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DATA EVALUATION RECORD

SAN 582H

Metabolism in Rats

STUDY IDENTIFICATION: Vollin, S. SAN 582H metabolism in the rat. (Unpublished study No. 12726/89 performed by Sandoz, Ltd., Basle, Switzerland; dated November 13, 1989.) MRID No. 415965-45.

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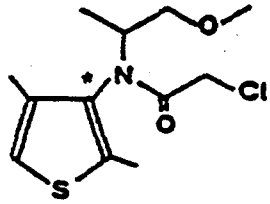
1. CHEMICAL: SAN 582H; 2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl) acetamide.

2. TEST MATERIAL: Unlabeled SAN 582H and SAN 582H labeled with ^{14}C in position 3 of the thiophene ring were used. The analytical-grade unlabeled test material was 99.8% pure, and [^{14}C]SAN 582H had a specific activity of 157 µCi/mg and a radiochemical purity of >99%. The structure and radiolabel position (*) of [^{14}C]SAN 582H are shown below:



3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Vollin, S. SAN 582H metabolism in the rat. (Unpublished study No. 12726/89 performed by Sandoz, Ltd., Basle, Switzerland; dated November 13, 1989.) MRID No. 415965-45.

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7. CONCLUSIONS:

A. Single low and single high oral doses of [^{14}C]SAN 582H and a single low oral dose of [^{14}C]SAN 582H following repeated low oral doses of unlabeled compound were readily absorbed and eliminated by male and female rats. Elimination of radioactivity was essentially complete within 3 days after dosing, and more than 90% of the ^{14}C dose was excreted in the urine and feces or urine, feces, and bile within 7 days. The total fraction of radioactivity eliminated was independent of sex and dose. However, females excreted more of the radioactive dose in the urine (47 to 63%) and less in the feces (26 to 48%) than males (about 30 and 62%, respectively). In addition, urinary levels of ^{14}C were much higher for high-dose rats (62 to 63% of the dose) than for all other groups (31 to 53%), indicating possible saturation of biliary excretion in the high-dose animals. Recovery of 75 to 82% of a single oral low dose of 10 mg [^{14}C]SAN 582H/kg in the bile demonstrated that the primary route of elimination of the parent compound and its metabolites is the liver.

SAN 582H was extensively metabolized by all groups within the first 3 days after administration of the ^{14}C-labeled test material. Less than 2.5% of the ^{14}C dose was recovered as unchanged parent compound, and 22 metabolites, 21 of which were found in both the urine and feces, were identified. The 22 metabolites plus unchanged SAN 582H accounted for approximately 22 to 39% of the orally administered radioactivity; no metabolite accounted for more than 10% of the ^{14}C dose, and most represented less than 2% (free and conjugated forms combined). An additional 18 to 30% of the orally administered radioactivity was excreted as "polar" and/or conjugated material that was not characterized further, and approximately 18 to 29% was extracted but not definable. Essentially all of the urinary radioactivity was extractable, but up to 12% of the ^{14}C dose remained bound to the feces.

On the basis of the metabolites identified in the urine and feces, the primary metabolic pathways for SAN 582H involve glutathione conjugation via displacement of the chlorine atom followed by (1) breakdown of glutathione to mercapturic acid or (2) hydrolysis of the thio bond of the mercaptan, which is then methylated and oxidized to the methylsulfoxide and methylsulfone. Additional major metabolic reactions include O-demethylation of the 2-methoxy-1-methylethyl moiety to form a 2-hydroxylated product and oxidation of the 2-methyl group on the thiophene ring to produce a hydroxymethyl. Dimerization, cyclization, and hydroxylation at the thiol also occur

following hydrolysis of the glutathione conjugate. Other minor pathways for SAN 582H involve direct biotransformation reactions such as reductive chlorination, oxidation of the sulfur on the thiophene ring to form a sulfoxide, oxidation of the 2- or 4-methyl group on the thiophene ring, and cyclization and hydroxylation of the thiol. Qualitatively, the metabolic pathways for SAN 582H appeared to be independent of sex and dose. Quantitatively, saturation of the glutathione conjugation pathway may have occurred at the higher dose.

