Metabolism of [^{14}C]Prometryn in Rats

Michael S. Maynard,*,† Diane Brumback,† William Itterly,† Thomas Capps,† and Randy Rose‡

Human Safety Department, Novartis Crop Protection, P.O. Box 18300, Greensboro, North Carolina 27419, and Department of Toxicology, North Carolina State University, Raleigh, North Carolina 27695

[^{14}C]Prometryn, 2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine, was orally administered to male and female rats at approximately 0.5 and 500 mg/kg; daily urine and feces were collected. After 3 or 7 days rats were sacrificed, and blood and selected tissues were isolated. The urine and feces extracts were characterized for metabolite similarity as well as for metabolite identification. Over 30 metabolites were observed, and of these, 28 were identified mostly by mass spectrometry and/or cochromatography with available reference standards. The metabolism of prometryn was shown to occur by N-demethylation, S-oxidation, S−S dimerization, OH substitution for NH_2 and SCH_3, and conjugation with glutathione or glucuronic acid. Rat liver microsomal incubations of prometryn were conducted and compared to the in vivo metabolism. Both in vivo and in vitro phase I metabolisms of prometryn were similar, with S-oxidation and N-dealkylation predominating. The involvement of cytochrome P-450 and flavin-containing monooxidase in the in vitro metabolism of prometryn was investigated.

Keywords: *Prometryn; metabolites; rats; in vivo; in vitro; cytochrome P-450; FMO*

INTRODUCTION

The metabolism of chloro-*s*-triazines by rats is known and has been previously described (Adams et al., 1990; Bakke et al., 1972; Boehme and Bar, 1967; Crayford and Hutson, 1972; Dauterman and Muecke, 1974; Wu et al., 1998). Only minimal information on the metabolism of methylthio-*s*-triazines by animals has previously been published (Boehme and Bar, 1967; Larsen and Bakke, 1978; Larsen et al., 1978; Wu et al., 1992). Prometryn is a methylthio-*s*-triazine herbicide registered as Caparol by Novartis Crop Protection for control of annual broadleaf and grass weeds in cotton, celery, pigeon peas, and corn crops. The major objective of this study was to describe the fate of ^{14}C-labeled prometryn in rats. The total ^{14}C residue levels in tissues and the amount of dose excreted were determined to evaluate the distribution of prometryn residues. The amount of prometryn and the amount and identity of the metabolites found in the urine and feces were determined. The effect of dose level, pretreatment with unlabeled prometryn, and gender of rats on the fate of prometryn was investigated. In addition, rat liver microsomes were prepared and incubated with [^{14}C]prometryn and an NADPH generating system at 37 °C for 20 min. An incubation of [^{14}C]prometryn with purified mouse liver flavin-containing monooxidase (FMO) was also conducted. A comparison of in vivo and in vitro metabolisms of prometryn by rats will be discussed as well as the involvement of cytochrome P-450 and FMO in the sulfur oxidation. From these results, the pathway of the metabolism of prometryn by rats is proposed.

* Author to whom correspondence should be addressed [fax (336) 632-6083; e-mail michael.maynard@cp.novartis.com].

† Novartis Crop Protection.

‡ North Carolina State University.

MATERIALS AND METHODS

Animal Treatment. Male (∼223 g) and female (∼202 g; nulliparous and nonpregnant) Crl;CD BR rats were obtained from Charles River Breeding Laboratories, Portage, MI. Each treatment group consisted of five male and five female rats. Animals were randomly selected for grouping, acclimated for 10 days, and administered a single oral (gavage) dose of uniformly ring labeled [^{14}C]prometryn. Two dosage levels, 0.5 and 500 mg/kg, were employed. At the 0.5 mg/kg dosage level, animals received [^{14}C]prometryn either alone (group 1) or preceded by 14 single daily oral doses of unlabeled prometryn at 0.5 mg/kg (group 3). One group (group 2) received a single oral dose of [^{14}C]prometryn at 470 mg/kg. Groups 1−3 were sacrificed 7 days post dose. Another group (group 4) received a single oral dose of [^{14}C]prometryn at 540 mg/kg and was sacrificed at 3 days post dose. Group 4 animals were used primarily for metabolite isolation and identification. The final specific activities of the [^{14}C]prometryn were 20.1 and 1.0 μCi/mg for the 0.5 and 500 mg/kg doses, respectively. Appropriate amounts of labeled and unlabeled prometryn were mixed with HiSil 233 silica gel at a 1.5:1 ratio (w/w) and diluted with aqueous 0.5% carboxymethyl cellulose solution. The concentrations of the dosing solutions were 0.1 and 110 mg/g for the 0.5 and 500 mg/kg dosages, respectively, and each rat received ∼1 g. Control animals received formulated solutions without prometryn. Rats were individually housed in cages that provided separate collection of urine and feces. Respired air was not collected for this study. Animals were given free access to food (Purina rat chow) and water. Under the experimental conditions of this study, there were no observable toxicological effects in the control or prometryn-treated animals.

Seven days after dosing (except 3 days for group 4), animals were anesthetized with ether and blood samples collected by cardiac puncture. The following samples were taken and individually analyzed: brain, bone (hind leg), fat (gonadal), ovaries/testes, heart, kidney, liver, lung, muscle (hind leg), and spleen. Urine and feces were individually collected at 4, 8, 12, and 24 h and daily thereafter.

The [^{14}C]prometryn standards were ring labeled and supplied by the Chemical Support Group, Development Department, Novartis Crop Protection, Greensboro, NC. Radiochemical purity was 98.6%. Labeled and unlabeled metabolite

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standards were supplied by Chemical Support Group or PTAS Group, Novartis Crop Protection, Greensboro, NC. All other chemicals and solvents were of reagent grade, HPLC grade, or better and purchased from commercial sources.

