on the concentration of III. However, as can be seen in Figure 5, the concentration of III was never extremely high, although from a maximum of 10 min, it continued to decrease until it could not be detected at 105 min. This tends to indicate that as quickly as metabolite III is formed, it is further metabolized very rapidly.

In the metabolic pathway indicated in Figure 1, route f which results in metabolite VI must be considered a possibility since VI is an acceptable transformation product of III. Biological conversion of VI to VII via route g would be expected to be the major process since the transformation of acetophenone to methylphenylcarbinol has been well documented (Smith et al., 1954). However, the formation of VII from IV is also quite possible.

In the present studies with the soluble fraction (105 000g) as the enzyme preparation, metabolites VIII, X, and XI were not detected. It seems unlikely that the oxidation steps in Figure 1, route c, take place. The study continues with an investigation of the enzymes in the mitochondrial and microsomal fractions of chicken liver homogenates. These should permit postulation of a metabolic pathway for the metabolism of tetrachlorvinphos in the laying hen which can be tested by in vivo studies.

ACKNOWLEDGMENT

The skilled technical assistance of N. Zabolotny is much appreciated. The mass spectrometric and NMR analyses by S. I. M. Skinner are gratefully acknowledged.

LITERATURE CITED

Akintonwa, D. A. A., Hutson, D. H., *J. Agric. Food Chem.* **15**, 632 (1967).
Baker, J. W., Tweed, W. T., *J. Chem. Soc.*, 796 (1942).
Donninger, C., Hutson, D. H., Pickering, B. A., *Biochem. J.* **126**, 701 (1972).
Fukami, J. I., Shishido, J., *J. Econ. Entomol.* **59**, 1338 (1966).
Gutenmann, W. H., St. John, L. E., Jr., Lisk, D. J., *J. Agric. Food Chem.* **19**, 1259 (1971).
Hutson, D. H., Pickering, B. A., Donninger, C., *Biochem. J.* **127**, 285 (1972).
Ivey, M. C., Hoffman, R. A., Claborn, H. V., *J. Econ. Entomol.* **61**, 1647 (1968).
Ivey, M. C., Hoffman, R. A., Claborn, H. V., Hogan, B. F., *J. Econ. Entomol.* **62**, 1003 (1969).
Johnson, M. K., *Biochem. J.* **98**, 44 (1966).
Miller, R. W., Gordon, C. H., *J. Econ. Entomol.* **66**, 135 (1973).
Oehler, D. D., Esche, J. L., Miller, J. A., Claborn, H. V., Ivey, M. C., *J. Econ. Entomol.* **66**, 1487 (1969).
Smith, J. N., Smithies, R. H., Williams, R. T., *Biochem. J.* **56**, 320 (1954).
Wasti, S. S., Shaw, F. R., *J. Econ. Entomol.* **63**, 224 (1971).
Whetstone, R. R., Phillips, D. D., Sun, Y. P., Ward, L. F., Shellenberger, T. E., *J. Agric. Food Chem.* **14**, 352 (1966).

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Mammalian Metabolism of Chlordimeform. Formation of Metabolites Containing the Urea Moiety

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The metabolism of chlordimeform-^{14}C following intraperitoneal treatment of mice and oral treatment of rats and following incubation with rat liver microsomes was examined. In addition to chlordimeform and other previously identified metabolites such as N'-(4-chloro-o-tolyl)-N-methylformamidine, 4′-chloro-o-formotoluidide, 4-chloro-o-toluidine, N-formyl-5-chloroanthranilic acid, and 5-chloroanthranilic acid, the following novel metabolites were present: N'-(4-chloro-o-tolyl)formamidine, 1,1-dimethyl-3-(4-chloro-o-tolyl)urea, 1-methyl-3-(4-chloro-o-tolyl)urea, and 3-(4-chloro-o-tolyl)urea. The N'-(4-chloro-o-tolyl)formamidine was formed by sequential N-demethylation of chlordimeform. It was suggested that at least one of the urea metabolites was formed by hydroxylation of the amidine carbon of one of the formamidines, followed by a shift to the keto tautomer. Apparently N-demethylation also was involved.

