shrubs. The salable conifers were removed in about 1955, leaving a deciduous stand ranging in age from 20+ to over 100 years. This stand is dominated by red alder and bitter cherry (*Prunus emarginata* Dougl. ex Eaton) and has an understory comprising two major shrub species, vine maple (*Acer circinatum* Pursh.) and salmonberry (*Rubus spectabilix* Pursh.), and one abundant fern, the swordfern [*Polystichum munitum* (Kaulf.) Presl.]. Younger hardwoods averaged about 22 m in height, with scattered older alder as tall as 35 m.

Pretreatment Sampling. Before herbicide treatment, samples of each type of material were collected as controls to ensure an adequate background basis against which treated samples could be compared. Samples were taken of vegetation (primarily leaves), of soil that was both covered (with litter) and uncovered (litter removed) at 0–7.5 cm, and of litter. Streamwater was sampled on the downstream edge of the site, where 300 coho salmon fin-

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