While a detailed investigation into the mechanisms of monoterpene glycoside hydrolyses has not been undertaken, it would seem that the water solubility of the compounds as well as the presence of an allylic glycosidic linkage greatly influences the reactivity of these substrates. The latter property would facilitate formation of a carbocation, which in turn appears to be an important early step in the hydrolytic process. In support of this it was found that when geranyl β-D-glucopyranoside was hydrogenated to 3,7-dimethyloctyl β-D-glucopyranoside, and also when the grape precursor material was hydrogenated, these reduced products were resistant to acid hydrolysis. Similarly, Croteau and Martinkus (1979) found that menthyl glucosides were also relatively stable and showed no aglycon rearrangement on acid hydrolysis.

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LITERATURE CITED

Baxter, R. L.; Laurie, W. A.; McHale, D. *Tetrahedron* **1978**, *34*, 2195.
Bunton, C. A.; Cori, O.; Hachey, D.; Leresche, J.-P. *J. Org. Chem.* **1979**, *44*, 3238.
Bunton, C. A.; Hachey, D.; Leresche, J.-P. *J. Org. Chem.* **1972**, *37*, 4036.
Coates, R. M. In "Progress in the Chemistry of Organic Natural Products"; Springer-Verlag: New York, 1976; p 73.
Corbier, B.; Teisseire, P. *Recherches* **1974**, *19*, 253.
Cordonnier, R.; Bayonove, C. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1974**, *278*, 3387.
Croteau, R.; Martinkus, C. *Plant Physiol.* **1979**, *64*, 169.
Heller, S. R.; Milne, G. W. A. "EPA/NIH Mass Spectral Data Base"; U.S. Department of Commerce and The National Bureau of Standards: Washington, DC, 1978; Vol. 1–4.
Jennings, W.; Shibamoto T. "Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography"; Academic Press: New York, 1980.
Klouwen, M. H.; Ter Heide, R. *J. Chromatogr.* **1962**, *7*, 297.
Pickett, J. A.; Coates, J.; Sharpe, F. R. *Chem. Ind. (London)* **1975**, 571.
Strikler, H.; Kovats, E. S. *Helv. Chim. Acta* **1966**, *49*, 2055.
Ter Heide, R.; de Valois, P. J.; Visser, J.; Jaegers, P. P.; Timmer, R. In "Analysis of Foods and Beverages"; Charalambous, G., Ed.; Academic Press: New York, 1978; p 249.
Thomas, A. F.; Willhalm, B. *Helv. Chim. Acta* **1964**, *47*, 475.
Van Den Dool, H.; Kratz, P. D. *J. Chromatogr.* **1963**, *11*, 463.
Williams, P. J.; Strauss, C. R.; Wilson B. *J. Agric. Food Chem.* **1980a**, *28*, 766.
Williams, P. J.; Strauss, C. R.; Wilson, B. *Phytochemistry* **1980b**, *19*, 1137.
Williams, P. J.; Strauss, C. R.; Wilson B. *Am. J. Enol. Vitic.* **1981**, *32*, 230.
Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. *J. Chromatogr.* **1982a**, *235*, 471.
Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westroop, R. A. *Phytochemistry* **1982b**, in press.

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Metabolism and Fate of Diflubenzuron in Swine

Joseph C. Opdycke, Richard W. Miller, and Robert E. Menzer*

^{14}C-Labeled diflubenzuron, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, was administered at 5 mg/kg to a female Duroc-Poland China pig as an oral dose. Analysis of feces for radioactivity revealed 82% of the administered dose, identified as diflubenzuron, while 5% of the dose was recovered in the urine. The highest [^{14}C]diflubenzuron residue present in pig tissues was 0.43 ppm in the gallbladder. Identification of the metabolic products found in the urine revealed (4-chlorophenyl)urea, 2,6-difluorobenzoic acid, 4-chloroaniline, and 2,6-difluorobenzamide. Cleavage of the urea moiety between the benzoyl carbon and urea nitrogen is indicated as the primary degradation pathway in swine.