The metabolic fate of chlordimeform pesticide has been studied in dogs and goats (Sen Gupta and Knowles, 1970) and in rats in vivo (Knowles and Sen Gupta, 1970; Morikawa et al., 1975) and in vitro (Ahmad and Knowles, 1971a; Morikawa et al., 1975). Organosoluble metabolites identified included N'-(4-chloro-o-tolyl)-N-methylformamidine or demethylchlordimeform, 4′-chloro-o-formotoluidide, 4-chloro-o-toluidine, N-formyl-5-chloroanthranilic acid, and 5-chloroanthranilic acid (Ahmad and Knowles, 1971a; Knowles and Sen Gupta, 1970; Sen Gupta and Knowles, 1970). Benezet and Knowles (1976), studying the metabolism of demethylchlordimeform-^{14}C in rats, reported the isolation and tentative identification of a new formamidine metabolite, N'-(4-chloro-o-tolyl)-formamidine or didemethylchlordimeform. Examination of autoradiographs of thin-layer chromatograms from urine of rats treated orally with chlordimeform (Knowles and Sen Gupta, 1970) and of autoradiographs of thin-layer chromatograms from in vitro studies of chlordimeform metabolism by rat liver subcellular fractions (Ahmad and Knowles, 1971a) indicated that an unknown metabolite with R_f coincident with that of didemethylchlordimeform was present. Subsequent to these observations it was learned that didemethylchlordimeform was toxic to rats (Benezet et al., 1977). Therefore, it was deemed necessary to reinvestigate the metabolism of chlordimeform in rats to confirm the identity of didemethylchlordimeform and to gain data relative to its concentration in rat urine. During the course of these experiments, three metabolites containing the urea moiety were identified. This paper

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Table I. Chromatographic Behavior of Chlordimeform and Potential Metabolites

Compound	R_f value in TLC solvent system[a] A	B	C
Chlordimeform (I)	0.52	0.00	0.74
Demethylchlordimeform (II)	0.29	0.00	0.51
Didemethylchlordimeform (III)	0.06	0.00	0.20
1,1-Dimethyl-3-(4-chloro-*o*-tolyl)-urea (IV)	0.29	0.40	0.47
1-Methyl-3-(4-chloro-*o*-tolyl)-urea (V)	0.06	0.40	0.20
3-(4-Chloro-*o*-tolyl)urea (VI)	0.00	0.33	0.05
4'-Chloro-*o*-formotoluidide (VII)	0.10	0.48	0.35
4-Chloro-*o*-toluidine (VIII)	0.42	0.63	0.63
N-Formyl-5-chloroanthranilic acid (IX)	0.00	0.33	0.00
5-Chloroanthranilic acid (X)	0.00	0.57	0.00

[a] TLC carried out on glass plates coated with a 500-μm layer of silica gel GF_{254}; solvent system A = benzene-diethylamine (95:5), B = benzene-dioxene-acetic acid (90:25:4), and C = benzene-acetone-diethylamine (75:20:5).

reports the results of our reassessment of chlordimeform metabolism in rats in vivo and in vitro and also includes the results of similar studies with mice.

MATERIALS AND METHODS

Compounds, Chromatography, and Radioisotope Methodology. Chlordimeform-^{14}C, radiolabeled at the tolyl carbon atom (sp act. 4.05 mCi/mmol), was provided by CIBA-GEIGY Corp., Greensboro, N.C. The following nonradioactive compounds were examined as potential chlordimeform metabolites: demethylchlordimeform, didemethylchlordimeform, 4'-chloro-*o*-formotoluidide, 4-chloro-*o*-toluidine, 5-chloroanthranilic acid, *N*-formyl-5-chloroanthranilic acid, 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea, 1-methyl-3-(4-chloro-*o*-tolyl)urea, and 3-(4-chloro-*o*-tolyl)urea. With the exception of the urea derivatives, the sources and properties of these compounds have been reported (Benezet and Knowles, 1976; Knowles and Sen Gupta, 1970; Sen Gupta and Knowles, 1970). 3-(4-Chloro-*o*-tolyl)urea was prepared by reacting potassium cyanate with an aqueous solution of 4-chloro-*o*-toluidine hydrochloride (Geissbühler et al., 1975). The insoluble urea was filtered, washed with water, and purified by TLC. The mass spectrum (direct probe, Consolidated Electrodynamics Corp. Model 1520) was consistent with the proposed structure. Further, measurement of the molecular ion peak (*m/e* 184) yielded a mass of 184.04136; calculated for $C_8H_9N_2OCl$ 184.04032 (Beynon and Williams, 1963). Methyl and dimethyl substituted ureas were prepared by transamidation of 3-(4-chloro-*o*-tolyl)urea with methylamine and dimethylamine, respectively, in dimethylformamide at 110 °C (Geissbühler et al., 1975). They were purified by TLC. Both compounds yielded mass spectra consistent with their proposed structure. Measurement of the molecular ion peak for the methyl urea (*m/e* 198) gave 198.05592; calculated for $C_9H_{11}N_2OCl$ 198.05603 (Beynon and Williams, 1963). For the dimethyl urea the molecular ion peak (*m/e* 212) measured 212.07117; calculated for $C_{10}H_{13}N_2OCl$ 212.07163 (Beynon and Williams, 1963).

