Comparative Metabolism of Sulfamidine and Chlordimeform in Rats

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To investigate the cause for differential toxicity between sulfamidine and chlordimeform, ^{14}C-labeled sulfamidine hydrochloride or chlordimeform hydrochloride was orally administered to male rats. In both cases, the dosed radioactivity was eliminated primarily into urine within the first 48 h. The identified urinary metabolites for sulfamidine were identical with that of chlordimeform, except for sulfamidine sulfoxide (III). Oxidation to sulfamidine sulfoxide is likely the initial metabolic step for sulfamidine, and metabolism of sulfamidine proceeded more rapidly than that of chlordimeform. On the other hand, the rate of formation of *N*-desmethylchlordimeform in vitro was higher in the preparation incubated with chlordimeform than that incubated with sulfamidine. *N*-Desmethylchlordimeform is more toxic against mammalian species as compared to sulfamidine, chlordimeform, and their metabolites. The major difference in metabolism between sulfamidine and chlordimeform appears to be the level of *N*-desmethylchlordimeform initially formed.

Sulfamidine hydrochloride [*N'*-(4-chloro-*o*-tolyl)-*N*-methyl-*N*-[(methylthio)methyl]formamidine hydrochloride (I·HCl), also known as HOKUPANON, Hokko Chemical Industry Co.] is a new insecticide–acaricide. This compound has been shown to be effective against the rice stem borer *Chilo supprealis* Walker (Takahashi et al., 1974), one of the most serious lepidopterous pest insect species in paddy fields in Japan. It is also effective against the two-spotted spider mite *Tetranychus urticae* Koch (Chang and Knowles, 1977), Kanzawa spider mite *Tetranychus kanzawai* (Kuwahara, 1978), and cattle tick *Boophilus microplus* (Atkinson and Knowles, 1974). Sulfamidine sulfoxide (III) and their common metabolite *N*-desmethylchlordimeform (V) also have marked activity against rice stem borer, two-spotted spider mite, and cattle tick (data of Hokko Chemical Industry; Morikawa et al., 1975; Chang and Knowles, 1977; Atkinson and Knowles, 1974).

Despite the their similar chemical structure of sulfamidine and chlordimeform, their mammalian toxicities are different. The acute oral LD_{50} of sulfamidine hydrochloride and sulfamidine sulfoxide were 1410 and 933 mg/kg for mice, respectively. The acute oral LD_{50} of chlordimeform, *N*-desmethylchlordimeform and *N,N*-didesmethylchlordimeform were 267, 163, and 78 mg/kg for mice, respectively (Hollingworth and Yim, 1980). Thus, sulfamidine has a lower mammalian toxicity in this regard. In the past the metabolism of chlordimeform has been extensively studied in white rats (Morikawa et al., 1975; Hornish, 1984; Knowles and Sen Gupta, 1970) and dogs and goats (Sen Gupta and Knowles, 1970). Its metabolism and toxicity in mammals have been reviewed by Hollingworth and Yim (1980). The existence of extensive metabolic information on this compound as well as its similarity to sulfamidine makes it as an ideal reference compound for a comparative study.

The purpose of the present study is to investigate the cause(s) for such differential toxicity in rats. More specifically, we have examined the differences in metabolism of sulfamidine and chlordimeform in rats to test a hypothesis that such a difference is due to metabolic activities.

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MATERIALS AND METHODS

Chemicals. [^{14}C]Sulfamidine hydrochloride (I·HCl) and [^{14}C]chlordimeform hydrochloride (II·HCl), both labeled with radiocarbon at the phenyl moiety, were purchased from New England Nuclear Co. Their specific activities were 4.25 and 4.25 mCi/mmol, respectively. Those chemicals have a purity greater than 99% as shown by thin-layer chromatography (Table V).

The following nonradioactive compounds were synthesized: sulfamidine hydrochloride (I·HCl), mp 195–196 °C; chlordimeform hydrochloride (II·HCl), mp 225–227 °C; *N'*-(4-chloro-*o*-tolyl)-*N*-methyl-*N*-[(methylsulfinyl)-methyl]formamidine or sulfamidine sulfoxide (III); *N'*-(4-chloro-*o*-tolyl)-*N*-methyl-*N*-[(methylsulfonyl)methyl]-formamidine or sulfamidine sulfone (IV); *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine or *N*-desmethylchlordimeform (V); *N'*-(4-chloro-*o*-tolyl)formamidine or *N,N*-didesmethylchlordimeform (VI); *N*-formyl-4-chloro-*o*-toluidine (VII); *N*-formyl-5-chloroanthranilic acid (IX); methyl 5-chloroanthranilate; methyl *N*-formyl-5-chloroanthranilate; 4-chloro-2-methylacetanilide (XI). 4-Chloro-*o*-toluidine (COT, VIII) and 5-chloroanthranilic acid (X) were purchased from Tokyo Chemical Industry Co. Analytical data of sulfamidine sulfoxide: 60-MHz NMR ($CDCl_3$, Me_4Si) δ 2.21 (s, 3 H), 2.61 (s, 3 H), 3.28 (s, 3 H), 4.86 (m, 2 H), 6.68 (w, 1 H), 7.02–7.55 (m, 3 H); IR (neat) cm^{-1} 1640 (C=N), 1040 (S=O); CI-MS (isobutane) *m/e* 259 $(M + 1)^+$, 183 (base peak). Analytical data of sulfamidine sulfone: 60-MHz NMR ($CDCl_3$, Me_4Si) δ 2.21 (s, 3 H), 2.98 (s, 3 H), 3.30 (s, 3 H, 4.80 (s, 2 H), 6.68 (w, 1 H), 7.02–7.55 (m, 3 H); IR (neat) cm^{-1} 1630 (C=N), 1310 (SO_2), 1120 (SO_2); EI-MS, *m/e* 274 $(M)^+$, 195, 182, 152, 141, 117 (base peak).