Diflubenzuron, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, is a potent broad spectrum insect growth regulator. Diflubenzuron interferes with insect cuticle formation (VanDaalen et al., 1972) and is effective in controlling immature stages of insects. Diflubenzuron has been shown to be effective in controlling diptera larvae in the manure of cattle and chickens when incorporated into their diet (Miller, 1974; Miller et al., 1975, 1976; Wright, 1974, 1975; Wright and Spates, 1976).

The metabolism of diflubenzuron has been reviewed by Ivie (1977) and Schooley and Quistad (1979). Diflubenzuron metabolism by sheep (Metcalf et al., 1975; Ivie, 1978), cattle (Ivie, 1978), chickens (Opdycke et al., 1982), and rats (Willems et al., 1980) indicates that hydroxylation, conjugation, and cleavage of the urea moiety of the diflubenzuron molecule are possible metabolic pathways.

This study is concerned with the amounts of diflubenzuron retained, metabolized, and/or excreted by a pig. Analysis of the metabolism of diflubenzuron by this economically important animal will help to clarify its potential as an insect feed-additive larvicide.

MATERIALS AND METHODS

Chemicals. [^{14}C]Diflubenzuron, uniformly radiolabeled in both rings (specific activity 17.42 mCi/mmol), and technical diflubenzuron were supplied by the Thompson-Hayward Chemical Co., Kansas City, KS. Compounds that are possible metabolites, (4-chlorophenyl)urea, 4-chloroaniline, 2,6-difluorobenzoic acid, *N*-[[(4-chloro-2-hydroxyphenyl)amino]carbonyl]-2,6-difluorobenzamide,

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and *N*-[[(4-chloro-3-hydroxyphenyl)amino]carbonyl]-2,6-difluorobenzamide, were also gifts from Thompson-Hayward.

2,6-Difluorobenzamide, *N*-(4-chlorophenyl)formamide, and *N,N'*-bis(4-chlorophenyl)urea were synthesized in this laboratory. 2,6-Difluorobenzamide was prepared by the method of Burton and Roe (1967), in which 2,6-difluorobenzoyl chloride was prepared from 1 g of 2,6-difluorobenzoic acid and 1 g of thionyl chloride. Ammonia gas was bubbled into the mixture to form the benzamide, which was extracted with ether. The product was recrystallized from ether to yield pure, white crystals, mp 123–127 °C. Anal. Calcd for $C_7H_5NOF_2$: C, 53.5; H, 3.2; N, 8.9. Found: C, 50.99; H, 3.17; N, 9.20. Infrared spectra showed two characteristic $-NH_2$ peaks at 3400 and 3200 cm^{-1}. The nuclear magnetic resource (NMR) spectrum showed a broad singlet at 3.0 ppm, a singlet at 4.5 ppm, and an unresolved multiplet at 6.7 ppm. Mass spectral analysis indicated the molecular ion at *m/e* 157 and the base peak at *m/e* 141 (loss of $-NH_2$).

N-(4-Chlorophenyl)formamide was prepared by heating 1.27 g of 4-chloroaniline at 65–70 °C with 0.46 g of formic acid (Haque et al., 1976). The product was purified by recrystallization from hexane–ether, mp 97–100 °C. Anal. Calcd for C_7H_6NOCl: C, 54.8; H, 3.8; N, 9.0. Found: C, 53.04; H, 3.72; N, 8.79. The infrared spectrum of the product was consistent with the proposed structure and did not contain the characteristic $-NH_2$ peak of the starting material. Mass spectral analysis yielded the molecular ion and base peak at *m/e* 155. A prominent peak was observed at *m/e* 127, representing 4-chloroaniline.