TLC was carried out on glass plates coated with a 500-μm layer of silica gel GF_{254}. Average R_f values for chlordimeform and potential metabolites in three solvent systems are given in Table I. Autoradiographs were prepared with no-screen x-ray film (Eastman Kodak Co., Rochester, N.Y.).

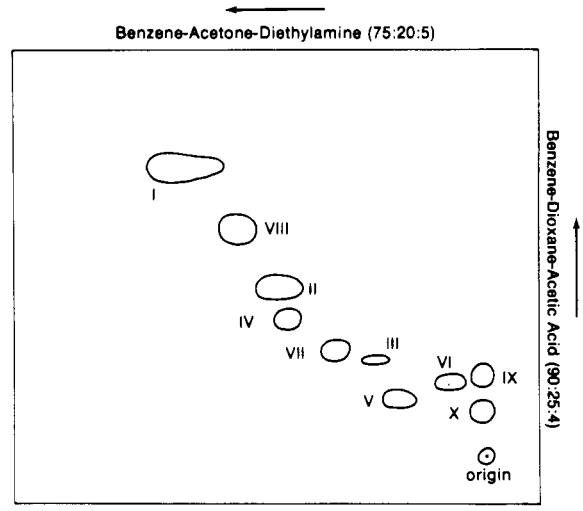

Figure 1. Diagrammatic representation of the chromatographic behavior of chlordimeform and potential metabolites. Legend: I = chlordimeform, II = demethylchlordimeform, III = didemethylchlordimeform, IV = 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea, V = 1-methyl-3-(4-chloro-*o*-tolyl)urea, VI = 3-(4-chloro-*o*-tolyl)urea, VII = 4'-chloro-*o*-formotoluidide, VIII = 4-chloro-*o*-toluidine, IX = *N*-formyl-5-chloroanthranilic acid, and X = 5-chloroanthranilic acid. Roman numeral designations for compounds correspond to the chemical names in the text and tables and the chemical formulae in the figures.

A Picker Liquimat 220 liquid scintillation spectrometer was used to measure the radiocarbon content in samples recovered from metabolism studies. Samples for radioassay were prepared as described previously (Sen Gupta and Knowles, 1970).

Metabolism Studies. Twelve male mice (Swiss-Webster) weighing about 20 g each were injected intraperitoneally with a saline solution of purified chlordimeform-^{14}C. Each mouse received about 0.6 μCi of the formamidine. Two groups of six mice were placed in Roth metabolism cages; food and water were provided ad libitum. Urine and feces samples were collected at 3, 8, 12, 24, 48, 72, and 96 h after treatment.

Urine and feces samples were analyzed for total radioactivity as described previously (Benezet and Knowles, 1976). The mouse urine was extracted according to Benezet and Knowles (1976), and the 0 to 96 h organic extracts were pooled for TLC. TLC in two directions (Figure 1) was used for resolution of the radioactive compounds in urine. Autoradiographs were prepared, and the radioactive components were quantitated (Benezet and Knowles, 1976).

Two male rats (Sprague Dawley) weighing about 150 g each were treated orally with 2 μCi of chlordimeform-^{14}C dissolved in acetone and corn oil (1:3). After treatment the rats were placed in Roth metabolism cages for 24 h. Urine samples were collected at 12 and 24 h after treatment. The total radioactivity in each sample was determined. The remaining samples were extracted, subjected to TLC and autoradiography, and the radioactive components were quantitated as described for mouse samples.