Chromatographic Analysis. For quantitative thin-layer chromatographic (TLC) studies, Whatman LK5F precoated plates with 250-μm silica gel layer were used. Separation, collection, and further identification steps were carried out on Merck precoated plates with 2-mm silica gel layer. A mixture of nonradiolabeled reference standards was applied to the side of the sample spot. Plates were prepared for one-dimensional development in two solvent systems: system A was 75:20:5 benzene–acetone–diethylamine, and system B was 90:25:4 benzene–dioxane–acetic acid. The radioactive spots were detected by

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autoradiography with no-screen X-ray film (Fuji Photo Film Co., Ltd., Tokyo), and spots of unlabeled reference compounds were visualized under ultraviolet irradiation. The scraped silica gel was wetted with 0.2 mL of 1:1 MeOH–H_2O and then slurried with scintillator for radioactive measurement. For further confirmation of the nature of metabolites, the TLC-purified sample fractions were analyzed on a gas–liquid chromatograph (GLC). A Shimadzu GC-7A gas chromatograph equipped with flame ionization detector and alkali flame ionization detector was used. The column packings used were 3% OV-17, 5% Apiezone grease L, 5% PEG-HT, and 5% FFAP + 1% KOH, all on Gas Chrom Q, 80–100 mesh. Those were packed in a glass column with a 3-mm i.d. Methyl esters of acids IX and X were used as authentic compounds. Electron impact and chemical ionization mass spectra (EI-MS, CI-MS) were obtained on a JMS-D300 mass spectrometer (JEOL Ltd., Tokyo) using isobutane as reagent gas.

Radioactive Measurement. Radioactive metabolites were partitioned in several fractions. Radioactivity found in organic solvents, aqueous phase, and silica gel was determined by conventional liquid scintillation spectroscopy. Radioactivities found in fecal material, body organs, and tissues were determined by using a Harvay Instrument Model OX-200 biological oxidizer, whereby ^{14}C was determined as $^{14}CO_2$ trapped in a scintillation solution consisting of 1 part Carbosorb II and 2 parts Permaflour V (Packerd Instrument Co.).

Treating and Handling of Rats. Male Sprague–Dawley rats, weighing approximately 150 g, were supplied by Spartan Research Animals, Inc., Haslett, MI. They were fasted during the period of 24 h, and each was given a single oral dose of [^{14}C]sulfamidine hydrochloride (10 μCi, 130 mg/kg) or five consecutive daily doses (2 μCi/day, 26 mg/kg). Each batch of three additional rats was treated with [^{14}C]chlordimeform hydrochloride by the identical procedure as for sulfamidine. After treatment, each rat was placed in a metabolic cage, and urine and feces were collected at regular intervals. Food and water were provided ad libitum. Tissue samples were recovered on sacrifice. For collection of large quantities of metabolites, rats were treated orally with unlabeled sulfamidine hydrochloride (500 mg/kg) or unlabeled chlordimeform hydrochloride (300 mg/kg). Urine and feces were collected during the 3 days posttreatment period.

Analysis of Excreta and Tissues. The feces were dried over P_2O_5 in a vacuum dessicator and ground with a mortar and a pestle. Portions (100–200 mg/sample) of the ground feces were subjected to combustion and liquid scintillation counting. Tissue samples were directly analyzed for total radiocarbon by combustion. The urine samples were analyzed directly by the latter method. They were transferred to a 10-mL glass-stopped centrifuge tube and adjusted to pH 8 with 1 N NaOH. Each sample was extracted three times with an equivalent volume of chloroform. The extracts were combined, dried over anhydrous sodium sulfate, and condensed. The aqueous solution was adjusted to pH 3 with 1 N HCl. This solution was extracted three times with chloroform as above. Aliquots of chloroform and water layer were transferred to a scintillation vial, chloroform was evaporated slowly under a gentle stream of dry air, and their radioactivities were determined. The remaining chloroform was evaporated to ca. 0.2 mL under a stream of dry air and spotted on a thin-layer plate. After the plate was developed in either solvent system A (in the case of pH 8 extract) or solvent system B (in the case of pH 3 extract), radioautograms were developed. The silica gel corresponding to the images on the film was scraped from the plate and radioassayed as mentioned.