N,N'-Bis(4-chlorophenyl)urea was prepared by adding an acetone solution of 0.5 g of 4-chlorophenyl isocyanate into a stirring solution of 0.5 g of formamide (Haque et al., 1976). The product was recrystallized from hot ether, mp 270–276 °C. Anal. Calcd for $C_{13}H_{10}N_2OCl_2$: C, 55.7; H, 3.5; N, 10.0. Found: C, 51.70; H, 3.23; N, 9.34. Infrared spectra showed absorption at 3300 cm^{-1} corresponding to an –NH stretch and four absorption peaks between 1760 and 1510 cm^{-1} corresponding to a primary amide bond. NMR showed a singlet at 2.9 ppm and a quintuplet at 7.5 ppm. Mass spectral analysis revealed the molecular ion at *m/e* 280, base peak at *m/e* 127 (corresponding to chloroaniline), a peak at *m/e* 153 corresponding to 4-chlorophenyl isocyanate, and a characteristic chlorine cluster indicating two chlorine atoms. Chemical and spectral data on the product obtained agree with that reported by Ivie et al. (1980) for a product isolated following treatment of water with diflubenzuron identified as *N,N'*-bis(4-chlorophenyl)urea. The type of product of this reaction reported by Haque et al. (1976) was not isolated under the reaction conditions used.

The purity of the radiolabeled diflubenzuron, 4-chloroaniline, 2,6-difluorobenzoic acid, the hydroxylated products, and technical diflubenzuron was found to be greater than 99% from thin-layer chromatographic analysis. Thin-layer chromatography was used to separate two minor contaminates from the (4-chlorophenyl)urea. All solvents were glass distilled or reagent grade. Aldrin and dieldrin chromatographic standards were obtained from Shell Chemical Co.

Animal Treatment. A Poland-China Duroc female pig (16 weeks old) was obtained from the Beltsville Agricultural Research Center, U.S. Department of Agriculture. The pig was housed in a stainless steel metabolism stall to facilitate collection of urine and feces. Commercial corn-based feed (16% protein) was given at 12-h intervals and water was available at all times. The pig was accli-

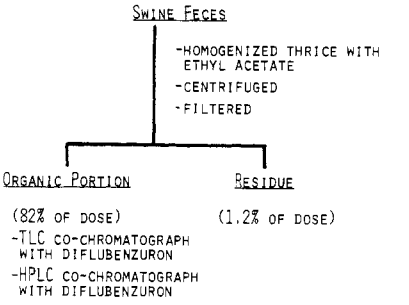

Figure 1. Scheme for extraction and analysis of swine feces.

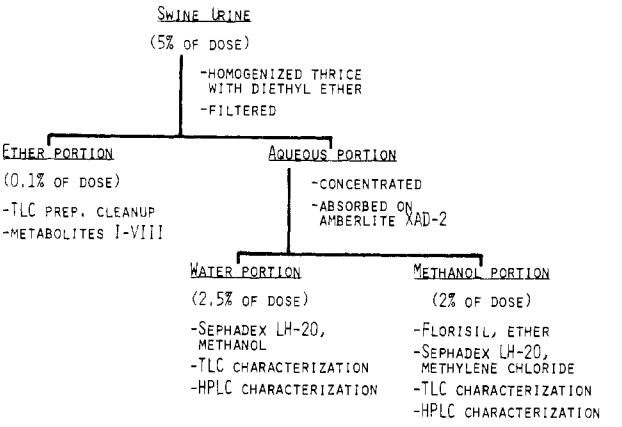

Figure 2. Scheme for the extraction and analysis of swine urine.

mated to the cage for 20 days prior to treatment and then kept off feed for 24 h before administration of diflubenzuron. On the day of diflubenzuron administration the pig weighed 46 kg. The dose was 5 mg/kg (405 μCi) and was prepared by dissolving appropriate amounts of technical and radiolabeled diflubenzuron in acetone and adding it to a capsule of feed. A jaw brace was placed in the pig's mouth, and the capsule was administered directly into the throat of the animal with the aid of mineral oil.