To obtain sufficient amounts of metabolites for spectral analyses, several rats were treated orally with 25 mg of purified nonradioactive chlordimeform and a small amount of chlordimeform-^{14}C. After 24 h the urine was collected and analyzed.

For in vitro studies rat liver microsomes were prepared as described by Chhabra and Fouts (1974). Metabolism of chlordimeform-^{14}C was investigated using the procedure of Ahmad and Knowles (1971). TLC of ethyl acetate

Table II. Chlordimeform-^{14}C Equivalents Eliminated in the Urine and Feces of Mice

Time after treatment, h	Cumulative % of dose		
	Urine	Feces	Total
3	27.3	16.4	43.7
8	35.3	34.6	69.9
12	37.3	38.1	75.4
18	38.0	43.3	81.3
24	39.6	45.0	84.6
48	41.8	49.7	91.5
72	42.3	51.5	93.8
96	42.5	53.0	95.5

extracts was carried out as mentioned for mouse urine.

RESULTS AND DISCUSSION

Chlordimeform-^{14}C equivalents eliminated in the urine and feces of mice are given in Table II. During the 96-h experimental period 42.5% of the radiocarbon was present in the urine and 53.0% was present in the feces. Thus 95.5% of the administered dose was eliminated in mouse urine and feces by 96 h (Table II). The vast majority of the radioactive material in mouse urine was water soluble. This material probably consisted mainly of conjugates, such as glucuronides and ethereal sulfates, based on analogy with previous studies of chlordimeform metabolism in rats (Knowles and Sen Gupta, 1970). An average of 14.0% of the urinary radioactivity in mouse urine was organosoluble, and the nature and relative amount of this material are given in Table III. The major organosoluble chlordimeform metabolites (>10%) were 4'-chloro-*o*-formotoluidide (VII), 4-chloro-*o*-toluidine (VIII), and *N*-formyl-5-chloroanthranilic acid (IX). Demethylchlordimeform (II), didemethylchlordimeform (III), 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea (IV), 1-methyl-3-(4-chloro-*o*-tolyl)urea (V), 3-(4-chloro-*o*-tolyl)urea (VI), and 5-chloroanthranilic acid (X) also were present. Identification was based on cochromatography with authentic standards (Table I, Figure 1).

These same metabolites were present in urine of rats treated orally with chlordimeform-^{14}C (Table III). Major organosoluble chlordimeform metabolites (>10%) in rat urine included 3-(4-chloro-*o*-tolyl)urea (VI), 4'-chloro-*o*-formotoluidide (VII), 4-chloro-*o*-toluidine (VIII), and

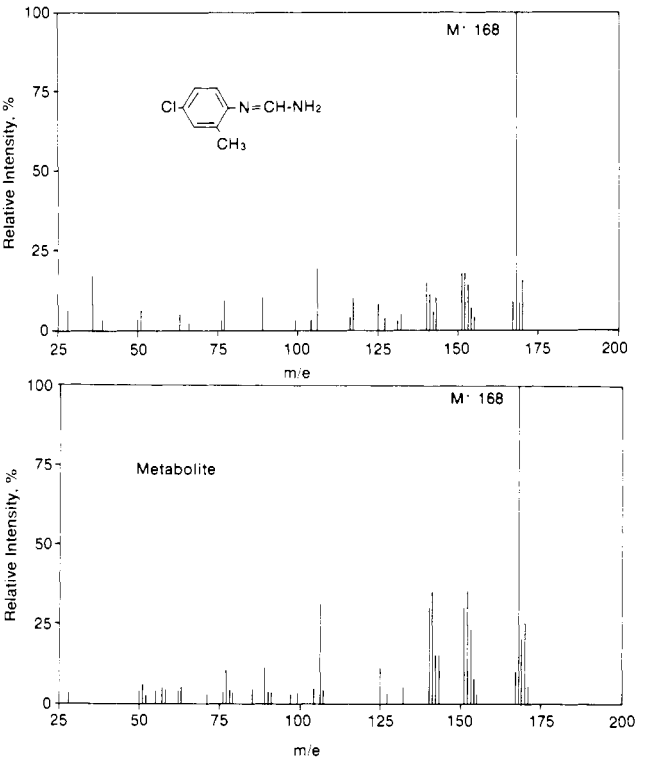

Figure 2. Partial mass spectra for didemethylchlordimeform (III) (top) and a metabolite (bottom) isolated from the urine of chlordimeform-treated rats.