The animal care, dosing, dissection, and analysis of ^{14}C residues were performed by WIL Research Laboratories, Ashland, OH. The samples were shipped frozen to Novartis Crop Protection, Greensboro, NC, where the metabolite isolation and identification were conducted.

The microsomal and purified FMO incubations were performed by R. Rose at North Carolina State University, Department of Toxicology, Raleigh, NC.

Instrumentation. *HPLC Systems.* The reversed phase high-performance liquid chromatography (RP-HPLC) systems employed included a DuPont Zorbax RX analytical (4.6 × 250 mm) or semipreparative (9.6 × 250 mm) column and a corresponding ODS precolumn with a mobile phase of ACN/H_2O (pH7) as follows: 0−5 min, 1:99; 5−60 min, linear to 100:0; 60−65 min, 100:0; and 67−72 min, 100% MeOH; all at 0.75 mL/min for the analytical column (S1) and 2.0 mL/min for the semipreparative column (S2). A Perkin-Elmer 4270 pump, a Rheodyne 7125 injector, an IN/US Radiomonitor, and an Isco Foxy fraction collector were used. An LKB 2140 photodiode array UV detector was utilized for UV absorbance spectra analysis of the metabolites during purification.

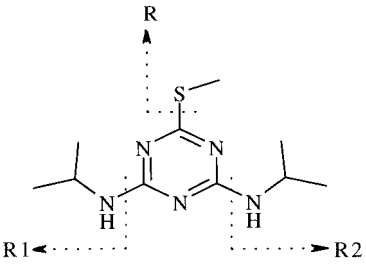
TLC Systems. All TLC analyses were conducted on silica gel 60 F-254 plates (E. Merck) with the following solvent systems: (SS1) MEK/ACN/HOAc/H_2O, 40:40:4:2; (SS2) MEK/ACN/HOAc/H_2O, 80:20:4:2; (SS3) CH_2Cl_2/MeOH/HOAc, 95:4:1; (SS4) 1-BuOH/HOAc/H_2O, 133:33:33; (SS5) CH_2Cl_2/MeOH/HOAc, 93:5:2; and (SS6) $CHCl_3$/MeOH/formic acid/H_2O, 70:25:2:4.

Mass Spectrometry Systems. The GC/MS data were obtained using a Hewlett-Packard 5890 GC with a 5970 MSD (70 eV and 200 °C source temperature). The column was a DB-5 (15 m × 0.23 mm i.d.), the injection temperature was 200 °C, with an oven temperature of 50 °C increased to 250 °C in 20 min. The solid probe and +/−FAB data were obtained using a VG70-SQ system equipped with the appropriate source. The VG70-SQ system was also used for EI+ and HREI+ acquisition at 70 eV and 200 °C source temperature, whereas the CI+ was acquired at 200 eV and 150 °C source temperature. The +/− FAB data were obtained using glycerol as matrix and 8 kV atom beam with ambient source temperature. The +thermospray data were obtained using a Finnigan TSQ-700 equipped with a thermospray II source with vaporizer set at 200 °C. If available, standards were analyzed for t_R, R_f, UV absorbance spectra, GC/MS, and mass spectrometry. The following unlabeled standards were available for these studies: prometryn, GS-11354, GS-26831, GS-16158, GS-16141, GS-14129, G-31435, GS-14626, GS-11526, GS-17794, GS-17791, GS-11957, GS-35713, GS-11955, NAC conjugates of prometryn, GS-11354, and GS-26831 (CGA-10582), cysteine conjugate of GS-11354, glutathionine conjugates of prometryn and GS-26831, S−S dimer of atrazine, and *S*-glucuronic acid conjugate of atrazine (Table 1).

Radioactivity Analysis. The radioactivity in the samples for a given treatment, time post dose, and gender was determined by combustion (Harvey oxidizer Model OX400) followed by liquid scintillation counting (LSC) using LKB Model 1217 or Beckman LS 5000TD. Urine and blood were counted directly. All samples were analyzed at least in duplicate and were ±10% of the mean value. For HPLC radioactivity analysis, 1 min fractions were collected and counted, or the column effluent was analyzed by the IN/US radioactivity monitor. For TLC analysis, the areas were scraped, eluted with methanol, and counted or analyzed by a linear analyzer (Raytest Rita TLC analyzer).

Urine Metabolite Characterization. For characterization of the ^{14}C residue in the urine, samples were applied to an XAD-4 column (2.5 × 15.5 cm) and eluted with H_2O (XAD H_2O fraction) and then methanol. The methanol eluate was concentrated to near dryness, brought up to volume with H_2O, and then partitioned with CH_2Cl_2 (XAD MeOH/H_2O and XAD MeOH/CH_2Cl_2 fractions). Each of the three subfractions was spotted on silica gel TLC plates, developed in two directions (2D-TLC), and injected on a reversed phase high-pressure liquid chromatograph (RP-HPLC). The metabolites were characterized on the basis of their t_R or R_f as compared to reference standards and quantitated as a percent of sample. Both systems produced similar results and recoveries were 90−110%. These analyses were used to compare the urine residue between high and low dose, male and female rats, and with and without preconditioning with unlabeled prometryn. These procedures were subsequently used for isolation of metabolites from the high-dose groups.