Pig urine and feces were collected at 12-h intervals starting at the time of [^{14}C]diflubenzuron administration and continuing until slaughter at 11 days postadministration. Pig feces were weighed and blended in a Waring blender 3 times with an equal volume of ethyl acetate each time, followed by suction filtration, to yield organic and residue fractions that were aliquoted and analyzed for carbon-14 (Figure 1). Pig urine was collected and aliquoted for quantitation of its radiocarbon content (Figure 2).

After 11 days the pig was slaughtered, and samples of brain, heart, lung, liver, gallbladder, kidney, blood, lymph, fat, ovary and oviduct, stomach wall, pancreas, small intestine, large intestine, internal and external muscle, skin, and bone were taken for [^{14}C]diflubenzuron residue quantitation. Each tissue was weighed wet, chopped up with a knife, and blended 3 times with equal volumes of ethyl acetate each time. The solvent was removed by suction filtration and aliquoted for counting. The residue was combusted for analysis of $^{14}CO_2$. The ppm of [^{14}C]-diflubenzuron equivalent residues were recorded for each tissue based on the wet weight, except for the blood, which was reported as micrograms of diflubenzuron equivalents per milliliter.

Radioassay Techniques. ^{14}C-Radiolabeled materials were detected with a Packard TriCarb liquid scintillation spectrometer (Model 3375) by using 5.0 g of PPO (2,5-diphenyloxazole) plus 3.0 g of dimethyl-POPOP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] per L of toluene as the organic scintillator and 5.5 g of PPO plus 0.2 g of

dimethyl-POPOP per L of 2:1 toluene–Triton X-100 (J. T. Baker Chemical Co.) as the aqueous scintillation cocktail. For radioassay of solids, 40.0 g of thixotropic gel (Cab-o-Sil, Packard Instrument Co.) and 5.0 g of PPO plus 2.8 g of dimethyl-POPOP per L of toluene were used as the scintillation mixture. [^{14}C]-*n*-Hexadecane, 1.13×10^6 dpm/g (Radiochemical Centre, Amersham, England), was used for internal standardization of counting efficiency. ^{14}C radioactivity was detected on thin-layer chromatograms by using both the Varian Aerograph Series 6000 thin-layer scanner and Kodak No-Screen X-ray film to which the plates were exposed for 4–10 days.

^{14}C-Labeled residues from fecal and tissue extractions were assayed by analysis of combustion products using a Packard Model 305 sample oxidizer. $^{14}CO_2$ was absorbed in monoethanolamine, which was then counted with 5.0 g of PPO plus 0.3 g of POPOP in 600 mL of toluene plus 330 mL of methyl-Cellosolve (ethylene glycol monomethyl ether) as the scintillation fluor. Up to 96% oxidation efficiency was obtained with sample sizes of less than 0.25 g as determined with [^{14}C]-*n*-hexadecane.

Chromatographic Procedures. Thin-layer chromatography was accomplished with 20 × 20 × 0.25 mm precoated silica gel 60 F-254 thin-layer plates (E. Merck Co.). Four solvent systems were used: (1) chloroform–ethanol–acetic acid, 85:10:5; (2) chloroform–methanol, 90:10; (3) benzene–dioxane–acetic acid, 90:30:1; (4) chloroform–methanol–water, 65:25:4, all in volume to volume ratios. Pure methanol was used to prewash the plates. Preparative plates were obtained by using silica gel G (E. Merck Co.), which was slurried with water (2:1 w/v), spread 2 mm thick on glass plates, and activated at 130 °C for 24 h. Ether was used as the eluant to separate interfering biological materials on preparative plates. Developing tanks were lined with filter paper to facilitate chamber saturation. All solvent systems were made fresh for each trial.

Column chromatography of aqueous materials was done with Amberlite XAD-2 (Mallinckrodt), slurried with methanol and poured into a 3.8 × 50 cm column. The column was precycled with methanol and water before adding the sample, after which it was eluted first with water and then with methanol.

