pellet mill was about 200 °C so the decline in methomyl levels was expected.

The average loss of methomyl residues during air curing was about 39% if the effect of rainfall is eliminated or about 46% if values for those samples that were rained upon are included. Thus, dehydration and pelletizing collectively had a much greater effect on residues than air curing.

ACKNOWLEDGMENT

We gratefully acknowledge the Du Pont chemists for assistance during adaptation of the method of analysis for methomyl.

LITERATURE CITED

Campbell, W. V.; Sheets, T. J. *J. Elisha Mitchell Sci. Soc.* **1976**, *92* (2), 68.

Dobson, S. H.; Kimbrough, E. L.; Baird, J. V.; Burns, J. C.; et al. *N.C. State Agric. Ext. Serv. Circ. 451*, **1974**, *No. 451*, 1-15.

Pease, H. L.; Kirkland, J. J. *J. Agric. Food Chem.* **1968**, *16*, 554.

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Fate of an Insect Growth Regulator EL-494 in Soybean Callus Tissue, Soybean Plants, and Gypsy Moth Larvae

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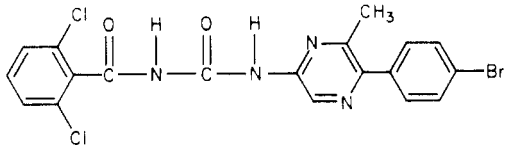
The metabolism of EL-494 [*N*-[[[5-(4-bromophenyl)-6-methyl-2-pyrazinyl]amino]carbonyl]-2,6-dichlorobenzamide], a potential insecticide which inhibits chitin synthesis, was investigated in soybean plant, soybean callus tissue, and gypsy moth larvae. Plants were incubated with [^{14}C]benzoyl-labeled EL-494 for 14 days. Callus tissues were incubated with [^{14}C]benzoyl- and 2-[^{14}C]pyrazinyl-labeled EL-494 for varying times (3, 6, 12, and 24 days). Gypsy moth larvae (fifth and sixth instars) were incubated with [^{14}C]benzoyl-labeled EL-494 for 7 days. Only two [^{14}C]benzoyl-labeled metabolites were found in the plant tissue or the gypsy moth larvae, and these were 2,6-dichlorobenzamide (6.1–16.2% in plant tissue and 5.6–8.3% in gypsy moth larvae) and 2,6-dichlorobenzoic acid (0.3–2.0% in plant tissue and ~0.3% in gypsy moth larvae). 2-Amino-5-(4-bromophenyl)-6-methylpyrazine was the only [^{14}C]-pyrazinyl-labeled metabolite of EL-494 found in the plant extracts. The metabolic degradation products generated by the soybean callus tissue were qualitatively and quantitatively similar to those of whole plants. Gypsy moth larvae eliminated ~27–31% of the [^{14}C]EL-494 by excretion.

EL-494 [*N*-[[[5-(4-bromophenyl)-6-methyl-2-pyrazinyl]amino]carbonyl]-2,6-dichlorobenzamide; I] is a



I

new chemical suggested for possible use as an insecticide.

It has molt-inhibiting properties and has been shown to have insecticidal properties against the southern armyworm, *Spodoptera eridania* (Cramer), tobacco budworm, *Heliothis virescens* (F.), fall armyworm, *Spodoptera frugiperda* (J. E. Smith), yellow fever mosquito, *Aedes aegypti* (L.), and housefly, *Musca domestica* (L.) (Lilly Research Laboratory, 1977). Retnakaran (1979) found that EL-494 was more active than Dimilin against the spruce budworm, *Choristoneura fumiferana* (Clemens), in laboratory tests. The EC_{50}, determined by diet tests, was 0.205 ppm for the third, 0.249 for the fourth, 0.287 for the fifth, and 0.486 for the sixth instars. In greenhouse tests he demonstrated that EL-494 was resistant to leaching and UV degradation and the compound remained active on potted balsam fir trees, *Abies balsamea* (L.), and white spruce trees, *Picea glauca* (Moench), for at least 15 days. In laboratory tests EL-494 reduced the survival of the pink bollworm larvae, *Pectinophora gossypiella* (Saunders), more than several other chitin synthetase inhibitors including Dimilin (Flint et al., 1978). Abdel-Monem et al. (1980) demonstrated that EL-494 affected the molting process of the gypsy moth larvae, *Lymantria dispar* (L.), at 0.39–0.88 ppm when larvae were fed an EL-494-treated diet for one stadium. It was more effective at longer feeding regimes. They also found that the incorporation of [^{14}C]glucose into chitin was reduced (77%) relative to controls when larvae were fed diets containing 20 ppm of EL-494.

The purpose of this investigation was to determine the fate of EL-494 in plants and in larvae of gypsy moth, *L. dispar*. Soybean plants and soybean cotyledon callus tissue were employed for metabolism studies because of their extensive use in similar studies (Mumma and Hamilton, 1979).

MATERIALS AND METHODS

Tissue Culture Technique and Incubation. Soybean (*Glycine max* (L.) Merrill var. Acme) cotyledon callus stock cultures were grown on an agar-solidified medium (Miller, 1963) with 3% sucrose, α-naphthaleneacetic acid (20 mg/L), and kinetin (0.5 mg/L) added. The stock cultures were maintained under low-intensity fluorescent light [0.5 μEinstein/(m^2·s)] at 25 °C and were routinely subcultured aseptically on the same medium once a month.