In Vitro Study. In this study the 20000*g* supernatant from male rat liver was used. The method for preparation of this subcellular fraction has been described by Conaway et al. (1977). In a typical experiment 0.05 μmol (0.2 μCi) of [^{14}C]sulfamidine hydrochloride or [^{14}C]chlordimeform hydrochloride in 10 μL of methyl cellosolve was incubated with 0.5 mL of supernatant plus various combination of cofactors and inhibitors in a 25-mL Erlenmeyer flask. The protein concentration in supernatant was adjusted to approximately 8 mg/mL as determined by the method of Lowry et al. (1951). The required cofactors such as GSH, NADPH, UDPGA, ATP, APS, and $MgCl_2$ were dissolved in the standard buffer (0.15 M phosphate buffer pH 7.4 and 0.05 M sucrose) at desired concentrations. By adding standard buffer, the incubation mixture was finally made up to 2.0 mL. The reaction mixtures were incubated aerobically at 37 °C by using a Lab-line shaking water bath for 2 h in the dark. At the end of the reaction period, 3 mL of ether was added to stop the reaction and the mixture was extracted twice with an equivalent volume of ether. The control was prepared by incubating [^{14}C]-sulfamidine hydrochloride or [^{14}C]chlordimeform hydrochloride with 0.5 mL of supernatant and 1.5 mL of standard buffer without cofactor or inhibitor. The combined ether phase was analyzed by TLC–autoradiography. The aqueous phase was directly radioassayed.

Identification of Metabolites. The metabolites were tentatively identified by TLC with appropriate authentic standards by the two solvent systems A and B. The chloroform extract of urine at pH 8 was mainly subjected to TLC in solvent system A; the chloroform extract from urine at pH 3 was subjected to TLC in solvent system B. For preparation of large amounts of metabolites, the pooled urine samples were extracted with chloroform (at pH 8 and 3), concentrated, and subjected to preparative TLC. The TLC-purified acids IX and X were methylated in ether with diazomethane at room temperature overnight. Purified metabolites and their derivatives were subjected to GLC and MS. Conjugates remaining in the aqueous phase were subjected to enzyme hydrolysis or acid hydrolysis as follows: 0.5 mL portions of aqueous phases were incubated with either 7000 units of β-glucuronidase type B-3 (Sigma Chemical Co., St. Louis), 150 units of arylsulfatase type VIII (Sigma Chemical Co., St. Louis) in 3.5 mL of acetate buffer at pH 5.0, or 3.5 mL of 1 N HCl (or 3.5 mL of buffer only for control). The reaction mixtures were incubated at 37 °C for 24 h. After the incubation mixture was adjusted to pH 3.0, the released aglycons were extracted twice each time with an equal volume of ether. The remainder was extracted twice with ether at pH 8. The extracts were combined for radioassay.

RESULTS

Excretion. The excretion of radioactivity from the rats orally administrated [^{14}C]sulfamidine hydrochloride (I·HCl) or [^{14}C]chlordimeform hydrochloride (II·HCl) is shown in Figure 1. Following a single oral administration of [^{14}C]sulfamidine hydrochloride the dosed radioactivity was rapidly eliminated into the urine (about 84%) and feces (about 10%) within 3 days. Most of the radioactivity was excreted within 2 days. When [^{14}C]chlordimeform hydrochloride was given to rats, the dosed radioactivity was also rapidly excreted into the urine (about 87%) and feces (about 8%) within 3 days. The overall results were similar to those obtained in the [^{14}]sulfamidine hydrochloride experiment. However, a small difference was found in

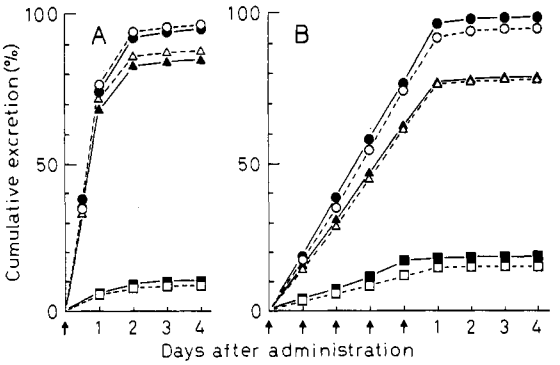

Figure 1. Cumulative excretion of radioactivity in feces and urine of rats: (A) single dose 130 mg/kg; (B) continuous dose 26 mg/kg, five times. Key: (↑) the point of administration. (●) [^{14}C]-sulfamidine hydrochloride; (▲) urine; (■) 7 feces. (○) total [^{14}C]chlordimeform hydrochloride; (△) urine; (□) feces.