Column chromatography of the organosoluble materials was done on Florisil, 60–100 mesh (Fisher Scientific Co.), poured dry into a 2.0 × 30 cm column and wet with 1 retention volume of anhydrous ether, followed by sample application and ether elution. Final isolation and cleanup of radiolabeled organic materials were done with Sephadex LH-20 (Pharmacia Fine Chemicals) that was presoaked in methylene chloride, which was the eluant. Similar columns using methanol and ethyl acetate as eluants were also used.

HPLC. High-pressure liquid chromatographic separations were conducted with a Du Pont Model 830 liquid chromatograph equipped with a solvent programmer and a 254-nm UV detector. The column used was a 1 m × 2.1 mm i.d. "Permaphase" octadecylsilane reverse-phase absorbent. Mobile phase was a concave exponential gradient from water to methanol at a rate of 2%/min. The mobile phase was pumped at 1000 psi with a flow rate of 0.3 mL/min while the column temperature was 48 °C. Response was recorded on a Varian A-25 recorder with the detector sensitivity set at 0.08–0.04 AUFS. Base-line separation of a standard mixture of possible diflubenzuron metabolites was achieved. Identification of [^{14}C]diflubenzuron and metabolites was done by chromatographing, collecting and radioassaying TLC plate extracts and Sephadex LH-20 column fractions that had similar retention volumes as authentic standards.

Table I. Recovery of [^{14}C]Diflubenzuron Equivalents following Administration of Five Milligrams per Kilogram to a Female Poland-China Duroc Pig[a]

days after administration	urine	feces extract	feces residue	total
2.0	0.04	0.01	0.00	0.04
3.0	0.58			0.58
3.5		0.01	0.00	0.01
4.0	1.00	76.36	0.32	77.68
5.0	0.87	2.78	0.12	3.77
5.5	0.40	0.63	0.05	1.09
6.0	0.39	0.87	0.14	1.40
6.5		0.18	0.04	0.22
7.0	0.80			0.80
7.5		0.19	0.07	0.25
8.0	0.67	0.25	0.12	1.04
8.5	0.27	0.17	0.07	0.51
9.0	0.06	0.10	0.08	0.23
9.5	0.19	0.04	0.06	0.28
10.0	0.41	0.04	0.03	0.47
10.5		0.05	0.04	0.09
11.0		0.04	0.03	0.07
total	5.68	81.69	1.18	88.57

[a] Excreted radioactivity is expressed as percent of the administered dose.

Metabolite Characterization. The swine feces ethyl acetate extract was concentrated and applied to TLC plates and eluted with the solvent systems described earlier. Quantitation was accomplished by counting radioactive zones scraped from the TLC plates. Swine urine adjusted to pH 5.0 was extracted with ether; the ether extract was characterized by TLC and the remaining water fraction was applied to an Amberlite XAD-2 column. The methanol and water fractions from the column were concentrated separately and applied to Florisil columns for elution with ether. The ether fraction was then applied to TLC plates for characterization of the metabolites. The combined washings from the Florisil columns were applied to Sephadex LH-20 columns for further purification and attempted characterization. Separate portions of the washings were also treated with β-glucuronidase for 24 h at 37 °C or in hydrochloric acid for 1 h at 50 °C. Radioactivity eluted or extracted from these systems was applied to TLC plates for characterization of metabolites. HPLC characterization of TLC-isolated metabolites and LH-20 metabolite fractions was also done.

Preparation and chromatography of methyl derivatives of carboxylic and hydroxylated metabolites were used for further characterization. Ethereal diazomethane was obtained by the method of Fales et al. (1973) by reacting 1 mmol of *N*-methyl-*N*-nitroso-*N'*-nitroguanidine and 0.6 mL of 5 N sodium hydroxide in 0.5 mL of water, while collecting the diazomethane in cold ether. TLC cochromatography of the methyl derivatives of reference standards with derivated metabolites isolated from swine excreta was used to further verify the identity of metabolites.