N-formyl-5-chloroanthranilic acid (IX) (Table III). Demethylchlordimeform (II), didemethylchlordimeform (III), 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea (IV), 1-methyl-3-(4-chloro-*o*-tolyl)urea (V), and 5-chloroanthranilic acid (X) were minor metabolites (Table III). The identity of didemethylchlordimeform (III) and 3-(4-chloro-*o*-tolyl)urea (VI) in rat urine was accomplished by cochromatography and mass spectral analysis (Figures 2 and 3). For comparison partial mass spectra for 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea (IV) and its *N*-demethyl derivative (V) are given in Figure 4.

Rat liver microsomes fortified with NADPH also rapidly degraded chlordimeform (Table III). The major metabolite at each analysis time was demethylchlordimeform (II); this

Table III. Metabolism of Chlordimeform-^{14}C following Intraperitoneal Treatment of Mice, Oral Treatment of Rats, and Incubation with Rat Liver Microsomes[a]

Compound	% identified organosoluble ratioactive material at indicated time interval, h					
	In vivo			In vitro		
	Mouse	Rat		Rat[e]		
	0 to 96[b]	0 to 12[c]	12 to 24[d]	1	2	6
Chlordimeform (I)	15.2	2.9	16.5	32.0	23.6	7.7
Demethylchlordimeform (II)	4.6	3.4	3.9	43.0	44.5	44.6
Didemethylchlordimeform (III)	4.2	2.5	6.4	3.1	3.4	4.7
1,1-Dimethyl-3-(4-chloro-*o*-tolyl)urea (IV)	0.9	0.5	0.9	2.8	4.2	1.3
1-Methyl-3-(4-chloro-*o*-tolyl)urea (V)	5.7	6.5	6.1	4.7	4.3	1.9
3-(4-Chloro-*o*-tolyl)urea (VI)	5.8	15.2	9.2	1.4	1.5	2.0
4'-Chloro-*o*-formotoluidide (VII)	13.5	16.4	14.8	1.9	5.0	25.8
4-Chloro-*o*-toluidine (VIII)	12.8	23.8	12.7	8.6	10.1	7.6
N-Formyl-5-chloroanthranilic acid (IX)	12.9	13.4	16.0	0.3	0.6	0.3
5-Chloroanthranilic acid (X)	2.5	8.2	5.5	1.8	2.4	3.3
Origin	21.9	7.2	8.0	0.4	0.4	0.8

[a] Organosoluble extracts were chromatographed on silica gel GF_{254} as given in Figure 1. [b] Organosoluble fractions from the eight sampling intervals were pooled for TLC analysis. About 14% of the urinary radioactivity was organosoluble. [c] Organosoluble fraction comprised 5.2% of urinary radioactivity from 0 to 12 h. [d] Organosoluble fraction comprised 2.3% of urinary radioactivity from 12 to 24 h. [e] Values were corrected for nonenzymatic degradation. An average of 96.6% of the total radioactive material was organosoluble at the three incubation periods.