Table I. Partition Behavior of Metabolites in Both Urine and Feces

	^{14}C radiocarbons[a]	
	sulfamidine	chlor-dimeform
urine, total recovery[a]	79.9	85.3
pH 8 chloroform extractable	13.0	27.2
pH 3 chloroform extractable	18.3	10.8
aqueous	41.9	38.9
feces, total recovery[a]	11.0	6.8
acetone phase	1.6	0.6
MeOH phase	3.4	2.1
solid phase	7.0	3.9

[a] Percent radioactivity calculated from the initial amount.

excretion pattern (Table I). At pH 8, chloroform could extract about 27% of chlordimeform-derived radioactive metabolites from the urine as compared with 13% from sulfamidine-treated samples. At pH 3, chloroform could extract about 11% of radioactivity from the urine of chlordimeform-treated rats as compared with 18% from that treated with sulfamidine. This chloroform-soluble fraction (pH 3) was considered to contain more extensively metabolized products than that extracted at pH 8. The rate of metabolism of sulfamidine appears to be faster than that of chlordimeform. The amounts of metabolites remaining in the aqueous phase of the urine and feces were not different from sulfamidine to chlordimeform.

The patterns of excretion of the radioactivity by the rats administered five daily doses of either [^{14}C]sulfamidine hydrochloride or [^{14}C]chlordimeform hydrochloride are shown in Figure 1. The rats administered [^{14}C]sulfamidine hydrochloride excreted 79% of the total dosed radioactivity into urine and 19% into feces, while the rats administered [^{14}C]chlordimeform hydrochloride excreted 78% and 15%, respectively. The pattern of excretion of sulfamidine was almost same as that of chlordimeform.

The amounts of the metabolites excreted in the urine of rats during the first 3-day period following a single oral administration of [^{14}C]sulfamidine hydrochloride or [^{14}C]chlordimeform hydrochloride are listed in Table II. Several urinary metabolites were identified in both cases. All of the identified sulfamidine metabolites were identical with those from chlordimeform, except sulfamidine sulfoxide (III). However, significant differences were observed in the levels of the metabolites between sulfamidine and chlordimeform treatment. 4-Chloro-*o*-toluidine (VIII) was obtained as a metabolite in both treatments. The 4-chloro-*o*-toluidine residues accounted for 2.8% and 8.6% of the originally administered sulfamidine and chlordimeform, respectively. *N*-Formyl-4-chloro-*o*-toludine (VII) was also identified as a metabolite from both treatments, the amounts being 3.0% and 8.5%, respectively. By comparison, the amounts of the more polar metabolites, 5-chloroanthranilic acid (X) and *N*-formyl-5-chloroanthranilic acid (IX), derived from sulfamidine were greater than those from chlordimeform. It is expected that at least some of these metabolites in the aqueous phase of the urine are conjugated. The treatments with deconjugation agents such as β-glucuronidase, arylsulfatase, or strong acid significantly increased the levels of radioactivity that could be extracted into ether in both cases. It is likely, therefore, that some of these metabolites are glucuronide(s) and sulfate(s). However, their levels appear to be almost identical in both pesticide treatment cases.

Identification of Metabolites. The partition behavior of metabolites in feces and urine from [^{14}C]sulfamidine hydrochloride or [^{14}C]chlordimeform hydrochloride administration is shown in Table I. The chloroform layers were concentrated and subjected to TLC using the solvent systems A and B. The metabolites were tentatively identified by TLC comparison with authentic standards. Several urinary metabolites from [^{14}C]sulfamidine hydrochloride and [^{14}C]chlordimeform hydrochloride were identified and quantitatively determined by TLC–autoradiography. TLC analysis with two solvent systems indicated that at least five metabolites were detected in chloroform-soluble extracts at pH 8 from the urine and two metabolites were found in the extracts at pH 3 from [^{14}C]sulfamidine and [^{14}C]chlordimeform samples. Nine metabolites from M-1 to M-9 were tentatively identified by TLC (Table II): sulfamidine (I), chlordimeform (II), 4-chloro-*o*-toluidine (VIII), *N*-desmethylchlordimeform

Table II. Relative Amounts of Radioactive Metabolites Present in Urine on Day 3 after Oral Administration of [^{14}C]Sulfamidine Hydrochloride or [^{14}C]Chlordimeform Hydrochloride