RESULTS

Swine Metabolism of [^{14}C]Diflubenzuron. Figures 1 and 2 illustrate the extraction of swine feces and urine while Table I gives the quantitative data on the recovery of [^{14}C]diflubenzuron-equivalent residues. More than 88% of the administered dose was accounted for, with over 82% in the feces and 5% in the urine. Extraction of the urine with ether resulted in only 0.1% of the administered dose in the ether phase. Fractionation of the remaining aqueous phase of urine on XAD-2 columns gave 2.5% of the ad-

Table II. [^{14}C]Diflubenzuron Residual Radioactivity in Swine Tissues

tissue	ppm
gallbladder	0.43
omental fat	0.30
liver	0.23
large intestine	0.20
subcutaneous fat	0.20
lymph (brachial node)	0.15
lung	0.11
kidney	0.11
brain	0.09
stomach wall	0.09
pancreas	0.08
small intestine	0.08
blood[a]	0.06
heart	0.05
longissimus dorsi muscle	0.05
latissimus dorsi muscle	0.04
ovary	0.04
bone	ND[b]

[a] Blood residues are calculated as micrograms of diflubenzuron equivalents per milliliter of blood. [b] ND = none detected.

ministered dose in the water portion and 2.0% in the methanol portion.

In Table II are reported the [^{14}C]diflubenzuron residues found in the swine tissues in parts per million. The highest residue, 0.43 ppm, was found in the gallbladder, while omental fat tissue was second with 0.30 ppm. All other tissue residues were found to be less than 0.20 ppm, indicating little [^{14}C]diflubenzuron residue buildup from a single oral dose.

Table III reports the swine urine [^{14}C]diflubenzuron metabolites. Comparative thin-layer characterization of the metabolites is shown for four solvent systems. β-Glucuronidase or HCl treatment of the aqueous urine fractions failed to release any extractable radioactivity. All of the [^{14}C]diflubenzuron residues extracted from the feces cochromatographed with known diflubenzuron.

Figure 3 illustrates the separation of diflubenzuron and several metabolites by HPLC. HPLC cochromatography was accomplished by collecting peaks with similar retention volumes as standards and assaying for ^{14}C. Identifications of the indicated metabolites in Table III are based on TLC cochromatography and HPLC cochromatography with authentic reference standards.

DISCUSSION

The pig in this study eliminated the bulk of administered diflubenzuron in the feces unchanged. The urine contained primarily metabolites, and the highest tissue level was found in the gallbladder. These findings indicate that most of the absorbed diflubenzuron is metabolized

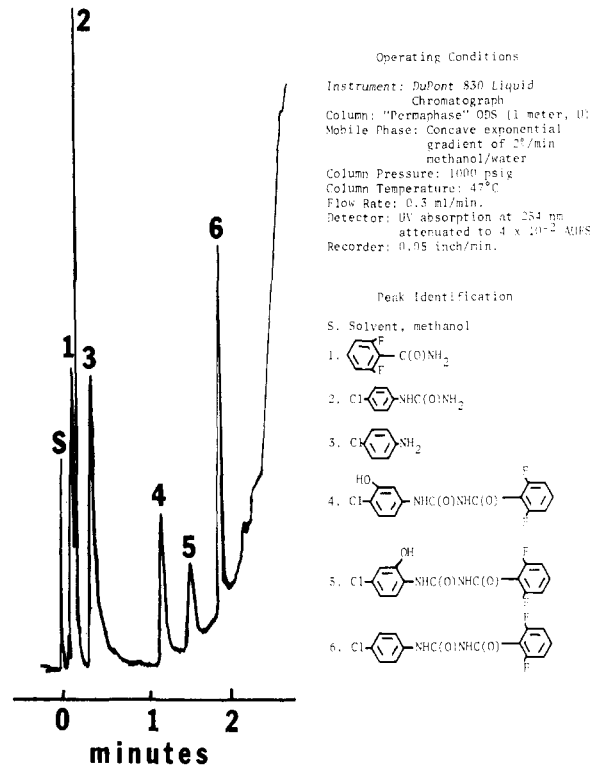

Figure 3. Separation of diflubenzuron and metabolites by high-pressure liquid chromatography.