Two-week-old callus clumps (four clumps per flask and three flasks per treatment) were injected with labeled EL-494 (~200 μg) dissolved in 60 μL of chloroform (5 μL/clump). The labeled EL-494 ([^{14}C]benzoyl, specific activity 12.33 μCi/mg; 2-[^{14}C]pyrazinyl, specific activity

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6.7 μCi/mg) was supplied by Elanco Products Co. The tissue was then incubated in a growth chamber at 26 ± 1 °C with a 14-h photoperiod 3, 6, 12, and 24 days. Three replicates were used for each treatment. Following incubation, the tissue was surface rinsed with cold distilled water on a filter paper in a Büchner funnel. The rinse solution was removed by suction and saved for analysis. The surface rinsed tissue was stored in plastic bags at −20 °C prior to extraction.

Application to Plants and Incubation. Two-week-old soybean plants were used in this study. All plants were grown in pots in a growth chamber at 26 ± 1 °C with a 14-h photoperiod. The ^{14}C-labeled EL-494 (~200 μg) was dissolved in 60 μL of acetone and injected into the stems of six plants (10 μL) above the cotyledon leaves. Following 2 weeks of incubation, the treated plants were gently pulled from the soil and briefly rinsed with cold distilled water. The rinsed plants were stored in separate plastic bags at −20 °C prior to extraction.

Application of EL-494 to Gypsy Moth Larvae and Incubation. Gypsy moth egg masses were obtained from the USDA-APHIS Gypsy Moth Laboratory and kept in the refrigerator (4 °C) prior to use. Egg masses were dehaired by using a camel-hair brush and a vacuum cleaner and then sterilized by dipping in a 10% formalin solution for 5 min, washed 3 times with distilled water, and air-dried. The dehaired sterilized eggs were kept in the growth chamber at 26 ± 1 °C, 70% relative humidity, and a 14-h photoperiod. After hatching the first instar larvae were transferred and maintained on artificial diet which was supplied by BioServ, Inc., and kept in secured 500-mL Dixie cups prior to treatment.

Five replicate groups of the fifth and sixth instars were injected in their first prolegs with 1.0 μL of an acetone solution of [^{14}C]benzoyl-labeled EL-494 per larva (0.1 μCi/μL). Each group consisted of three larvae. The treated larvae were fed the diet for 7 days, then chilled, and stored in plastic bags at −20 °C prior to extraction.

Extraction and Fractionation of EL-494 and Metabolites from Plant Tissues. The frozen callus and plants were ground in 95% ethanol with a Virtis homogenizer. The homogenate was filtered in a Büchner funnel with suction, and the residue was repeatedly rinsed with 80% ethanol. The procedure of extraction and fractionation were the same as those described for 2,4-D metabolites (Hamilton et al., 1971).

Extraction and Fractionation of the EL-494 Metabolites from Gypsy Moth Larvae. The procedure for extraction of gypsy moth larvae is a modification of the Bligh–Dyer procedure for extraction of lipids (Bligh and Dyer, 1959; Still and Leopold, 1978). For determination of recoveries, known standards of [^{14}C]EL-494 were incorporated with larvae and extracted. The insect frass and the artificial diet were extracted as described by Hamilton et al. (1971).

Detection, Separation, and Characterization of the EL-494 Metabolites. The radioactivity in each fraction was measured by liquid scintillation counting (Aquasol). The residue was combusted by a Packard Tri-Carb oxidizer, Model 306, and the $^{14}CO_2$ was determined by liquid scintillation counting. All counts were corrected for quenching by the external standards channels ratio method. Initial separation of the ether-soluble metabolites was accomplished by thin-layer chromatography (TLC) with the solvent system hexane–ethyl acetate (3:2 v/v). The relative amount of each radioactive metabolite, as detected by autoradiography and X-ray film, was determined by eluting the appropriate regions of chromatograms and counting the radioactivity of the eluent. Standards and metabolites were characterized by mass spectrometry (Kratos M59/50).

Radiocarbon Assay. All the solid samples, including gypsy moth larvae, were prepared for liquid scintillation assay by combustion in a Packard Tri-Carb oxidizer, Model 306. All liquid samples were assayed by direct liquid scintillation counting in a Beckman liquid scintillation counter, Model LS8000.

High-Pressure Liquid Chromatography. A Model ALC/GPC 244 high-pressure liquid chromatograph equipped with a 6000-A pump, U6K injector, and a 440 UV detector (Waters Associates, Inc.) was used. Absorbance of eluted compounds was recorded as 254 nm. A 30 cm × 4 mm i.d. μBondapak C_{18} column was used with a methanol–water (80:20 v/v) solvent at a flow rate of 1.0 mL/min (Abdel Monem and Mumma, 1981). Chromatography was conducted at room temperature with a pressure setting of 1000 psi. The same column was used with an acetonitrile–water (55:45 v/v) solvent at a flow rate of 1.0 mL/min to identify the pyrazinyl metabolite.

RESULTS AND DISCUSSION

Soybean Plants and Callus Tissue. The procedure used to extract the soybean plants or callus tissue is presented in Figure 1. The amount of radiolabel in the ethyl ether, water, and residue fractions from both [^{14}C]-benzoyl-labeled and 2-[^{14}C]pyrazinyl-labeled EL-494 incubations with soybean callus tissue is shown in Figures 2 and 3. The ethyl ether fraction contained most of the ^{14}C label (86.7–95.7%), the water fraction contained much less (1.8–8.1%), and the residue fraction contained very little (0.4–2.1%). The ^{14}C-labeled metabolites of the ether fraction were separated into three bands on TLC and were arbitrarily designated Et_1 through Et_3. Et_3 was the only major band, Et_2 was a minor band, and Et_1 was found in trace quantities. Similar results were obtained with incubations with whole plants. Et_3 (~82–88%) cochromatographs on TLC and HPLC with standard EL-494, and therefore, Et_3 represents unmetabolized EL-494 in the plant tissue. This is consistent with previous studies in which significant amounts of free diflubenzuron were found in cotton plant extracts (Mansager et al., 1979).