Table 1. Chemical Structures of Prometryn Standards and Metabolites



standard/metabolite[a]	R	R1	R2
prometryn	SCH_3	Nhipr	NHipr
GS-11354	SCH_3	NH_2	NHipr
GS-26831	SCH_3	NH_2	NH_2
GS-16141 (sulfoxide)	$SOCH_3$	NHipr	NHipr
GS-16158 (sulfone)	SO_2CH_3	NHipr	NHipr
GS-14129	$SOCH_3$	NH_2	NHipr
sulfoxide GS-26831	$SOCH_3$	NH_2	NH_2
GS-11955	SH	Nhipr	NHipr
disulfide prometryn	GS-11955	NHipr	NHipr
disulfide GS-11354	SH-GS-11354	NH_2	NHipr
disulfide prometryn/GS-11354	GS-11955	NH_2	NHipr
GS-11526	OH	Nhipr	NHipr
GS-17794	OH	NH_2	NHipr
GS-11791	OH	NH_2	NH_2
GS-11957	OH	OH	NHipr
GS-35713	OH	OH	NH_2
G-31435	OCH_3	NHipr	NHipr
GS-14626	OCH_3	NH_2	NHipr
6-NH_2 prometryn	NH_2	NHipr	NHipr
6-NH_2 GS-11354	NH_2	NH_2	NHipr
S-GLUT prometryn	S-GLUT	NHipr	NHipr
S-GLUT GS-11354	S-GLUT	NH_2	NHipr
S-NAC prometryn	S-NAC	NHipr	NHipr
S-NAC GS-11354	S-NAC	NH_2	NHipr
S-NAC GS-26831 (CGA-10582)	S-NAC	NH_2	NH_2
S-CYST prometryn	S-CYST	Nhipr	NHipr
S-CYST GS- 11354	S-CYST	NH_2	NHipr
S-CYST GS-26831	S-CYST	NH_2	NH_2
S-GLUC prometryn	S-GLUC	NHipr	NHipr
S-GLUC GS-11354	S-GLUC	NH_2	NHipr

[a] ipr = isopropyl; GLUT = glutathione; NAC = *N*-acetylcysteine; CYST = cysteine; GLUC = glucuronide.

Urine Metabolite Isolation. Metabolite isolation and purification were conducted with the high-dose (groups 2 and 4) 24 and 48 h composite male and female urine samples. The XAD H_2O fractions were applied to a Sephadex G-10 column (2.5 × 54 cm) and eluted with H_2O. All of the ^{14}C eluted as one major and three minor peaks. The three minor peaks were purified by TLC (SS1). The ^{14}C metabolites were eluted off the TLC plates with methanol, mixed with a corresponding selected unlabeled standard, and applied to another TLC system (SS1). The ^{14}C from the metabolite and the UV from the standard were compared for cochromatography.

The major peak from the G-10 column was purified by RP-HPLC (S2). The subsequent HPLC peaks were collected and analyzed by TLC (SS1). The ^{14}C metabolites were eluted off the TLC plate with methanol, mixed with a selected unlabeled standard, and applied to a second TLC system (SS1). The ^{14}C

from the metabolite and the UV from the standard were compared for cochromatography.

The XAD MeOH/H_2O fractions were purified by TLC (SS2). The two major bands (>80% of ^{14}C) were eluted off the TLC plate and purified by 2D-TLC (SS2, SS4). The metabolites were eluted from the TLC plate and further purified by TLC (SS2) or RP-HPLC (S1). The resulting major metabolites were analyzed by MS and/or cochromatography with standards by RP-HPLC. The minor metabolites were tentatively identified by t_R and/or R_f comparison to standards on several systems.

The XAD MeOH/CH_2Cl_2 fractions were purified by TLC (SS3). Five metabolite bands were eluted and each further purified by another TLC (SS1 or SS2). The two least polar bands were eluted off the second TLC and injected on a GC/MS system. The t_R and MS analyses of the metabolites (and prometryn) were compared to standards on the GC/MS system. The metabolites from the other three bands were eluted off the second TLC and purified by RP-HPLC (S1). Because an LKB 2140 UV photodiode array detector was used throughout all HPLC purifications, the UV absorbance spectra of the metabolites were obtained and compared to standards on the same system, as well as their t_R. These metabolites were further purified by successive RP-HPLC, and the major metabolites were isolated and analyzed by MS. When available, MS analysis of standards was conducted.

Feces Analysis. The feces samples for all groups were sequentially extracted with ethanol, ACN/H_2O (90:10 v/v), and H_2O. The extracts were purified and characterized by two TLC systems (SS5 and SS6) and the metabolites characterized by R_f comparison to standards.

Microsomal and FMO Incubations. Liver microsomes were prepared from three Fisher male rats (6 weeks old). Livers were excised and minced in 50 mM potassium phosphate buffer (pH 7.5) containing 0.1 mM EDTA and 1.5% KCl and then homogenized with a Polytron homogenizer. The homogenate was centrifuged at 10000*g* for 10 min; the resulting supernate was passed through glass wool and then centrifuged at 100000*g* for 60 min. The microsomal pellet was resuspended in buffer and centrifuged again at 100000*g* for 60 min. The microsomal pellet was suspended in 50 mM phosphate buffer containing 0.1 mM EDTA and 0.25 M sucrose. Cytochrome P-450 content was determined by measuring the dithionite reduced CO difference spectrum using an SLM-Amino DW-2C spectrophotometer (Omura and Sato, 1964). Protein determination was accomplished using the Coomassie blue dye as prepared by Bio-Rad.