B. Classification: (Core Supplementary) Unacceptable - see 2-11-88

This study provides supplementary information on the metabolism of SAN 582H in male and female rats for all treatment regimens required by EPA (Guideline 85-1): single low, single high, and repeated low oral dosing. The study does not meet EPA requirements, however, because between 61 and 78% of the ^{14}C dose was not identified or was characterized only as polar material. Information on tissue ^{14}C distribution and retention also was not included in this report but was part of another study (see footnote 2 of this DER). The study could be upgraded through the inclusion of (1) tissue distribution/retention data and complete chromatographic results; (2) dose selection rationale; and (3) further identification of metabolites, or at least an explanation as to why more of the excreted material was not identified.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1) [^{14}C]SAN 582H (batch No. 1, synthesis No. RA683), labeled in position 3 of the thiophene ring, had a specific activity of 157 μCi/mg and a radiochemical purity of >99%. Unlabeled analytical-grade SAN 582H was 99.8% pure (analysis No. 20278).

2) Male and female Kfm:WIST rats (purchased from Madörin, Switzerland) were used. Information on body weights, acclimatization period, and feed/fasted state of the animals at the time of dosing was not given.

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[1]Only items appropriate to this DER have been included.

3) Information on the preparation of the dosing solution was not provided.

4) This report describes the isolation, identification, and quantification of the metabolites of [^{14}C]SAN 582H in the urine, feces, and bile of rats used in a previously conducted pharmacokinetics study.[2] Five experiments were conducted. Groups of six males and six females were assigned to one of the following: (1) the low-dose group, in which animals were given a single oral dose of 10 mg [^{14}C]SAN 582H/kg; (2) the high-dose group, in which rats received a single oral dose of 1000 mg [^{14}C]SAN 582H/kg; (3) the repeated-dose group, in which rats were given a single oral dose of 10 mg unlabeled SAN 582H/kg/day for 14 days followed by a single oral dose of 10 mg [^{14}C]SAN 582H/kg on day 15; or (4) the intravenous (iv)-dose group, in which animals received a single injection of 10 mg [^{14}C]SAN 582H/kg. A fifth group of six bile duct-cannulated rats (three/sex) were given a single oral dose of 10 mg [^{14}C]SAN 582H/kg.

Urine, feces, and bile were collected for 7 days (168 hours) after administration of the radiolabeled test material. Since excretion of ^{14}C by all routes was essentially complete within 72 hours after dosing, excreta collected between 0 and 72 hours postdosing were pooled separately by sex for each experiment.

5) a. Aliquots of acidified (pH 2 to 3), pooled urine samples from all but the bile duct-cannulated animals were desalted on an Amberlite XAD-2 column. Before use, the XAD-2 resin was kept in methanol overnight, passed through a fritted glass filter, washed several times with deionized water, added to the column, and rinsed with dilute (0.1 N) HCl. After the acidified urine was added, the column was washed with dilute HCl and eluted with methanol; the washings and eluate were analyzed for ^{14}C content by liquid scintillation counting (LSC).

The methanolic eluate was concentrated, and the remaining aqueous solution was transferred to a separatory funnel, diluted with water, and

[2]Schweitzer, A. SAN 582H: Adsorption, distribution, and excretion in rats after single and multiple doses of ^{14}C SAN 582H. Unpublished study performed by Sandoz, Ltd., Basle, Switzerland, February 1988.

adjusted to pH 7 using dilute (1 N) NaOH. The transferred material was extracted twice with dichloromethane; the remaining aqueous phase was adjusted to pH 2 using 1 N HCl and extracted as before. Extracts were combined by pH, dried over anhydrous sodium sulfate, and concentrated. The concentrates were transferred to 10-mL flasks and diluted to volume. Aliquots of the dichloromethane extracts and the remaining aqueous phase were counted.

The aqueous phase was neutralized to pH 7 with NaOH, divided, and lyophilized. The powdered material was dissolved in sodium acetate buffer (0.1 M, pH 5.0). One reconstituted sample was incubated with ß-glucuronidase and arylsulfatase for 24 hours; the other sample was held under the same incubation conditions but was not treated with enzymes (control). After incubation, the samples were diluted with water and extracted with dichloromethane (three times at pH 7 and then three times at pH 2). The extracts were concentrated, and aliquots of the organic extracts and the aqueous phase were analyzed for ^{14}C content. The amount of enzyme-hydrolyzed metabolites was calculated from the difference in the radioactive content between the treated and control samples.