Et_1 and Et_2 were not present when 2-[^{14}C]pyrazinyl-labeled EL-494 was used, thus indicating that Et_1 and Et_2 are associated only with the benzoyl portion of the parent molecule. Both Et_1 and Et_2 remained in the ethyl ether when extracted with acidic water (pH 3), but Et_1 dissolved in a $NaHCO_3$ wash, implying that Et_2 is a neutral molecule and Et_1 is acidic and presumably an acid. These data suggested Et_1 and Et_2 might be 2,6-dichlorobenzoic acid and 2,6-dichlorobenzamide, respectively, and these standards were purchased or prepared. Et_3 (EL-494) was acid hydrolized (6 N HCl; 100 °C; 24 h) and a product corresponding to Et_2 was obtained. The TLC mobility of Et_1 and Et_2 corresponded with the mobility of 2,6-dichlorobenzoic acid and 2,6-dichlorobenzamide, respectively, in four solvent systems: chloroform–methanol–water–acetic acid (130:70:12:1 v/v/v/v), benzene–acetic acid (4:1 v/v), benzene–acetone–acetic acid (9:1:1 v/v/v), and benzene–ethyl acetate (9:1 v/v).

EL-494 and its metabolites were separated by HPLC on a μBondapak C_{18} column with a methanol–water (80:20 v/v) solvent. The eluate was sequentially collected (25 s) and the radioactivity was determined in each fraction. Standard EL-494, 2,6-dichlorobenzoic acid, and 2,6-dichlorobenzamide were added to the sample prior to analysis. Figure 4 shows a plot of the counts per minute and the UV absorption vs. the elution time. The radio-

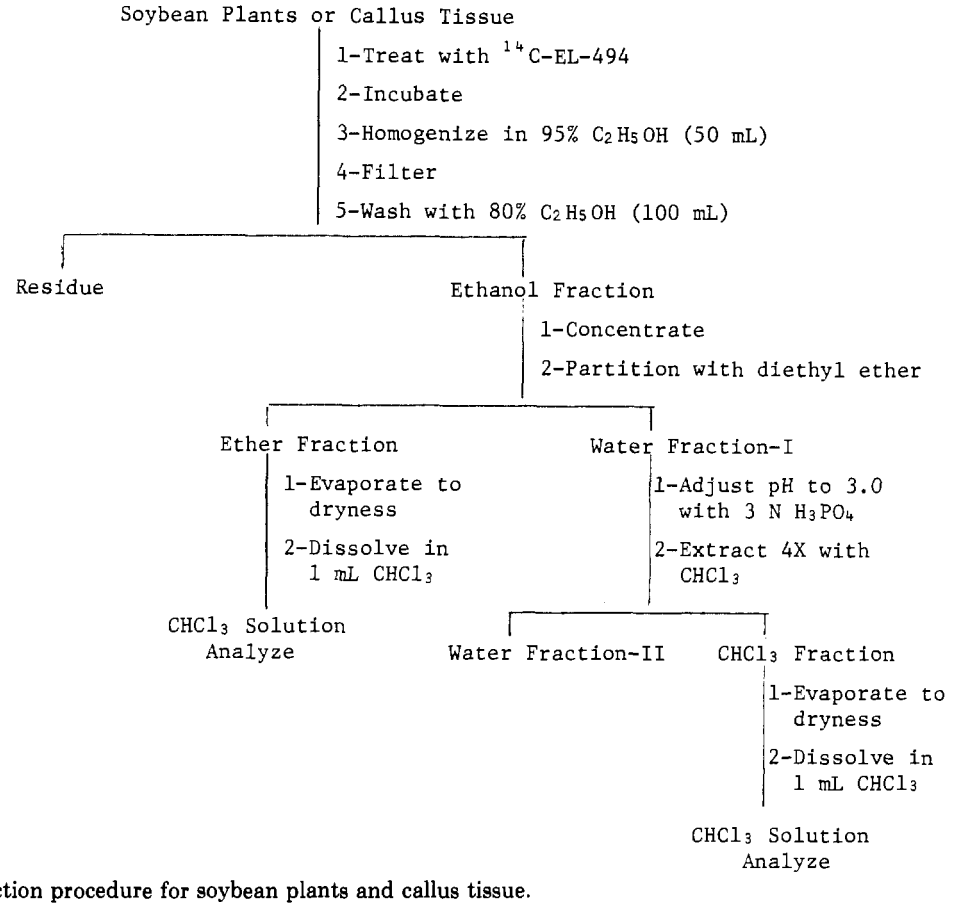

Figure 1. Extraction procedure for soybean plants and callus tissue.

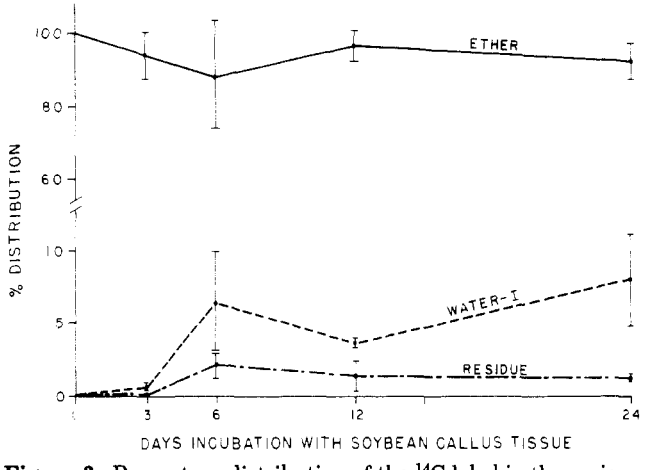

Figure 2. Percentage distribution of the ^{14}C label in the various fractions when soybean callus tissue was incubated with [^{14}C]-benzoyl-labeled EL-494.