		R_f value		amount, % radioactivity			
				I·HCl		II·HCl	
	metabolite	A[a]	B[a]	pH 8 (A)[a]	pH 3 (B)[a]	pH 8 (A)[a]	pH 3 (B)[a]
M-1	sulfamidine	0.89	0.80	0.2	nd		
M-2	chlordimeform	0.88	0			1.0	nd
M-3	4-chloro-*o*-toluidine	0.75	0.75	2.8	nd	8.6	nd
M-4	*N*-desmethylchlordimeform	0.62	0.05	1.1	nd	1.1	nd
M-5	*N*-formyl-4-chloro-*o*-toluidine	0.41	0.55	3.0	nd	8.5	nd
M-6	sulfamidine sulfoxide	0.34	0.19	0.3	nd		
M-7	*N*,*N*-didesmethylchlordimeform	0.25	0.04	0.9	nd	1.1	nd
M-8	5-chloroanthranilic acid	0	0.59	nd	6.4	nd	3.2
M-9	*N*-formyl-5-chloroanthranilic acid	0	0.28	nd	2.5	nd	1.2
	others			4.7	9.4	6.6	6.4
	total			13.0	18.3	27.2	10.8

[a] Solvent systems: (A) benzene–acetone–diethylamine (75:20:5); (B) benzene–dioxane–acetic acid (90:25:4).

Table III. Residue Levels in Tissues of Rats after Oral Administration of [^{14}C]Sulfamidine Hydrochloride (130 mg/kg) or [^{14}C]Chlordimeform Hydrochloride (130 mg/kg)

	level, μg equiv/g wet tissues (ppm)									
	I·HCl					II·HCl				
tissue	30 min	1 h	6 h	1 day	10 days	30 min	1 h	6 h	1 day	10 days
blood	22.83	20.95	10.33	15.22	1.37	19.39	13.37	19.49	11.97	1.00
brain	47.41	35.20	14.78	9.75	0.03	73.13	67.71	72.23	13.18	0.04
heart	81.82	34.35	12.13	8.44	0.25	60.27	58.92	57.10	9.35	0.27
lung	43.85	46.20	32.25	9.33	0.67	122.79	180.97	131.55	12.69	0.69
liver	121.50	65.23	36.95	43.39	2.71	187.41	118.58	119.70	36.01	2.62
kidney	69.80	56.57	27.50	20.77	0.41	114.00	89.27	113.09	20.69	0.49
spleen	38.00	31.74	14.28	7.68	<0.03	82.64	66.93	68.93	7.81	0.43
adipose tissue	44.08	55.11	15.63	6.18	0.10	79.49	120.75	90.32	6.70	0.09
testicle	25.25	24.65	9.65	4.21	0.04	31.80	39.65	43.81	4.19	0.03
muscle	33.21	26.77	8.86	6.13	0.12	34.00	33.17	34.84	6.68	0.07

(V), *N*-formyl-4-chloro-*o*-toluidine (VII), sulfamidine sulfoxide (III), *N*,*N*-didesmethylchlordimeform (VI), 5-chloroanthranilic acid (X), and *N*-formyl-5-chloroanthranilic acid (IX). In addition, the metabolites were partially purified by preparative TLC in solvent systems A and B, and they were analyzed on GLC and MS. GLC retention times and mass spectra of M-3 coincided with those the authentic 4-chloro-*o*-toluidine. The EI-MS fragments of M-3 appeared at m/e 143 $(M + 2)^+$, 141 $(M)^+$, and 106 $(M - Cl)^+$. On the basis of these data, M-3 was identified as 4-chloro-*o*-toluidine (III). The GLC–EI-MS fragments of M-4 appeared at m/e 184 $(M + 2)^+$, 182 $(M)^+$, 167 $(M - CH_3)^+$, 152 $(M - NHCH_3)^+$, and 141 $(COT)^+$. M-4 was identified as *N*-desmethylchlordimeform (V). The GLC–EI-MS fragments of M-5 appeared at m/e 169 $(M)^+$, 141, 140, and 106. M-5 was identified as *N*-formyl-4-chloro-*o*-toluidine (VII). The identifications of sulfamidine sulfoxide (M-6) and *N*,*N*-didesmethylchlordimeform (M-7) were also established by TLC, GLC, and mass spectroscopic analysis. The metabolite M-8 was methylated by reaction with diazomethane overnight at room temperature to give one product. The EI-MS fragments of the methylated M-8 appeared at m/e 187 $(M + 2)^+$, 185 $(M)^+$, and 153 $(M - OCH_3)^+$; M-8 was identified as 5-chloroanthranilic acid (X). M-9 was also identified as *N*-formyl-5-chloroanthranilic acid (IX) through GLC and MS.