The aqueous phase remaining after dichloromethane extraction of the enzyme-hydrolyzed urine sample was treated with 3N NaOH and stirred for 72 hours at 100°C. The sample was then neutralized with 1N HCl, the precipitated material was filtered, and the remaining material was washed twice with water. The filtrate was extracted with dichloromethane at pH 7 and pH 2, and the extracts were assayed for radioactivity via LSC. Material remaining in the filter was air dried and weighed; aliquots were combusted and then counted.

Aliquots of the aqueous phases remaining after enzymatic or chemical hydrolysis were acetylated and methylated and analyzed for ^{14}C content.

Methods used to process the urine of bile duct-cannulated rats were not described.

b. Aqueous suspensions of homogenized feces from all rats were centrifuged; the supernatant was collected, and the precipitated material was resuspended in methanol and homogenized. The

homogenates were filtered, and the filter cake was extracted twice with methanol. The combined methanol extracts were concentrated, and the remaining aqueous material was added to the supernatant collected after centrifugation of the homogenized feces. Aliquots of the extracts were analyzed for ^{14}C content. Extracted feces were air dried and weighed; aliquots were combusted prior to counting.

The aqueous extracts were extracted with dichloromethane three times at pH 7 and three times at pH 2 as described for methanolic eluates of urine. Extracts were combined by pH, dried with anhydrous sodium sulfate, and concentrated. Aliquots of the dichloromethane extracts and of the remaining aqueous phase were assayed for radioactivity.

The extracted aqueous phase was treated with 3N NaOH, stirred for 72 hours (100°C), neutralized with 1N HCl, and processed as described for the aqueous fecal extracts (immediately preceding paragraph). Aliquots of the extracted air-dried feces were hydrolyzed under the same conditions. Before extraction, the neutralized samples were filtered, and the filter cakes were air dried, combusted, and analyzed for ^{14}C content by LSC.

c. Methods for the partitioning, enzymatic and chemical hydrolysis, and ^{14}C determination of bile samples were the same as for those of urine collected from all but the bile duct-cannulated animals, with the following exception: for partitioning, aliquots of bile samples were first diluted with water; samples then underwent two series of three extractions with dichloromethane, first at pH 7 and then at pH 2.

d. Appropriate measures were taken to determine counting efficiencies and to monitor color quenching during assaying of radioactivity.

6) Structural characterization of metabolites of SAN 582H was done using urine from high-dose rats. The metabolites in high-dose urine were separated via chromatographic methods [preparative thin-layer liquid chromatography (TLC) and high-performance liquid chromatography (HPLC)] and characterized by spectroscopic methods [nuclear magnetic resonance (NMR) and mass spectrometry (MS)]. Prior to these analyses, high-dose urine samples were pooled and extracted as described in

section (5)(a) of this DER. Methanolic extracts were concentrated, and the aqueous concentrate was extracted with dichloromethane at pH 7 and pH 2. The remaining aqueous phase was hydrolyzed with ß-glucuronidase and arylsulfatase and then extracted as described for enzyme-hydrolyzed urine samples (section (5)(a) of this DER). These extracts were concentrated to a small volume for separation by chromatographic methods.

Extracts were applied to silica gel TLC plates and developed in various solvent systems (Table 1). Spots and bands were scraped from the TLC plates and extracted with methanol. The extracts were concentrated and used for further separation by TLC and/or by reverse-phase HPLC. After the separation steps were completed, fractions were concentrated, applied to a prewashed silica gel TLC plate, and developed. Metabolites were eluted from the plates using an Eluchrom apparatus.

Characterization of metabolite structure was obtained by Fourier transform NMR (360 MHz); prior to analysis, samples were dissolved in deuterochloroform, and tetramethylsilane was added as an internal standard. Fast atom bombardment (FAB) mass spectrometry was used to verify molecular ion mass, and electron impact (EI) was used to confirm structures on the basis of fragmentation. For FAB, samples were dissolved in a thioglycerine or 3-nitrobenzylalcohol matrix and then bombarded with Xenon atoms. Spectrometry data obtained from the metabolites were compared with spectral data from synthesized reference compounds (Appendix A).