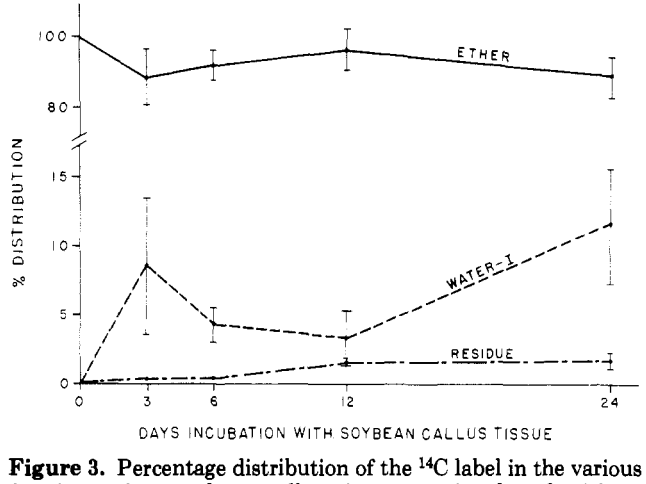

Figure 3. Percentage distribution of the ^{14}C label in the various fractions when soybean callus tissue was incubated with 2-[^{14}C]pyrazinyl-labeled EL-494.

active peaks are superimposed with the UV peaks of the standards. The HPLC was subsequently used to isolate the metabolites from the callus tissue incubated for various times prior to quantification by liquid scintillation counting and analysis by mass spectrometry. The mass spectrum of EL-494 (Et_3) is presented in Figure 5, and the interpretation of this fragmentation is shown in Figure 6. The molecular ion is present (m/e 478) but of low intensity (1.5%). Major fragments, m/e 289 (51.9%), 263 (28.4%), 189 (19.2%), and 173 (100%, base), arise from fragmentation of the —C(=O)NH— linkage and are easily recognized due to the isotopic contribution of their halogen atoms. Other ions arise from further fragmentation of the major ions. The mass spectrum of Et_1 showed major fragments at m/e 190 (87%, M^+), 173 (100%, base), 145 (16%), 109 (12%), 84 (11%), 75 (28%), and 74 (21%) with characteristic chlorine isotope peaks and corresponded to the mass spectrum of standard 2,6-dichlorobenzoic acid. The mass spectrum of Et_2 exhibited major mass spectra ions at m/e 189 (36%, M^+), 173 (100%, base), 145 (20%), 109 (20%), 84 (31%), 75 (26%), and 74 (23%) and also corresponded to the spectrum of standard 2,6-dichlorobenzamide.

The percentage composition of ^{14}C label in the ethyl ether and water fractions of soybean plants and callus tissue incubated with [^{14}C]benzoyl-labeled EL-494 is shown in Table I. Unchanged EL-494 represents ~82–88% of the recovered ^{14}C label. The amount of 2,6-dichlorobenzamide increased with incubation, 6.1% at 3 days to 16.2% at 24 days, while the amount of 2,6-dichlorobenzoic acid remained low and did not increase. It is important to note that the metabolites identified in the soybean callus

Table I. Percentage Composition of ^{14}C Label in the Ethyl Ether and Water Fractions of Soybean Plant and Callus Tissue Incubated with [^{14}C]Benzoyl-Labeled EL-494[a]

	callus tissue								plants, 14 days	
	3 days		6 days		12 days		24 days			
components	ether	water	ether	water	ether	water	ether	water	ether	water
Et_3, EL-494	85.9	0.6	82.1	2.7	85.8	0.6	83.2	0.4	87.9	0.5
Et_2, 2,6-dichlorobenzamide	5.8	0.3	5.8	3.6	10.4	2.9	8.8	7.4	7.2	2.1
Et_1, 2,5-dichlorobenzoic acid	2.0	trace	0.4	0.2	0.1	0.6	0.3	0.2	0.2	0.1
residue	0.3		2.1		1.4		1.1		2.0	

[a] Data represent averages of three replicates based on recovered ^{14}C.

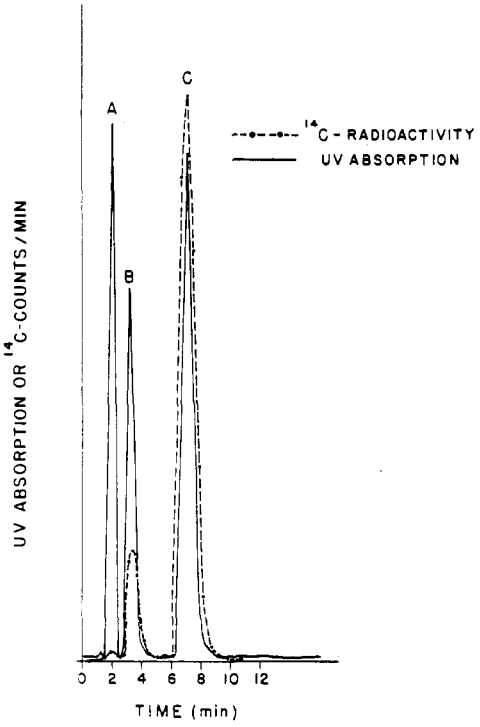

Figure 4. Counts per minute of ^{14}C-labeled metabolites and UV absorption vs. the elution time of a high-pressure liquid chromatograph. The letters represented standards: A = 2,6-dichlorobenzoic acid, B = 2,6-dichlorobenzamide, and C = EL-494.

tissue are identical with those metabolites in the whole plant. These data support the hypothesis that metabolism studies with plant tissue cultures are qualitatively and quantitatively similar to the metabolism studies with the whole plant and are useful as predictors of plant metabolism (Mumma and Hamilton, 1979).