Distribution. The distribution of the radioactivity in selected tissues of rats, after single oral administration of the [^{14}C]sulfamidine hydrochloride or [^{14}C]chlorodimeform hydrochloride, is shown with its concentration (ppm) equivalent to sulfamidine hydrochloride or chlordimeform hydrochloride (Table III). Within 30 min after a single oral dose [^{14}C]sulfamidine hydrochloride the concentration of sulfamidine hydrochloride in the blood, the kidney, and the liver reached the maximum levels of 23, 70, and 122 ppm, respectively, and rapidly decreased thereafter. Relatively high levels of radioactivity were found in the liver, kidney, and blood at 1 day after administration, the actual values being 43, 21, and 15 ppm, respectively. The residue levels at 10 days were much lower than those obtained after 1 day. When [^{14}C]chlordimeform hydrochloride was given to rats, relatively high concentrations of the radioactivity were found in the liver (187 ppm), lung (123 ppm), and kidney (114 ppm) 30 min after administration. Its disappearance rates from other tissues were also rapid. The disappearance from the liver was slowest among them. About 2.6 ppm of [^{14}C]chlordimeform hydrochloride equivalent remained in the liver after 10 days. A difference was found between the tissue residue levels of radiocarbons derived from [^{14}C]sulfamidine hydrochloride treated and [^{14}C]chlordimeform hydrochloride treated rats. The levels of radioactivity remaining in tissues from [^{14}C]chlordimeform hydrochloride treated rats were generally higher than that from [^{14}C]sulfamidine hydrochloride treated ones at the 6-h time point.

Table IV. Effect of Various Cofactors and Inhibitors[a] upon the Production of Water-Soluble Metabolites in Vitro Using 20000*g* Crude Supernatant

	% water-sol metab from added sulfamidine and chlordimeform	
treatment	sulfamidine	chlordimeform
control	0.49 ± 0.06	0.42 ± 0.07
control + SKF 525-A	0.40 ± 0.03	0.35 ± 0.02
control + GSH	0.54 ± 0.02	0.36 ± 0.06
control + GSH + NEM	0.45 ± 0.03	0.34 ± 0.04
control + GSH + DTT	0.48 ± 0.04	0.35 ± 0.04
control + GSH + PCMB	0.34 ± 0.02	0.27 ± 0.01
control + NADPH	11.80 ± 1.01	6.89 ± 0.54
control + NADPH + SKF 525-A	5.17 ± 0.23	1.54 ± 0.06
control + UDPGA	0.61 ± 0.06	0.56 ± 0.06
control + UDPGA + NADPH	12.18 ± 1.31	8.19 ± 0.59
control + ASP,ATP,Mg^{2+}	0.52 ± 0.12	0.39 ± 0.11
control + ASP,ATP,Mg^{2+} + NADPH	15.34 ± 2.49	9.24 ± 1.06

[a] Concentrations: reduced glutathion (GSH), *N*-ethylmaleimide (NEM), dithiothreitol (DTT), and *p*-chloromercuribenzoate (PCMB) were 4 mM; reduced nicotinamide adenine dinucleotide phosphate (NADPH), uridine 5′-diphosphoglucuronic acid (UDPGA), adenosine 5′-phosphosulfate (APS), and adenosine 5′-triphosphate (ATP) were 2 mM; $MgCl_2$ was 10 mM; SKF 525-A was 0.125 mM.

In Vitro Studies. To understand the general mechanism of metabolism, [^{14}C]sulfamidine hydrochloride and [^{14}C]chlordimeform were incubated with the 20000*g* crude supernatant from the rat liver with various cofactors and inhibitors (Table IV). As judged by the levels of production of water-soluble metabolites, NADPH was the most important activator. Since its action is antagonized by SKF 525-A, it may be concluded that the main enzyme system responsible for their metabolism is mixed-function oxidase. It must be noted here that in all cases sulfamidine metabolism appears to be more extensive than that of chlordimeform.

When the content of the ether phase was examined (Table V), it became evident that in the NADPH-fortified preparations most of the radioactivities are due to metabolic products for both pesticides. The most conspicuous pattern was that as much as 43.8% of radioactivity was due to *N*-desmethylchlordimeform in the case of [^{14}C]-chlordimeform-derived metabolites, while the corresponding figure for sulfamidine metabolites was 19.7%. Also the level of *N*,*N*-didesmethylchlordimeform was much higher in the former preparation than the latter. In the latter cases both *N*-formyl-4-chloro-*o*-toluidine (25.0%) and sulfamidine sulfoxide (22.4%) were predominant. A

Table V. Analysis of Ether-Extractable Metabolites in Vitro Using 20000*g* Crude Supernatant

	% recovered radioactivity							
	I·HCl				II·HCl			
compound	std	control	control + NADPH	control + NADPH + SKF 525-A	std	control	control + NADPH	control + NADPH + SKF 525-A
sulfamidine	99.4	72.3	2.0	3.5				
chlordimeform					99.3	85.0	2.4	54.3
4-chloro-*o*-toluidine	0.3	1.2	4.5	4.6	0.3	1.6	2.8	2.7
N-desmethylchlordimeform	<0.1	1.3	19.7	13.9	<0.1	1.0	43.8	23.7
N-formyl-4-chloro-*o*-toluidine	<0.1	3.1	25.0	4.2	<0.1	5.0	12.1	6.8
sulfamidine sulfoxide	<0.1	11.3	22.4	54.4				
N,N-didesmethylchlordimeform	0.1	0.1	1.9	0.2	<0.1	0.2	10.8	0.2
others	0.2	0.5	4.8	1.8	0.4	2.0	8.9	1.5
ether soluble total		89.8	80.3	82.6		94.8	80.8	89.2
aqueous fraction		0.7	11.3	6.3		0.4	8.1	2.0
total	100.0	90.6	91.4	88.9	100.0	95.2	88.9	91.2