Qualitative and quantitative analysis of SAN 582H metabolites present in urine, feces, and bile involved mixing extracted excreta samples with a solution of the reference standards and applying the mixture to TLC plates. A two-dimensional (2-D) TLC system was used to identify compounds corresponding to unchanged parent compound and to metabolites M1, M3 through M15, and M20 through M22; plates were developed twice with solvent system 6 [diethyl ether:dichloromethane:methanol:ammonium hydroxide (25% aqueous solution), 50:50:1:1 v/v/v/v] and then twice with solvent system 11 (dichloromethane:methanol:formic acid, 98:2:0.5 v/v/v). Metabolites M2 and M16 were identified via one-dimensional TLC using a single solvent system (solvent system 4, diethyl ether:ethyl acetate, 50:50 v/v). For metabolites M17, M18, and M19, TLC plates were developed in one direction with solvent system 12 (dichloromethane:methanol:acetic acid, 70:70:0.5 v/v/v). Radioactive spots on TLC plates were detected

TABLE 1. TLC Solvent Systems Used for the Separation and Isolation of Urinary and Fecal Metabolites of Rats Dosed Orally with SAN 582H

solvent system no.	composition
SS 1	diethylether
SS 2	diethylether - ethyl acetate; 70 : 30 (v/v)
SS 3	diethylether - ethyl acetate; 50 : 50 (v/v)
SS 4	diethylether - methanol ; 95 : 5 (v/v)
SS 5	diethylether - dichloromethane - methanol; 60 : 35 : 5 (v/v)
SS 6	diethylether - dichloromethane - methanol - ammonium hydroxyde (25 % aqueous solution); 50 : 50 : 1 : 1 (v/v)
SS 7	ethyl acetate - n-hexane; 70 : 30 (v/v)
SS 8	ethyl acetate - methanol - formic acid; 95 : 5 : 0.5 (v/v)
SS 9	ethyl acetate - methanol - formic acid; 90 : 10 : 1 (v/v)
SS 10	dichloromethane - methanol; 95 : 5 (v/v)
SS 11	dichloromethane - methanol - formic acid; 98 : 2 : 0.5 (v/v)
SS 12	dichloromethane - methanol - acetic acid; 70 : 30 : 0.5 (v/v)

Source: CBI Table 10, CBI p. 41.

using photographic methods; spots of unlabeled reference material were visualized with a UV lamp (254 nm) and/or by spraying the plates with a mixture of 4-dimethylaminobenzaldehyde and sulfuric acid in an aqueous iron (III) chloride solution. Radiolabeled metabolites were quantified by scanning the TLC plates. For samples analyzed by 2-D TLC, the entire plate was scanned; for other samples, only one line (i.e., one sample) was scanned at a time. Data were analyzed using the software package NSCAN.

B. Protocols: A protocol was not included with this report.

12. REPORTED RESULTS:

A. [^{14}C]SAN 582H was readily absorbed and eliminated by all orally dosed rats (Tables 2 and 3). Elimination of radioactivity was essentially complete within 3 days after oral dosing for all groups, accounting for approximately 85 to 91% of the ^{14}C dose. Within 7 days, approximately 89 to 96.5% of the ^{14}C administered was recovered from the urine and feces (Table 2) or urine, feces, and bile (Table 3) of these animals. The iv-dosed rats excreted approximately 80 to 83 and 86 to 88% of the ^{14}C dose in the urine and feces within 3 and 7 days postinjection, respectively (Table 2). Urinary levels of ^{14}C were somewhat lower for high-dose rats than for other animals during the first 24 hours after compound administration. Urinary elimination of radioactivity was higher in female rats (47 to 63% of the ^{14}C dose) than in male rats (31 to 62%) (excluding the bile duct-cannulated animals); fecal levels of radioactivity, in turn, were lower in females (26 to 48%) when compared with males (30 to 62%) (Table 2). Urinary elimination of ^{14}C was highest in high-dose animals (62% males; 63% females); low- and repeated-dose animals excreted approximately 35% (males) and 48 to 53% (females) of the ^{14}C dose in the urine. Most of the radioactivity eliminated by iv-dosed females was recovered from the urine (49% of the ^{14}C dose versus 37% in the feces); in contrast, the urine and feces of iv-dosed males contained approximately 31 and 56% of the radioactivity administered, respectively. Biliary excretion of radioactivity was high, accounting for 82 and 75% of the ^{14}C dose given to males and females, respectively, at 72 hours after compound administration (Table 3).

B. [^{14}C]SAN 582H was extensively metabolized by rats. Twenty-two metabolites plus unchanged parent compound were isolated from the excreta of high-dose rats; all but one (fecal metabolite M22) were found in both the feces and the urine (Tables 4 to 7).