by the pig. The small amount of unmetabolized diflubenzuron may have resulted from fecal contamination of the urine during collection. Ivie (1978) reported that all radioactivity found in the urine of cows or sheep was metabolized diflubenzuron. Ivie (1978) also demonstrated that up to 50% of the oral dose was excreted unchanged in the feces of the cow. This study seems to be consistent with the results from sheep, cattle (Ivie, 1978), and rats (Willems et al., 1980) in that diflubenzuron is eliminated rapidly and does not accumulate in the tissues. The extent of metabolism of diflubenzuron by the pig was considerably less than in rats, cattle, or sheep but equal or greater than in chickens (Opdycke et al., 1982).

It is reasonable to conclude on the basis of studies by Ivie (1978) and Willems et al. (1980) that rats and cattle are better able to hydroxylate intact diflubenzuron than the pig, sheep, or chickens. Analysis of urinary metabolites indicates cleavage of the urea bridge as the main pathway of metabolism in the pig. The presence of unidentified polar metabolites suggests the possibility that the pig may be able to hydroxylate diflubenzuron.

The fact that most of the administered diflubenzuron is excreted by the pig unchanged supports its use as a

Table III. Quantitation and Thin-Layer Chromatographic Characterization of [^{14}C]Diflubenzuron and Metabolites in Swine Urine

metabolite	% of dose	R_f values in solvent systems[a]			
		1	2	3	4
I, diflubenzuron	0.45	0.94	0.62	0.61	0.95
II, unknown	0.68	0.80	0.48	0.39	0.91
III, unknown	1.48	0.73	0.54	0.23	0.83
V, 4-chloroaniline	1.03	0.62	0.45	0.24	0.77
VI, 2,6-difluorobenzamide	0.83	0.53	0.28	0.16	0.68
VIII, (4-chlorophenyl)urea	0.82	0.39	0.34	0.22	0.62
X, unknown	0.31	0.43	0.06	0.11	0.47
XI, 2,6-difluorobenzoic acid	0.29	0.06	0.11	origin	0.32
XII, origin	0.1	origin	origin	origin	origin

[a] Solvent systems: 1, chloroform-ethanol-acetic acid (85:10:5); 2, chloroform-methanol (90:10); 3, benzene-dioxane-acetic acid (90:30:1); 4, chloroform-methanol-water (65:25:4).

feed-through insect larvicide. The detection of only small residues of [^{14}C]diflubenzuron in the tissues also lends confidence to the use of this compound for this purpose.

LITERATURE CITED

Burton, R. A.; Roe, A. M. British Patent 1 080 167, 1967.
Fales, H. M.; Jaouni, T. M.; Babshek, J. F. *Anal. Chem.* **1973**, *45*, 2302.
Haque, A.; Weisgerber, I.; Kotzias, P.; Klein, W.; Korte, F. *J. Environ. Sci. Health, Part B* **1976**, *B11*, 211.
Ivie, G. W. In "Fate of Pesticides in Large Animals"; Ivie, G. W.; Dorough, H. W., Eds.; Academic Press: New York, 1977.
Ivie, G. W. *J. Agric. Food Chem.* **1978**, *26*, 81.
Ivie, G. W.; Bull, D. L.; Veech, J. A. *J. Agric. Food Chem.* **1980**, *28*, 330.
Metcalf, R. L.; Lu, P. Y.; Bowlus, S. *J. Agric. Food Chem.* **1975**, *23*, 359.
Miller, R. W. *J. Econ. Entomol.* **1974**, *67*, 697.
Miller, R. W.; Corley, G.; Hill, K. R. *J. Econ. Entomol.* **1975**, *68*, 181.
Miller, R. W.; Corley, G.; Oehler, D. D.; Pickens, L. G. *J. Agric. Food Chem.* **1976**, *24*, 687.
Opdycke, J. C.; Miller, R. W.; Menzer, R. E. *J. Agric. Food Chem.* **1982**, following paper in this issue.
Schooley, D. A.; Quistad, G. B. *ACS Symp. Ser.* **1979**, *No. 10*, 161.
VanDaalen, J. J.; Meltzer, J.; Mulder, R.; Wellinga, K. *Naturwissenschaften* **1972**, *7*, 312.
Willems, A. G. M.; Overmars, H.; Scherpenisse, P.; DeLange, N.; Post, L. C. *Xenobiotica* **1980**, *10*, 103.
Wright, J. E. *J. Econ. Entomol.* **1974**, *67*, 746.
Wright, J. E. *J. Econ. Entomol.* **1975**, *68*, 322.
Wright, J. E.; Spates, G. E. *J. Econ. Entomol.* **1976**, *69*, 365.