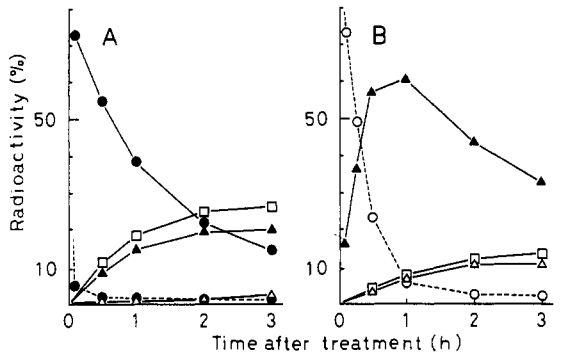

Figure 2. Relationship between the reaction time and the level of metabolite formed from [^{14}C]sulfamidine hydrochloride or [^{14}C]chlordimeform hydrochloride in vitro using 20000*g* crude supernatant with NADPH: (A) [^{14}C]sulfamidine; (B) [^{14}C]-chlordimeform. Key: (●—●) sulfamidine hydrochloride; (○) chlordimeform hydrochloride; (●—●) sulfamidine sulfoxide (III); (▲) *N*-desmethylchlordimeform (V); (△) *N,N*-didesmethyl-chlordimeform (VI); (□) *N*-formyl-4-chloro-*o*-toluidine (VII).

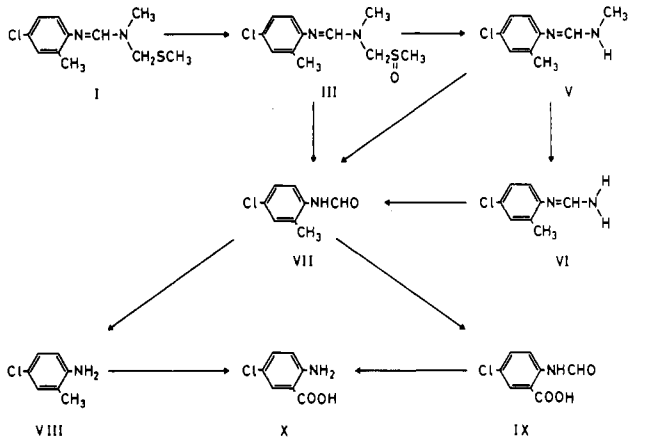

Figure 3. Proposed metabolic pathway for sulfamidine.

treatment with SKF 525-A greatly increased the level of unmetabolized chlordimeform (2.4–54.3%), but the same did not occur for sulfamidine. Instead the level of sulfamidine sulfoxide increased. Thus, the oxidation step involved in the formation of sulfoxide may be less dependent on mixed-function oxidase.

The time course of formation of these metabolites was then studied. In the presence of NADPH the formation of sulfoxide from sulfamidine is extremly rapid (Figure 2). Formation of *N*-desmethylchlordimeform from sulfamidine is gradual, but in the case of chlordimeform metabolism the level of this toxic metabolite reached a maximum at ca. 60% to 1 h (Figure 2). The level of *N,N*-didesmethylchlordimeform derived from chlordimeform is also higher than that derived from sulfamidine.

DISCUSSION

When administered orally to rats, sulfamidine was extensively degraded and rapidly eliminated within the first 48 h. The primary mode of excretion was via the urinary route. The proposed metabolic pathway for sulfamidine in male rats is given in Figure 3. It appears that the initial step of oxidation of the thionyl moiety of sulfamidine gives rise to sulfamidine sulfoxide (III). On the other hand, the corresponding sulfone was not detected as a metabolite. It is clear from the current study results that the next step of reaction is conversion of sulfamidine sulfoxide (III) to *N*-desmethylchlordimeform (V). Once *N*-desmethylchlordimeform is formed, the rest of the metabolic process may be postulated from the studies by Ahmad and Knowles (1971), Morikawa et al. (1975), and Knowles and Benezet (1977). Deformylation of *N*-formyl-4-chloro-*o*-toluidine (VII) should yield 4-chloro-*o*-toluiine (VIII). The 4-chloro-*o*-toluidine is subsequently metabolized to 5-chloroanthranilic acid (X) and 4-chloro-2-methyl-acetanilide (Figure 3). Acetylation of the free group of 4-chloro-*o*-toluidine appears to be a minor pathway for sulfamidine as we have detected a small amounts of this metabolite only in the case of large dosing.