Microsomal incubations consisted of 100 mM sodium phosphate buffer (pH 7.5, the determined optimal pH), an NADPH generating system (0.25 mM NADP, 2.5 mM glucose-6-phosphate, 1.0 unit of glucose-6-phosphate dehydrogenase), 50 μM prometryn (1.1 μCi/μmol), and 0.1−1 mg of microsomal protein. The reaction was started by the addition of prometryn in 10 μL of ethanol. Incubations were carried out at 37 °C for 20 min. Control incubations consisted of either boiled microsomes (2 min) or 0 min controls. Termination of the incubations was conducted by the addition of 2 mL of acetone with vortexing. Incubations were extracted twice with 2 volumes of CH_2Cl_2, and the solvent was removed with N_2. Recovery of ^{14}C from these incubations was >90%. The dried residue was dissolved in 150 μL of CH_2Cl_2, applied to silica gel TLC plates, and developed with either 9:1 benzene/ethanol or 9:1:0.1 benzene/ethanol/HOAc. Quantitation was with a Berthold linear analyzer, and identification was by comparison of TLC R_f to reference standards.

FMO incubations were conducted using purified mouse liver FMO (courtesy of Dr. K. Venkatesh, NCSU) in a manner previously reported (Smyser et al., 1985; Sabourin and Hodgson, 1984). Incubations of 1 mM prometryn with mouse liver FMO and NADPH at 37 °C for 20 min were conducted. The oxidation of NADPH (Smyser et al., 1985; Sabourin and Hodgson, 1984) and the formation of prometryn metabolites (described above for microsomes) were investigated.

Table 2. Distribution of [^{14}C]Prometryn Residues Expressed as a Percentage of Dose[a]

dosage group[b]	sex	urine	feces	tissues	blood	cage wash	total
1	M	47.1	43.6	0.4	1.2	1.0	93.2
1	F	49.6	39.7	0.4	1.3	0.5	91.5
2	M	46.5	44.6	0.6	1.5	0.8	93.9
2	F	52.7	33.1	0.6	1.9	1.1	89.4
3	M	53.3	44.2	0.5	1.5	0.4	99.9
3	F	51.8	45.5	0.4	1.5	1.0	100.1

[a] Values reported are the means of five rats per group at 7 days post dose. [b] Dosage groups are described under Materials and Methods.

RESULTS AND DISCUSSION

The chemical structures of prometryn and standards and/or metabolites are shown in Table 1. The distribution of the [^{14}C]prometryn residues for the various dose groups is shown in Table 2. Most of the dose was eliminated within 7 days with the 90−98% of the recovered dose found in the urine and feces. Low amounts of residue were recovered in the tissues and accounted for up to 0.6% of the dose. A significant amount (1.2−1.9%) was recovered in the blood fraction that was previously observed for methylthio-*s*-triazine compounds (Hamboeck et al., 1981).

Selected tissue residue levels at 7 days post dose are shown in Table 3. The residue levels in these tissues were observed in the following order: fat < muscle < kidney < liver. The residue levels in tissue were dose dependent, and the levels in female tissue appeared to be higher than in male tissue (Table 3). The rats pretreated with unlabeled prometryn for 14 days (group 3) appeared to have slightly higher tissue residue levels than non-pretreated rats (group 1). In general, only minor, if any, differences were observed in the distribution and elimination of [^{14}C]prometryn between doses, with or without pretreatment, and gender.

Purification and Identification of Metabolites in Urine. Purification of the urine samples by an XAD-4 column and the resulting distribution of ^{14}C residue are shown in Table 4. The residue recovered in these fractions expressed as a range and (mean) are XAD H_2O, 17−44% (29%); XAD MeOH/H_2O, 34−65% (51%); and XAD MeOH/CH_2Cl_2, 9−44% (23%). The metabolites identified in these fractions will be presented below and account for the polarity differences observed here.

Because the XAD MeOH/H_2O fraction contained the greatest amount of ^{14}C residue, the metabolites were identified and compared for groups 1, 3, and 4, male and female rats. The results are shown in Table 5. Comparison of this fraction with the greatest amount of residues provides metabolite qualification and quantitation among dosage groups and gender. The major metabolite observed in all samples (13−26% of urine) was the *N*-acetylcysteine conjugate of GS-11354. The majority of the metabolites observed were conjugates: cysteine, *N*-acetylcysteine, or *S*-glucuronic acid conjugates of GS-11354 and prometryn (24−45% of urine). These results are consistent with the XAD column properties whereby the polar conjugates elute in the water fraction (Table 4). The remaining metabolites are free polar metabolites resulting from S-oxidation, N-dealkylation, and OH substitution of the NH_2 group. In general, the metabolites observed in this XAD fraction were qualitatively and quantitatively similar between male and female rats and among the dosage groups. Furthermore, between 92 and 100% of the ^{14}C residue

Table 3. [^{14}C]Prometryn Residue Levels in Selected Tissues

	dosage group[a]							
tissue	1M	1F	2M	2F	3M	3F	4M[b]	4F[b]
liver	0.021	0.025	21.045	33.752	0.028	0.037	54.841	68.072
kidney	0.011	0.015	10.925	20.285	0.014	0.016	34.909	41.451
fat	<0.024	<0.026	3.506	3.820	<0.017	<0.017	6.743	5.778
muscle	0.003	0.004	4.365	7.053	0.004	0.005	8.021	8.144

[a] Dosage groups are described under Materials and Methods; number refers to group (groups 1−4) and letter to sex (M, male; F, female). Values are expressed as ppm (μg/g) and are the mean of five rats with SD <10%. (<) values represent the level of detection for a sample series for the day of quantitation. [b] Animals sacrificed 3 days post dose.