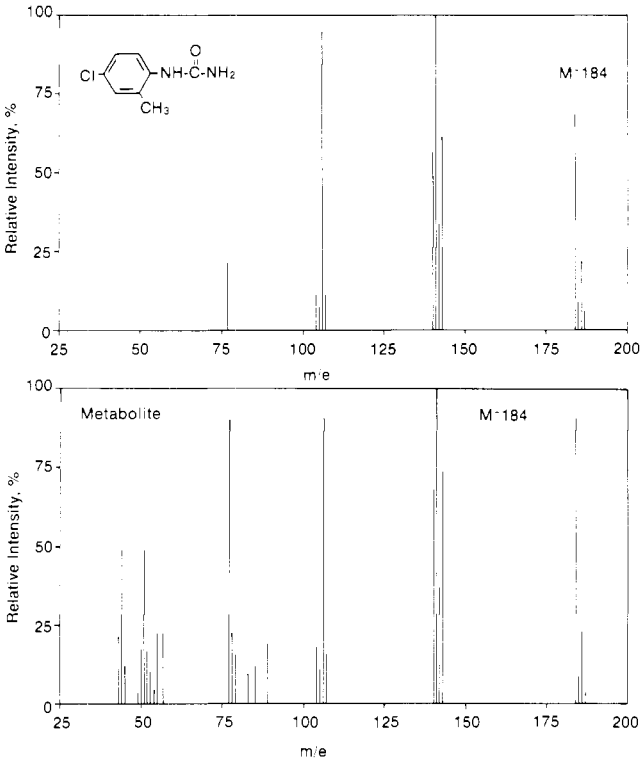

Figure 3. Partial mass spectra for 3-(4-chloro-*o*-tolyl)urea (VI) (top) and a metabolite (bottom) isolated from the urine of chlordimeform-treated rats.

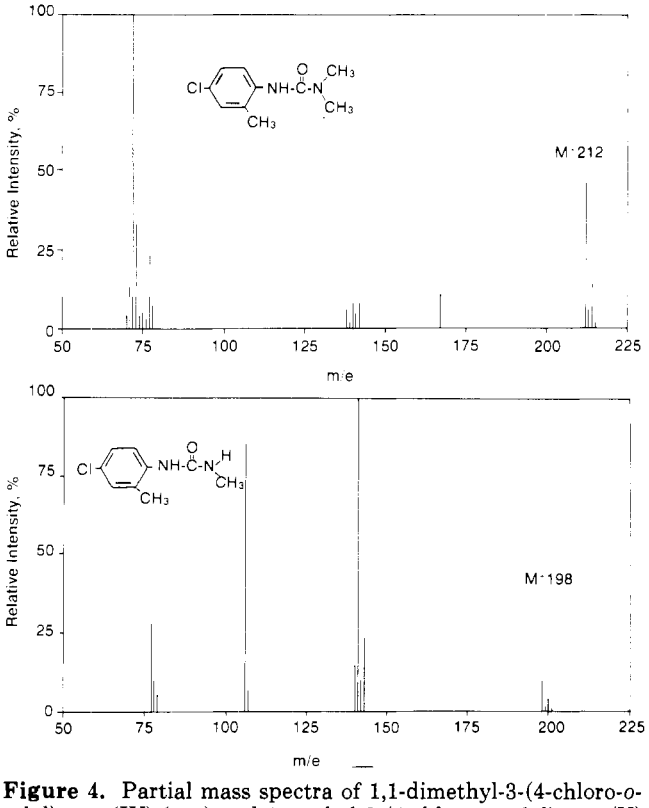

Figure 4. Partial mass spectra of 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea (IV) (top) and 1-methyl-3-(4-chloro-*o*-tolyl)urea (V) (bottom).

finding was consistent with previous studies (Ahmad and Knowles, 1971a). 4′-Chloro-*o*-formotoluidide (VII) and 4-chloro-*o*-toluidine (VIII) also were present in appreciable amounts.

In previous in vivo and in vitro studies of chlordimeform metabolism by mammals (Ahmad and Knowles, 1971a; Knowles and Sen Gupta, 1970; Morikawa et al., 1975; Sen Gupta and Knowles, 1970) benzene–diethylamine (95:5) was the TLC solvent system of choice. When using this solvent system (Table I) it was not possible to resolve demethylchlordimeform (II) from 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea (IV) and didemethylchlordimeform (III) from 1-methyl-3-(4-chloro-*o*-tolyl)urea (V) as their R_f values were coincident. The data in Table III indicated that in mice and rats demethylchlordimeform (II) was present in larger amounts than 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea (IV) and that didemethylchlordimeform (III) and 1-methyl-3-(4-chloro-*o*-tolyl)urea (V) were present in about equal amounts. However, neither of the four compounds was a major organosoluble chlordimeform metabolite in mouse and rat urine (Table III). 3-(4-Chloro-*o*-tolyl)urea (VI) was a major urinary metabolite. It chromatographed at the origin in the benzene diethylamine system and heretofore was not isolated and quantitated.