As for the toxicities of sulfamidine and sulfamidine sulfoxide (the data by Dr. S. Kobayashi of Hokko Chemical Industry) and chlordimeform and its metabolites (Hollingworth and Yim, 1980), the LD_{50} dosed in oral administration to mice (mortality expressed in mg/kg) are as follows: sulfamidine hydrochloride (I·HCl), 1410; sulfamidine sulfoxide (III), 933; chlordimeform hydrochloride (II·HCl), 267; *N*-desmethylchlordimeform (V), 163; *N,N*-didesmethylchlordimeform (VI), 78; *N*-formyl-4-chloro-*o*-toluidine (VII), 750; 4-chloro-*o*-toluidine (VIII), >1000.

It is generally considered that the successive N-desmethylation steps yield increasingly more toxic metabolites, the LD_{50} for *N,N*-didesmethylchlordimeform being 78 mg/kg (i.e. it is 18× as toxic as sulfamidine hydrochloride). The order of toxicity (i.e. sulfamidine hydrochloride < sulfamidine sulfoxide < chlordimeform hydrochloride < *N*-desmethylchlordimeform < *N,N*-didesmethylchlordimeform) suggests that much of the toxicity of sulfamidine is due to its desmethylation products. Yet, the level of *N*-desmethylchlordimeform found in the urine from the [^{14}C]sulfamidine hydrochloride administered rats was similar to that from the [^{14}C]chlordimeform hydrochloride treated ones (see Table II) on day 3 of treatment. However, the levels of radioactivity found in tissues of rats administered [^{14}C]chlordimeform hydrochloride were generally higher than that of [^{14}C]sulf-

amidine hydrochloride treated ones at the 6-h time point (Table III). Furthermore, the results of in vitro study indicate that the formation of *N*-desmethylchlordimeform and *N,N*-didesmethylchlordimeform is much more pronounced in the preparation incubated with chlordimeform than that incubated with sulfamidine (Figure 2).

Throughout the study it has been consistently observed that metabolism of sulfamidine is more rapid than that of chlordimeform. A logical explanation for this phenomenon would be that the rate of enzymatic hydrolytic degradation of chlordimeform is less than that for sulfamidine or sulfamidine sulfoxide. It must be noted that the rate of nonenzymatic hydrolysis for chlordimeform itself was found to be identical with that for sulfamidine and its sulfoxide (data not shown). Also, once *N*-desmethylchlordimeform (V) or *N*-formyl-4-chloro-*o*-toluidine (VII) is formed as a result of oxidative reactions, the subsequent metabolic fate should be identical for both compounds. Since the key difference in metabolism between these two compounds appears to be in the levels of *N*-desmethylchlordimeform (V) formed as judged by the distribution and the in vitro study, it appears to be reasonable to ascribe the overall metabolic difference to the differential degradation of sulfamidine sulfoxide and chlordimeform.

In the data shown in Table V, we can observe that in the presence of NADPH and SKF 525-A the levels of these two precursors for *N*-desmethylchlordimeform (V) are almost identical (54%). Yet, the level of *N*-desmethylchlordimeform (V) was higher in the test with chlordimeform (II) than that with sulfamidine sulfoxide (III). At the same time the level of very polar metabolites (aqueous) in the former test was only one-third as much.

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Comparative Aerobic Soil Metabolism of Fenvalerate Isomers

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An aerobic soil metabolism study was conducted to determine the degradation rate of individual isomers of fenvalerate and to assess the potential influence of the *RS*, *SR*, and *RR* isomers to the metabolism of the most insecticidally active *SS* isomer. Individual [*phenoxyphenyl*-^{14}C]fenvalerate isomers degraded at different rates. The calculated half-lives for the *SR*, *RS*, *SS*, and *RR* isomers in fenvalerate (racemic mixture) were 155, 89, 108, and 178 days, respectively. The resolved *SS* isomer degraded at a faster rate with a calculated half-life of 74 days. Racemization of the resolved *SS* isomer did not occur. A qualitative difference in the chemical nature of soil metabolites between fenvalerate and the resolved *SS* isomer was not observed. Soil degradation products, phenoxybenzoic acid, 3-(4-hydroxyphenoxy)benzoic acid, and 4′-OH- and $CONH_2$-fenvalerate, each accounted for less than 2% of the applied radioactivity. Extensive degradation of these soil metabolites was evident since approximately 50% of the applied radioactivity was recovered as $^{14}CO_2$ and as unextractable bound residues.

Fenvalerate [cyano(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate, PYDRIN insecticide] is an effective broad-spectrum pyrethroid insecticide. In addition to its highly selective insecticidal activities, fenvalerate exhibits photolytic stability and an extended field residual activity (Miyamoto and Mikami, 1983; Reed et al., 1983).

Fenvalerate contains two asymmetric carbon atoms and the most insecticidally active stereoisomer has the 2*S*,α*S* (*SS*) configuration (Yoshioka, 1978). Significant qualitative or quantitative difference in soil metabolism between the racemic mixture and the resolved *S*-acid (*SR*, *SS*) diastereomer was not observed (Ohkawa et al., 1978). The primary objective of this investigation was to determine the soil degradation rate of individual fenvalerate isomers

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