Table 4. Distribution of [^{14}C]Prometryn Residues from Urine Processed by XAD-4 Chromatography[a]

		% of urine		
dosage group	sex	XAD H_2O	XAD MeOH/H_2O	XAD MeOH/CH_2Cl_2
1	M	23	34	44
1	F	26	53	20
2	M	26	65	9
2	F	17	62	21
3	M	44	46	10
3	F	29	56	15
4	M	36	48	27
4	F	32	41	36

[a] Dosage groups are described under Materials and Methods. Values are expressed as a percentage of urine from large preparations of 24 and 48 h composite samples.

Table 5. Metabolite Identification in XAD MeOH/H_2O Fraction for Male and Female Rat Urine from Groups 1, 3, and 4[a]

	dosage group (% of urine)					
metabolite	4M	4F	1M	1F	3M	3F
6-NH_2 prometryn	2.3	3.4	−	−	−	−
S-NAC prometryn	3.2	4.3	2.8	5.6	6.5	4.6
S-NAC GS-11354	12.6	15.6	12.9	17.8	14.1	26.3
S-CYST prometryn	3.6	1.5	1.0	2.0	−	−
S-CYST GS-11354	1.2	1.0	1.8	2.9	3.7	1.6
unknown 1	4.1	3.2	2.3	2.9	1.2	3.4
S-GLUC prometryn	1.5	0.9	−	1.9	−	−
S-GLUC GS-11354	9.3	5.2	5.3	5.6	7.8	12.0
GS-17794	−	−	0.7	2.4	−	−
GS-26831	0.9	1.0	1.2	2.4	3.8	1.2
GS-14129	−	−	−	−	1.6	1.8
GS-14626	2.0	1.8	−	−	−	1.5
GS-16158	0.5	0.5	1.1	2.7	2.1	1.1
GS-11354	0.6	0.5	1.0	2.0	1.7	1.0
GS-16141	1.3	0.7	−	1.4	−	−
prometon	0.8	0.8	1.2	2.2	−	−
prometryn	0.3	0.6	−	−	−	−
disulfide prometrvyn	0.1	0.1	−	−	−	−
total	44.3	41.1	31.3	51.8	42.5	54.5

[a] Dosage groups are described under Materials and Methods. Dashes (−) indicate the presence of a metabolite, but its levels were too low to quantitate. See legend of Table 3. Results are presented here to demonstrate comparison of metabolites among dosage groups and gender from the greatest residue fraction. Metabolites are presented in order of elution.

in these XAD fractions (Tables 4 and 5) was identified and represents 17 metabolites plus prometryn.

Because no significant qualitative differences were observed in the metabolite patterns between male and female rats and high and low dosage (data not shown), the XAD H_2O fraction from the high-dose male rats (group 2; 48 h sample) was purified for metabolite identification and is shown in Table 6. For the XAD H_2O fraction the major metabolites are the glutathione conjugates of prometryn, GS-11354, and GS-26831 (CGA-10582); GS-17791; and GS-17794. These five

Table 6. Metabolite Identification in XAD H_2O and XAD MeOH/CH_2Cl_2 Fraction for High-Dose Rats[a]

	% of urine	
metabolite	XAD H_2O	XAD MeOH/CH_2Cl_2
GS-17794	3.7	0.0
GS-17791	2.0	0.5
GS-11526	0.0	1.0
S-GLUT GS-11354	0.5	0.0
S-GLUT prometryn	2.7	0.0
S-NAC GS-26831 (CGA-10582)	6.0	0.0
S-NAC GS-11354	0.8	0.0
S-NAC prometryn	0.4	3.5
S-CYST GS-11354	0.1	0.0
S-CYST prometryn	0.4	0.0
GS-11354	0.1	2.4
GS-26831	0.5	2.0
GS-14129	0.2	1.0
GS-16141	0.2	0.5
GS-16158	0.0	1.0-
GS-11955	0.2	0.0
GS-11957	0.1	0.5
GS-35713	0.8	0.0
disulfide prometryn	0.0	6.0
disulfide GS-11354	0.0	9.0
disulfide prometryn/GS-11354	0.0	3.5
prometryn	0.6	3.5
total	22.6	31.0

[a] Values represent the fraction of a specific compound as a percent of the total HPLC analysis times of the fraction of the subsample of the total urine residue. Dosage groups and procedures are described under Materials and Methods. The XAD H_2O fraction is from group 2 male, whereas the XAD MeOH/CH_2Cl_2 fraction is from group 4 female. Due to established similarity between male and female, the fractions with the greatest residues and least amount of natural products were selected for metabolite isolation. Metabolites are presented in order of elution.

metabolites represent 14.9% of the urine or 66% of the XAD H_2O fraction. The remaining metabolites are free polar metabolites involving S-oxidation, N-dealkylation, and conjugates of cysteine and *N*-acetylcysteine. As for the XAD MeOH/H_2O fraction (Table 5), the XAD H_2O fraction provided similar metabolites independent of dosage and gender. Furthermore, ~87% of the ^{14}C residue in the XAD H_2O fraction (Tables 4 and 6) was identified as 16 metabolites plus prometryn.