Thin-layer chromatography of the ethyl ether and water-I extracts from the 2-[^{14}C]pyrazinyl-labeled EL-494 incubations (Figure 3) revealed only one radiolabeled compound (EL-494) that moved from the origin. Both extract fractions possessed a small amount of radiolabeled material staying at the origin. Since 2,6-dichlorobenzoic acid and 2,6-dichlorobenzamide had been shown to be the only detectable metabolites of [^{14}C]benzoyl-labeled EL-494 incubations and they undoubtedly arose from a hydrolytic mechanism, we expected to find the [^{14}C]pyrazinyl-labeled portion of EL-494 in our extracts. The pyrazinyl portion of EL-494 is basic and would remain at the origin if protonated. Therefore, we hydrolyzed standard EL-494 (6 N HCl; 168 h; 107 °C) and separated the neutralized products of hydrolysis on thin-layer chromatography (benzene–acetic acid), 80:20 v/v). All TLC bands were isolated and identified through mass spectrometry. 2-Amino-5-(4-bromophenyl)-6-methylpyrazine was isolated as a band at R_f = 0.2 and gave prominent mass spectra ions of m/e 264 (100%, base), 262 (92%, M^+), 183 (35%), 157 (20%), and

Table II. Percentage Distribution of Radioactivity When Fifth and Sixth Larval Instars of the Gypsy Moth Were Injected with [^{14}C]Benzoyl-Labeled EL-494 and Incubated for Seven Days[a]

component	5th instar, recovered	6th instar,[b] recovered
within insects		
gypsy moth	71.0	62.1
external to insects		
cage wash	1.2	1.3
diet	0.8	5.2
frass	27.0	31.4

[a] Data are averages of five replicates: total ^{14}C recovered was 93.4 and 97.6% of the injected ^{14}C in the fifth and sixth instar larvae, respectively. [b] Only female larvae.

Table III. Percentage Distribution of Radioactivity in Various Fractions When Fifth and Sixth Larval Instars of the Gypsy Moth Were Injected with [^{14}C]Benzoyl-Labeled EL-494 and Incubated for Seven Days[a]

	5th instar		6th instar[b]	
fraction	% of ^{14}C recovered	% ^{14}C in fraction	% of ^{14}C recovered	% ^{14}C in fraction
nonpolar extract				
acetonitrile	59.5	83.9	54.0	86.9
hexane	0.7	1.0	0.4	0.7
polar extract				
water	1.2	1.7	1.4	2.2
residues	9.5	13.4	6.3	10.2
total	70.9	100.0	62.1	100.0

[a] Data are averages of five replicates based on recovered ^{14}C. [b] Female larvae.

129 (13%) and additional bromine isotope peaks.

Unlabeled 2-amino-5-(4-bromophenyl)-6-methylpyrazine was added to an aliquot of the original ethanol extract of the [^{14}C]pyrazinyl-labeled 3-day incubation with soybean callus tissue. This ethanol aliquot was analyzed each 60 s by HPLC utilizing UV detection and liquid scintillation counting of samples (Figure 4). Only two radiochemical peaks were observed. They correspond to EL-494 and 2-amino-5-(4-bromophenyl)-6-methylpyrazine in an 8:1 ratio, respectively.

Gypsy Moth Larvae. The treated gypsy moth larvae were extracted as shown in Figure 7. The percentage distribution of the [^{14}C]benzoyl label in the various fractions in the fifth and sixth instar is shown in Table II. After 7 days the gypsy moths contained ~62–71% of the radiolabel, and the frass accounted for most of the rest of the radiolabel, ~27–31%. The diet and cage washings contained 1–5%. These data suggest that excretion could play a significant role in the defense mechanism of the gypsy moth larvae against EL-494. Still and Leopold (1978) also found that 24% of the recovered ^{14}C from [^{14}C]diflubenzuron-injected boll weevils was in the frass after 6-day incubations. The radiolabel in the diet and the frass of the sixth instar was higher than in the fifth instar,