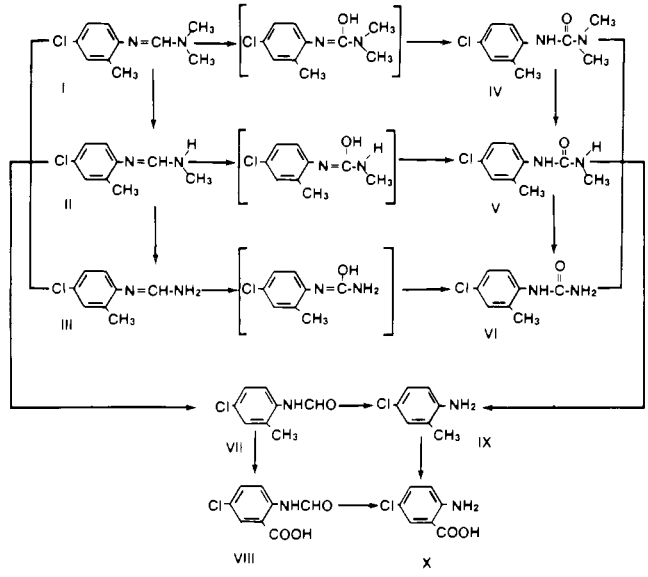

Figure 5. Proposed metabolic paths for chlordimeform (I) metabolism in rats and mice.

Chlordimeform was rapidly and extensively degraded by mice and rats; proposed paths for formation of organosoluble metabolites are given in Figure 5. The present study and other investigations (Ahmad and Knowles, 1971a; Knowles, 1970; Knowles and Sen Gupta, 1970; Sen Gupta and Knowles, 1970) have indicated that chlordimeform (I) was demethylated oxidatively to demethylchlordimeform (II). It seems probable that didemethylchlordimeform (III) was formed by oxidative *N*-demethylation of demethylchlordimeform (II). 1,1-Dimethyl-3-(4-chloro-*o*-tolyl)urea (IV) probably was formed by hydroxylation of the amidine carbon of chlordimeform (I), followed by a shift to the keto tautomer. The other two urea metabolites (V and VI) could have formed by a similar mechanism from their respective formamidines (II and III) or by sequential oxidative N-demethylation. Oxidative N-dealkylation is an important pathway for mammalian metabolism of substituted phenylurea herbicides (Geissbühler et al., 1975).

4′-Chloro-*o*-formotoluidide (VII) probably was formed from each of the formamidines (I, II, and III) by direct hydrolytic cleavage (Knowles, 1970). However, the high levels of this compound (VII) in the in vitro studies also indicated an oxidative mode for its formation. 4-Chloro-*o*-toluidine (VIII) could have been produced by direct cleavage of the ureas (IV, V, and VI) or by defor-

mylation of 4'-chloro-*o*-formotoluidide (VII). Direct cleavage of substituted phenylurea herbicides in mammals to yield substituted anilines has been postulated but apparently is not a major pathway. Therefore, it seemed likely that most of the 4-chloro-*o*-toluidine (VIII) was derived from 4'-chloro-*o*-formotoluidide (VII). This conversion has been well documented (Ahmad and Knowles, 1971a; Knowles, 1970), and an enzyme capable of catalyzing the deformylating reaction was partially purified from rat liver (Ahmad and Knowles, 1971b).

N-Formyl-5-chloroanthranilic acid (IX) was formed by oxidation of the tolyl methyl moiety of 4'-chloro-*o*-formotoluidide (VII), and the 5-chloroanthranilic acid (X) was formed by deformylation of *N*-formyl-5-chloroanthranilic acid (IX) and/or by oxidation of the tolyl methyl moiety of 4-chloro-*o*-toluidine (VIII) (Knowles, 1970; Knowles and Sen Gupta, 1970).