Because no significant qualitative differences were observed in the metabolite patterns between male and female rats and high and low dosage (data not shown), the XAD MeOH/CH_2Cl_2 fraction from the high-dose female rats (group 4; 48 h samples) was purified for metabolite identification and is shown in Table 6. For this fraction, the major metabolites were the disulfides of prometryn and GS-11354, representing 18.5% of the urine. Two homo disulfides consisting of either prometryn or GS-11354 were identified as the major metabolites. A hetero disulfide containing one prometryn and one GS-11354 molecule was also identified. Prometryn and the mono- and di-dealkylated products

Table 7. Prometryn Metabolites Identified from Urine of High-Dose Rats[a]

metabolite	% of urine
prometryn	5
GS-11354	4
GS-26831	3
S-NAC prometryn	8
S-NAC GS-11354	16
S-NAC GS-26831	6
S-CYST prometryn	2
S-CYST GS-11354	1
S-GLUT prometryn	3
S-GLUT GS-26831	1
S-GLUC prometryn	1
S-GLUC GS-11354	4
6-NH_2 prometryn	3
6-NH_2 GS-11354	<1
disulfide prometryn	6
disulfide GS-11354	9
disulfide prometryn/GS-11354	4
sulfoxide prometryn (GS-16141)	1
sulfoxide GS-11354 (GS-14129)	1
sulfoxide GS-26831	<1
sulfone prometryn (GS-16158)	2
GS-11955	<1
6-OH prometryn (GS-11526)	<1
6-OH GS-11354 (GS-17794)	3
6-OH GS-26831 (GS-17791)	3
2,6-di-OH prometryn (GS-11957)	1
2,6-di-OH GS-26831 (GS-35713)	1
6-OCH_3 prometryn	1
6-OCH_3 GS-11354 (GS-14626)	2
polar unknowns (2)	5
total	96%

[a] Results are composite summary for all fractions (Tables 5 and 6) and represent the metabolite profile from high-dose rat urine. Due to previous determined similarities between dosage and gender (see Results), profile is representative.

(GS-11354 and GS-26831) were identified as 8% of this fraction (2.5% of urine). The remaining metabolites included the *N*-acetylcysteine conjugate of prometryn, S-oxidation metabolites, and OH substitution of the NH_2 group products. Furthermore, ~97% of the ^{14}C residue in the XAD MeOH/CH_2Cl_2 fraction (Tables 4 and 6) was identified as 12 metabolites plus prometryn.

The identification of the ^{14}C residue in total urine is shown in Table 7. The metabolites identified from the three XAD fractions were used (Tables 5 and 6) to produce the composite Table 7. Because no significant qualitative difference was observed among the dosage groups, this metabolite profile for urine is representative for rats administered prometryn. From these results, the metabolism of prometryn by rats primarily occurs by N-dealkylation, conjugation through the sulfur group, sulfur oxidation; and disulfide formation. It was observed that lower percentages of disulfides were found in the urine from the low-dose compared to the high-dose rats. These lower levels of disulfides are presumed to occur from less formation of SH metabolites. From these results, 96% of the total ^{14}C residue in urine was identified as prometryn and 28 metabolites.

The analyses by mass spectrometry of the isolated metabolites and standards are shown in Table 8. The order of ion abundance is presented, as well as the molecular ion. Isolated metabolites were compared to reference standards when available.

The UV absorbance spectra, recorded from the HPLC photodiode array detector, of prometryn and GS-11354 are shown in Figure 1. The absorbance maximum for prometryn is 222 nm. The loss of an isopropyl group (GS-11354) produces a hyposchromic shift to 213 nm. It was observed that all S-conjugation with prometryn or N-deisopropyl prometryn (GS-11354) maintained these same UV absorbance maxima and overall absorbance shapes. The UV maximum of the disulfides also followed this phenomenon. The homo disulfide of prometryn had a maximum at 222 nm, whereas the homo disulfide of GS-11354 had a maximum at 213 nm. The hetero disulfide, containing one prometryn and one GS-11354 molecule, had an intermediate maximum at 218 nm. The UV absorbance properties greatly assisted our HPLC purification efforts by providing early characterization of metabolites.

In summary, metabolite identification from urine was accomplished by several methods. The HPLC t_R, GC t_R, and/or TLC R_f of metabolites were compared to reference standards if available. Major and most minor metabolites were isolated from urine and analyzed by MS. Most isolated metabolites were also cochromatographed by HPLC and/or TLC by mixing with unlabeled standards and separately analyzing the ^{14}C and UV. Lastly, the UV absorbance spectra of metabolites were obtained and compared to standards. Identification of metabolites was consistent among these methods, and the pathway of the metabolism of prometryn in rats is shown in Figure 2.

Purification and Identification of Metabolites in Feces Extracts. The metabolites identified from the ^{14}C residue extracted from the feces are shown in Table 9. An additional two to four unknown metabolites were observed; however, all metabolites observed in feces were also observed in urine. Urine samples were eluted side-by-side with feces extracts on these TLC systems for comparison (data not shown). Identification in feces extracts was made only by TLC t_R in these two systems.

Identification of in Vitro Metabolites. Prometryn was readily metabolized by control rat liver microsomes. At the determined pH optimum (7.5), the rate of prometryn metabolism was 1.8 nmol/min/mg of protein or 2.4 nmol/min/nmol of cytochrome P-450. The major metabolites were GS-11354 and GS-16141 (both at 0.8 nmol/min/mg of protein) with lesser amounts of GS-26831 (0.2 nmol/min/mg of protein). No metabolism of prometryn occurred in the absence of an NADPH generating system or with boiled microsomes. These results indicate that the primary sites of metabolism of prometryn are oxidation of the 6-sulfur moiety and N-dealkyalation.

To test for FMO (flavin containing monooxygenase) involvement, liver microsomes were heated at 50 °C for 90 s (Kinsler et al., 1988) and the prometryn metabolites investigated. Heating the microsomes under these conditions reduced the formation of all three metabolites by ~10%. No selective loss in S-oxidation was observed. The NADPH oxidation and prometryn metabolites were also monitored in incubations of prometryn and purified mouse liver FMO. Incubation with purified FMO did not result in the metabolism of prometryn or the oxidation of NADPH. From these results, FMO does not appear to be responsible for the formation of GS-16141, the sulfur oxidation product of prometryn. Therefore, it appears that cytochrome P-450 is responsible for the S-oxidation of prometryn to generate GS-16141.