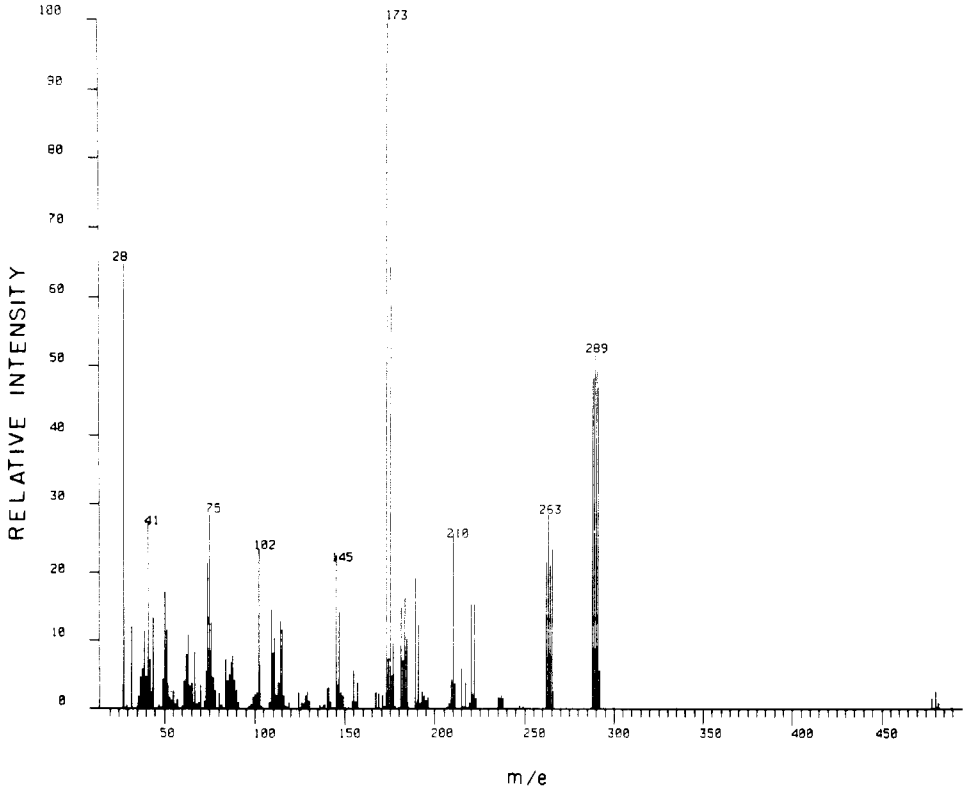

Figure 5. Mass spectrum of EL-494.

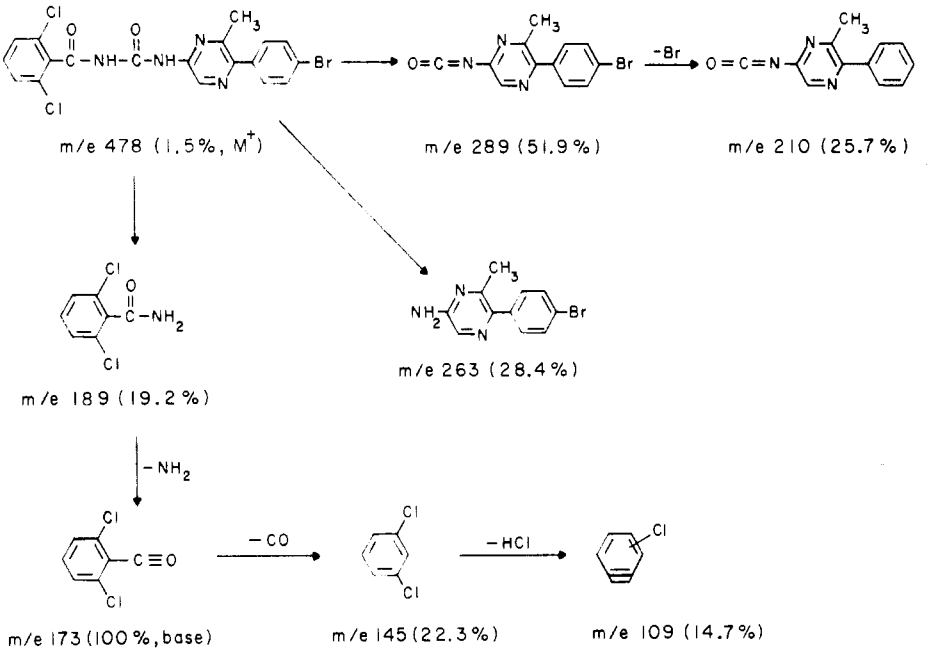

Figure 6. Interpretation of mass spectral fragmentation pattern of EL-494.

Table IV. Percentage Composition of Radioactivity in the Various Solvent Fractions and Frass When Fifth and Sixth Larval Instars of the Gypsy Moth Were Injected with [^{14}C]Benzoyl-Labeled EL-494 and Incubated for Seven Days[a]

	5th instar				6th instar[b]			
	solvent fraction				solvent fraction			
component	acetonitrile	hexane	water	frass	acetonitrile	hexane	water	frass
EL-494	57.6	0.4	0.1	24.3	50.3	0.3	0.2	27.9
2,6-dichlorobenzamide	1.7	0.2	1.1	2.6	3.5	0.1	1.2	3.5
2,6-dichlorobenzoic acid	0.2	0.1	trace	trace	0.2	0.1	trace	trace
total % recovered	59.5	0.7	1.2	26.9	54.0	0.5	1.4	31.4

[a] Data are averages of five replicates based on recovered ^{14}C. [b] Female larvae.