LITERATURE CITED

Ahmad, S., Knowles, C. O., *Comp. Gen. Pharmacol.* **2**, 189 (1971a).
Ahmad, S., Knowles, C. O., *J. Econ. Entomol.* **64**, 792 (1971b).
Benezet, H. J., Knowles, C. O., *J. Agric. Food Chem.* **24**, 152 (1976).
Benezet, H. J., Chang, K. -M., Knowles, C. O., in "Neurotoxic Actions of Pesticides and Venoms," Shankland, C. L., Flattum, R. F., Hollingworth, R. M., Smyth, T., Ed., Plenum Press, New York, N.Y., in press, 1977.
Beynon, J. H., Williams, A. E., "Mass and Abundance Tables for Use in Mass Spectrometry," Elsevier, New York, N.Y., 1963.
Chhabra, R. S., Fouts, R. J., *Drug Metab. Dispos.* **2**, 375 (1974).
Geissbühler, H., Martin, H., Voss, G., in "Herbicides, Chemistry, Degradation and Mode of Action", Vol. 1, Kearney, P. C., Kaufman, D. D., Ed., Marcel Dekker, New York, N.Y., 1975.
Knowles, C. O., *J. Agric. Food Chem.* **18**, 1038 (1970).
Knowles, C. O., Sen Gupta, A. K., *J. Econ. Entomol.* **63**, 856 (1970).
Morikawa, M., Yokoyama, S., Fukami, J., *Bochu-Kagaku* **40**, 162 (1975).
Sen Gupta, A. K., Knowles, C. O., *J. Econ. Entomol.* **63**, 951 (1970).

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Residues of Diflubenzuron [1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl)urea] in Pasture Soil, Vegetation, and Water following Aerial Applications

Charles H. Schaefer* and Emil F. Dupras, Jr.

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] was applied to irrigated pastures using liquid (25% wettable powder in water) and granular (1% Attaclay and 1% sand) formulations. Multiple applications of the wettable powder produced high and long-lasting residues on vegetation; these residue levels were reduced but not eliminated using the 1% Attaclay granules. One percent sand granules achieved control of mosquito larvae and no high or persistent residues on vegetation resulted. None of these formulations caused high or persistent residues in soils.

Diflubenzuron, TH6040, or Dimilin [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] shows high potential as a mosquito control agent (Schaefer et al., 1975). Since diflubenzuron is projected to be used as a commercial larvicide, its persistence in mosquito breeding habitats is of high interest. It has been demonstrated that its persistence in pasture waters is limited to a few days following aerial applications of a 25% wettable powder formulation containing 0.02 to 0.04 lb of active ingredient (AI)/acre; hydrolysis, and adsorption onto organic matter, appear to limit persistence in water (Schaefer and Dupras, 1976). As it is anticipated that diflubenzuron will be used numerous times (each irrigation cycle) on the same fields in a given year, for controlling pasture mosquitoes (*Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar) in California, determinations of residues in water, soil, and vegetation following multiple applications were made.

MATERIALS AND METHODS

1975 Studies. Two 40-acre pastures were treated with four consecutive applications of diflubenzuron each during the 1975 field season. One field (Colosso pasture in western Stanislaus County) was treated at 0.02 lb of AI/acre and the other (Monteiro pasture in Tulare County) at 0.04 lb of AI/acre. These rates cover the projected range for operational use of diflubenzuron for pasture mosquito control. A 25% wettable powder formulation was mixed so that the aircraft dispersed the active ingredient in 1 gal of water/acre. No other chemical treatments were made to these fields during the study period.

Before and at 1 h, 1, 3, and 7 days following each of the first three treatments and 1 h, 1, 3, 7, 28, and 56 days following the fourth treatment, soil and vegetation samples were collected from each field. Soil cores (3 in. diameter and ca. 3 in. depth) were collected, using a sharpened steel cylinder, from areas not having vegetative cover. Cores were collected from areas over the entire field until a composite sample of 5–6 lbs was obtained. Vegetation samples were cut from areas over the entire field until 3–4 lbs, fresh weight, was obtained. Soil and vegetation samples were stored at −20 °C until analysis. Water samples (4 × 600 mL) were collected before and at 1, 24, 48, and 72 h after each treatment.

1976 Studies. A comparative study of residues in water, soil, and vegetation was made using four multiple aerial applications of 0.04 lb of AI/acre of a liquid (25% wettable powder in 1 gal of water/acre) and a dry (1% diflubenzuron on 16/30 mesh Attaclay granules) formulation. One-half of a 12-acre pasture (File pasture, Fresno County) was treated with the wettable powder and one-half with

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