Table 8. Mass Spectrometry Fragmentation of Prometryn Metabolites

metabolite/standard	MS mode	*m/z* fragments[a]
prometryn	GC/MS/EI+	**241** (**M**+), 184, 226, 142, 199
GS-11354	GC/MS/EI+	**199** (**M**+), 184, 157, 142
GS-11354	TSP+	**200** (**M** + **1**)
GS-26831	EI+	**157** (**M**+), 142
GS-16141	GC/MS/EI+	225, 210, 168, 183, **257** (**M**+)
GS-14129	TSP+	**216** (**M** + **1**)
sulfoxide GS-26831	GC/MS/EI+	**173** (**M**+), 127
GS-16158	GC/MS/EI+	227, 196, 184, **273** (**M**+)
GS-11526	TSP+	**212** (**M** + **1**)
GS-17791	EI+	**126** (**M**+)
GS-17794	TSP+	**169** (**M** + **1**)
G-31435	GC/MS/EI+	210, **225** (**M**+), 168, 183, 141, 153, 126
GS-14626	GC/MS/EI+	168, **183** (**M**+), 141, 126
GS-11955	EI+	**227** (**M**+), 186
disulfide prometryn	TSP+	228, 196, **453** (**M** + **1**)
disulfide GS-11354	TSP+	186, **369** (**M** + **1**)
disulfide prometryn/GS-11354	TSP+	186, 228, **411** (**M** + **1**)
S-NAC prometryn	TSP+	227, **357** (**M** + **1**)
S-NAC GS-11354	FAB+	**315** (**M**+), 239, 186
S-NAC GS-26831	TSP+	143; **273** (**M** + **1**)
S-GLUC prometryn	TSP+	362, **404** (**M** + **1**)
S-GLUC GS-11354	TSP+	320, **362** (**M** + **1**)
6-NH_2 prometryn	HREI+	153, 168, 126, 111, 195, **210** (**M**+)
6-NH_2 GS-11354	TSP+	**168** (**M** + **1**)

[a] Intensity of ions presented in order; **M**+ or **M** + **1** ion designated.

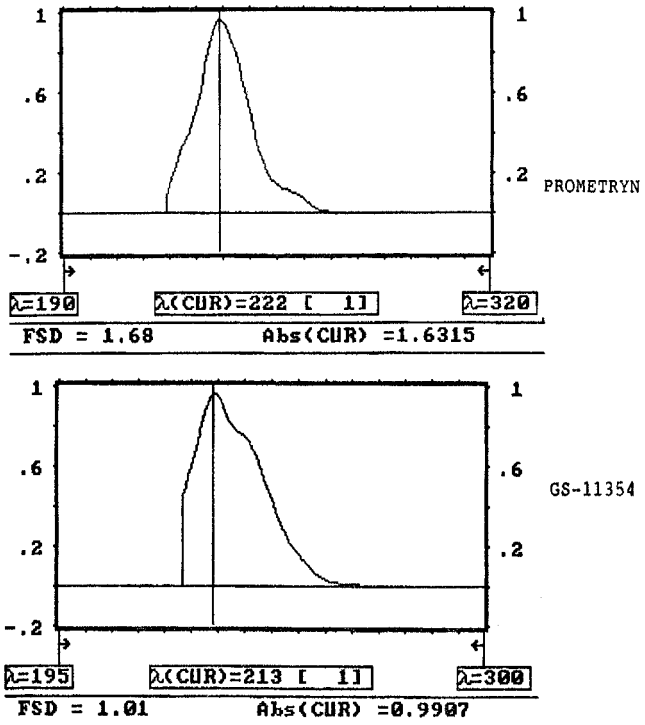


Figure 1. UV absorbance spectra of metabolites and standards obtained from a photodiode array HPLC detector. Compounds and absorbance maxima are prometryn (222 nm) and GS-11354 (213 nm).

Metabolism of Prometryn by Rats. The in vitro and in vivo metabolisms of prometryn are consistent. It appears that the major metabolism pathways of prometryn involve N-dealkylation (GS-11354) and S-oxidation (GS-16141). Subsequent N-dealkylation of GS-11354 produces GS-26831. Due to the extent of metabolism of prometryn, the other metabolites modified initially at the sulfur atom (e.g., S-oxidation, S-conjugation, etc.) can also subsequently undergo N-dealkylation and form metabolites by alternative pathways. The initial oxidation of the sulfur atom appears to result in a susceptible compound that undergoes subsequent transformations (e.g., oxidation to the sulfone, GS-16158; S-conjugation; or OH substitution, GS-11526, GS-17794, GS-17791). Because the sulfone GS-16158 would be a better leaving group, it is proposed that the sulfoxide (GS-16141) is readily oxidized to the sulfone (GS-16158), which then conjugates with *S*-glutathione or *S*-glucuronic acid, producing extensive S-conjugation that was observed in these studies. The disulfides are apparently produced from dimerization of the thio metabolites (GS-11955 and SH-GS-11354). The disulfide of prometryn was previously reported (Boehme and Bar, 1967). However, thio formation and its precursor are unknown and future research is necessary.

Table 9. Prometryn Metabolites Identified from Feces Extracts[a]

metabolite	male	female (% of dose)
prometryn	3.2	4.6
GS-11354	6.9	7.8
GS-26831	1.2	1.4
GS-16141	1.4	2.4
GS-16158	0.7	0.7
GS-11955	3.4	3.4
CGA-10582	5.1	5.4
GS-11526	3.3	3.9
GS-11957	2.1	2.2
GS-17794	5.1	5.0
S-GLUT GS-26831	1.6	1.6

[a] Fecal extracts from high-dose rats are presented; extracts for low-dose rats were similar.