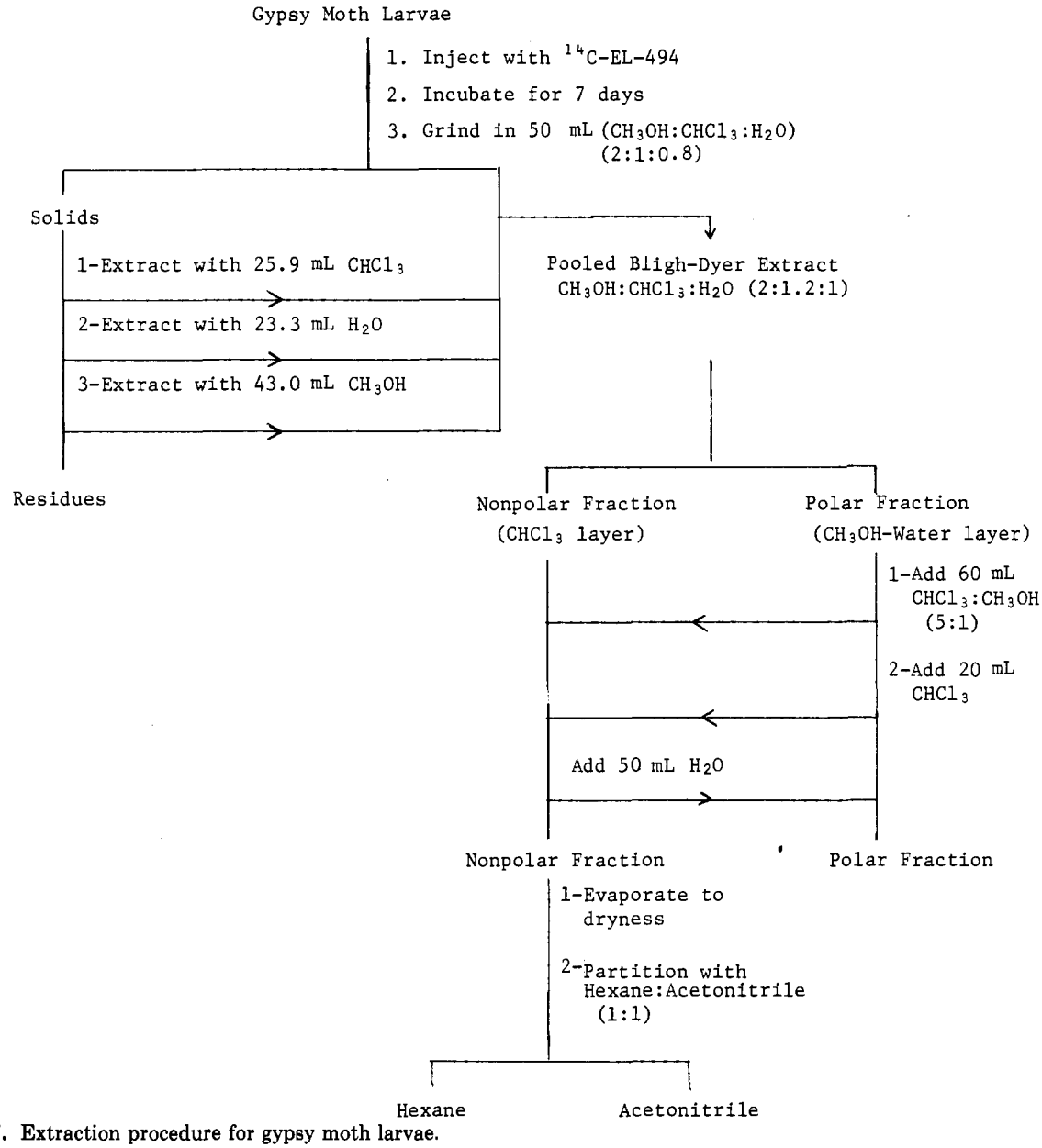

Figure 7. Extraction procedure for gypsy moth larvae.

reflecting the greater amounts of frass of the sixth instar or actual instar differences.

Table III shows the distribution of ^{14}C in the various extracts of the larvae. Most of the radioactivity was in the acetonitrile extract, ~54–59%; however, a significant amount (~6–9%) remained in the residue. Repeated extraction of the residue with acetone, a good solvent for EL-494, slowly removed the ^{14}C label. Analysis of this hard-to-recover ^{14}C label indicated it was unchanged [^{14}C]EL-494. Since EL-494 presumably interferes with the terminal polymerization step of chitin synthetase (Abdel-Monem et al., 1980), it may be trapped within the newly formed chitin and thus difficult to remove.

Each fraction was analyzed by TLC and HPLC and the same two metabolites that were found in the plant tissue, 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid, were identified.

The relative composition of the various solvent fractions and frass is presented in Table IV. Most of the acetonitrile fraction (~93–97%) consists of unchanged [^{14}C]-EL-494. 2,6-Dichlorobenzamide is the major metabolite (5.6–8.3%) and is present in higher amounts in the sixth instar and in the frass. More than 42% of the 2,6-dichlorobenzamide was excreted in the frass while only

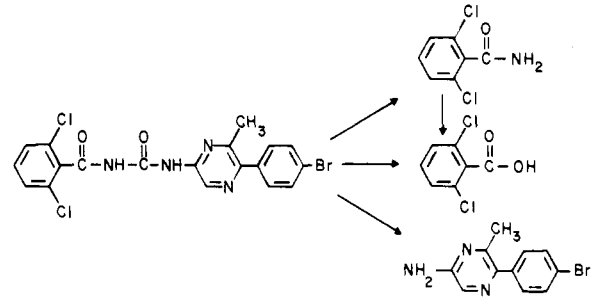

Figure 8. Summary of metabolism of EL-494 in soybean plants, soybean callus tissue, and gypsy moth larvae.

~29–35% of the EL-494 and 8–15% of the 2,6-dichlorobenzoic acid were found in the frass. This indicates that the gypsy moth larvae selectively excretes the major metabolite. This mechanism could play a role in defense. However, the excretion of unchanged EL-494 is much greater than the conversion to metabolites; therefore, excretion of unchanged EL-494 is an important part in the defense mechanism.