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In Vivo and Liver Microsomal Metabolism of Diflubenzuron by Two Breeds of Chickens

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The metabolism and fate of [^{14}C]diflubenzuron, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, in White Leghorn (WL) and Rhode Island Red/Barred Plymouth Rock (RIR/BPR) chickens were studied after an oral dose of 5 mg/kg. Quantitation of [^{14}C]diflubenzuron equivalents confirmed a breed-related 2-fold higher egg residue level in WL chickens. Biotransformation of only small amounts of the administered compound with rapid excretion of unaltered diflubenzuron demonstrated its resistance to in vivo metabolism. WL chickens were shown to produce a higher percentage and greater number of metabolites in the excreta, but the breed-related egg residue difference was not completely a result of different in vivo metabolic rates. In vitro incubations of diflubenzuron using WL and RIR/BPR liver microsomal preparations showed less than 10% conversion to metabolites. Neither induction nor inhibition of mixed-function oxidase (MFO) activity altered diflubenzuron metabolism. The breed-related egg residue difference was thus not the result of MFO activity.

Diflubenzuron, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, is a potent, broad spectrum insecticide that inhibits the formation of insect cuticle (VanDaalen et al., 1972). Diflubenzuron has been registered as Dimilin for use against the gypsy moth and conditionally registered to control the boll weevil. It has been demonstrated that diflubenzuron will control fly larvae breeding in the manure of chickens and cattle when added to their diet (Miller, 1974; Miller et al., 1975, 1976; Wright, 1974, 1975; Wright and Spates, 1976). In the course of these "feed-through" investigations Miller et al. (1975), pointed out a 2-fold higher diflubenzuron egg residue level in White Leghorn (egg production type) chickens than in New Hampshire (meat production type) hens after 3 weeks of 50 ppm of diflubenzuron in the diet. Further investigation (Miller et al., 1976) indicated that diflubenzuron residue levels were higher in White Leghorn (WL) fat tissues than crossed Rhode Island Red/Barred Plymouth Rock (RIR/BPR) hen tissues after receiving 10 ppm of diflubenzuron in the diet for 15 weeks. The present study was undertaken to describe the in vivo metabolism and liver microsomal metabolism of [^{14}C]diflubenzuron in these two types of chickens as its relates to the demonstrated 2-fold egg residue level difference.

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MATERIALS AND METHODS

Chemicals. [^{14}C]Diflubenzuron, uniformly radiolabeled in both rings (specific activity 17.42 mCi/mmol), and technical diflubenzuron were supplied by the Thompson-Hayward Chemical Co., Kansas City, KS. Possible hydrolysis products, (4-chlorophenyl)urea, 4-chloroaniline, and 2,6-difluorobenzoic acid, as well as the hydroxylated materials, *N*-[[(4-chloro-2-hydroxyphenyl)amino]-carbonyl]-2,6-difluorobenzamide and *N*-[[(4-chloro-3-

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