Identification of the mono- and di-OH metabolites (e.g., GS-11526, GS-17794, GS-17791, GS-11957, and GS-35713) suggests a substitution of the −S and −NH_2 by OH as a possible reaction. Identification of G-31435 and GS-14626 substantiates that methylation of the OH group also could occur. The mechanism is unknown from these studies as to whether the methylation was enzymatic or chemical during sample preparation. Lastly, the formation of the 6-NH_2 metabolites was observed

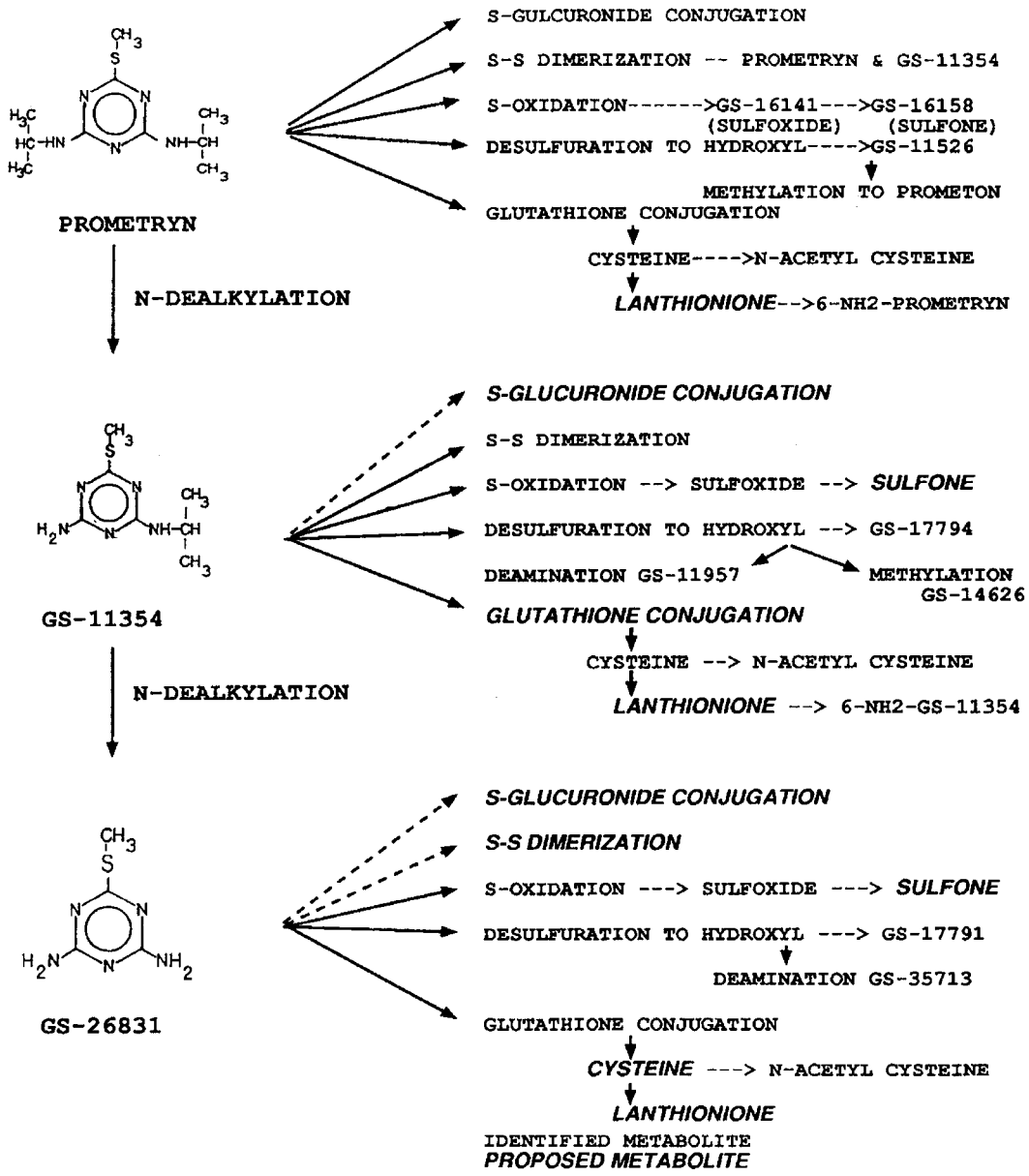


Figure 2. Degradative pathway of prometryn in rats.

for prometryn and GS-11354. No melamine was observed in these studies. The formation of the 6-NH_2 metabolites could occur by formation of a lanthionine conjugate (N-conjugated cysteine) by rearrangement of the cysteine conjugate, which subsequently cleaves to form the resulting NH_2 metabolite. Consistent with this, a lanthionine conjugate was reported with atrazine in sorghum and sugarcane (Lamoureux et al., 1972; Larson et al., 1994). Moreover, the corresponding NH_2 atrazine metabolite was also found in the same sugarcane studies (Larson et al., 1994). The mechanisms of formation of the disulfides, S-conjugates, thios, OCH_3, and NH_2 metabolites are worth further investigation. The metabolism of prometryn by rats is extensive and complicated, resulting in the formation of >30 metabolites, of which 28 were identified here. A summary pathway of metabolism is shown in Figure 2. Due to multiple transformation sites and routes of formation for many metabolites, various pathway are possible and require additional study to delineate.

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