The metabolism of EL-494 by soybean plants and gypsy moth larvae appears to be identical and is summarized in

Figure 8. Hydrolytic cleavage gives rise to 2,6-dichlorobenzoic acid, 2,6-dichlorobenzamide, and 2-amino-5-(4-bromophenyl)-6-methylpyrazine. Hydrolytic cleavage has also been shown to be an important detoxification pathway in the metabolism of diflubenzuron (Ivie et al., 1980).

LITERATURE CITED

Abdel-Monem, A. H.; Cameron, E. A.; Mumma. R. O. *J. Econ. Entomol.* **1980**, *73*, 22-25.
Abdel Monem, A. H.; Mumma, R. O. *J. Agric. Food Chem.* **1981**, *29*, 75-78.
Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911-917.
Flint, H. M.; Smith, R. L.; Noble, J. M.; Shaw, D.; DeMilo, A. B.; Khalil, E. *J. Econ. Entomol.* **1978**, *71*, 616-619.
Hamilton, R. H.; Hurter, J. Hall, J. K.; Ercegovich, D. C. *J. Agric. Food Chem.* **1971**, *19*, 480-483.
Ivie, G. W.; Bull, D. L.; Veech, J. A. *J. Agric. Food Chem.* **1980**, *28*, 330-337.
Lilly Research Laboratory, Indianapolis, IN, 1977, Technical Report on EL-494, 1-4.
Mansanger, E. R.; Still, G. G.; Frear, D. S. *Pestic. Biochem. Physiol.* **1979**, *12*, 172-182.
Miller, C. O. In "Moderne Methoden der Pflanzenanalyze"; Linskens, H. F.; Tracey, M. V., Eds.; Springer-Verlag: Berlin, 1963; Vol. 6, p 194.
Mumma, R. O.; Hamilton, R. H. *ACS Symp. Ser.* **1979**, *No. 98*, 35-76.
Retnakaran, A. *Can. Entomol.* **1979**, *111*, 847-850.
Still, G. G.; Leopold, R. A. *Pestic. Biochem. Physiol.* **1978**, *9*, 304-312.

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Interactions of Pyrethroid Insecticides and Toxaphene in Cotton

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Effects of toxaphene on pyrethroid insecticide residues on cotton foliage were determined by spraying aqueous emulsions, extracting leaves with methylene chloride, separating the pyrethroid from toxaphene by preparative thin-layer chromatography, and quantitating by gas chromatography. Fenvalerate residues were increased up to 2-fold due to enhanced persistence in the presence of toxaphene. Permethrin residues were reduced by toxaphene, the effect being greater in 1*RS*,trans isomers after several applications. Toxaphene synergized fenvalerate and permethrin 2-fold in *Spodoptera frugiperda*; susceptibility of *Heliothis virescens* was increased 1.5-fold to both pyrethroids.

Combinations of insecticides for cotton insect control have had a successful history which includes the use of DDT/toxaphene, EPN/methyl parathion, and toxaphene/methyl parathion. While the first and second examples acted as insecticidally synergistic mixtures (Brown, 1971), addition of toxaphene was found to increase the deposit and residual persistence of methyl parathion sprays in cotton (Ware et al., 1979). Since photostable pyrethroid insecticides have replaced methyl parathion for protecting cotton, there is interest in possible combinations of insecticides with pyrethroids.

This report examines toxaphene/pyrethroid mixtures and describes the effects of toxaphene on the residual concentrations of fenvalerate and permethrin on cotton leaves. The combinations were tested further for toxicological interactions in the cotton pests *Heliothis virescens* (Fabricius), the tobacco budworm, and *Spodoptera frugiperda* (J. E. Smith), the fall armyworm, and these results are also reported.

MATERIALS AND METHODS

Applications of Insecticides. Combinations of toxaphene/permethrin and toxaphene/fenvalerate and each pyrethroid alone were sprayed on cotton of the Coker 304 variety in Sumter, SC, in 1978. Each treatment was applied in quadruplicate to randomized 141-m^2 plots of cotton by using a conventional high-clearance sprayer calibrated to deliver 79.5 L/ha. Insecticides were commercial emulsifiable concentrates of permethrin as Pounce, fenvalerate as Pydrin, and toxaphene as formulated by FCX, Inc. Insecticide residue samples consisted of 35 mature leaves gathered from the top portions of plants while walking diagonally across the plots; samples were frozen immediately in sealed plastic bags and later transported to the laboratory under dry ice.

Confirmatory experiments were performed with toxaphene/fenvalerate in 1979 and toxaphene/permethrin in 1980 in Florence, SC, by using plots of Coker 310 cotton which were 324 and 202 m^2, respectively. Application methods were similar to those used in Sumter, and samples were collected and frozen as above.

Determination of Residual Pyrethroid Insecticides. Extraction of pyrethroids from leaves followed a procedure modified from George et al. (1977). Each thawed sample was chopped and a 20-g lot blended for 1 min with pesticide-grade methylene chloride in a Sorvall Omnimixer while cooling with an ice bath. This extract and two subsequent rinses were filtered through granular, anhydrous Na_2SO_4 and concentrated in vacuo at 45 °C.

Preparative thin-layer chromatography (TLC) removed plant lipids and toxaphene from pyrethroids which were further analyzed. A 750-μm layer of silica gel PF-254 (E. Merck, Darmstadt) was spread on glass plates which were air-dried and then activated at 110 °C for 30 min. One-tenth of the extract and the pyrethroid standard were chromatographed to 10 cm in 10% (v/v) ethyl acetate in hexane. Ultraviolet light revealed the standard, and the pyrethroid zone was removed and eluted with 50% (v/v) ethyl acetate in hexane which gave >95% recovery. R_f

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