TABLE 2. Cumulative 7-Day Recovery of Radioactivity in the Urine and Feces of Rats Dosed Orally or Intravenously with [^{14}C]SAN 582H[a,b,c]

Exp.no.	1 p.o. low		2 i.v. low		3 p.o. high		4 multiple	
	m	f	m	f	m	f	m	f
URINE:								
0- 7 h	6.5	14.1	12.9	17.7	1.7	2.2	10.0	12.1
0- 24 h	23.2	35.5	24.4	36.5	11.9	16.4	24.5	38.5
0- 48 h	30.2	41.6	27.9	43.1	45.3	47.9	30.2	46.5
0- 72 h	32.8	44.6	29.4	46.2	59.7	60.3	32.3	50.2
0-168 h	35.3	46.9	31.2	49.4	61.6	63.1	34.9	53.3
FAECES:								
0- 24 h	34.0	32.1	45.1	18.3	4.5	2.7	36.1	20.3
0- 48 h	50.5	41.1	52.0	28.9	20.0	12.8	48.7	33.8
0- 72 h	54.2	44.8	54.0	33.7	28.9	24.3	58.4	37.1
0-168 h	57.7	47.6	56.4	36.6	30.1	26.1	61.6	39.9
TOTAL:								
0-168 h	93.0	94.6	87.6	85.9	91.6	89.2	96.5	93.2

[a]Animals were given a single oral (p.o.) dose of 10 or 1000 mg [^{14}C]SAN 582H/kg, a single intravenous (i.v.) dose of 10 mg [^{14}C]SAN 582H/kg, or a single oral dose of 10 mg unlabeled SAN 582H/kg/day for 14 days followed by a single oral dose of 10 mg [^{14}C]SAN 582H/kg (multiple).

[b]m = males; f = females.

[c]Values represent the mean percent of the ^{14}C dose administered to groups of six animals.

Source: CBI Table 2, CBI p. 26.

TABLE 3. Cumulative 7-Day Recovery of Radioactivity in the Urine, Feces, and Bile of Bile Duct-Cannulated Rats Given a Single Oral Dose of 10 mg [^{14}C]SAN 582H/kg[a]

	male rats	female rats
URINE:		
0- 7 h	3.2	4.2
0- 24 h	5.8	9.9
0- 48 h	6.9	11.6
0- 72 h	7.3	11.9
0-168 h	7.6	12.4
FAECES:		
0- 24 h	-	-
0- 48 h	1.9	3.4
0- 72 h	2.1	3.6
0-168 h	2.2	3.7
BILE:		
0- 7 h	63.7	45.1
0- 24 h	74.5	72.0
0- 48 h	81.6	74.8
0- 72 h	82.0	74.9
0-168 h	82.2	75.1
TOTAL:		
0-168 h	92.0	91.2

[a]Values represent the mean percent of the ^{14}C dose administered to groups of three rats.

Source: CBI Table 3, CBI p. 27.

TABLE 4. Composition of the Total Excreted Radioactivity (0–72 hr) After Administration of a Single Oral Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	urine	faeces	total	urine	faeces	total
a.i.	0.2	1.0	1.2	0.6	1.2	1.8
1/7	0.4	2.7	3.1	1.6	1.7	3.3
2	3.3	<0.1	3.3	6.4	<0.1	6.4
3	0.2	0.6	0.8	0.1	0.3	0.4
4	0.2	---	0.2	---	---	---
5	0.2	0.4	0.6	1.1	0.3	1.4
6	0.1	0.8	0.9	0.3	0.3	0.6
8	---	0.2	0.2	---	---	---
9	<0.1	---	<0.1	0.1	---	0.1
10	<0.1	0.7	0.7	0.2	0.2	0.4
11	<0.1	0.3	0.3	0.2	0.2	0.4
12	0.4	---	0.4	0.4	---	0.4
13	1.0	1.5	2.5	2.8	1.8	4.6
14	1.0	1.4	2.4	2.2	1.7	3.9
15	---	0.1	0.1	---	---	---
16	0.9	2.9	3.8	1.2	3.2	4.4
17	0.4	0.9	1.3	3.3	0.2	3.5
18	0.4	0.5	0.9	0.6	0.4	1.0
19	0.5	0.5	1.0	0.3	0.3	0.6
20	---	0.5	0.5	---	0.3	0.3
21	0.2	0.5	0.7	0.5	0.3	0.8
22	---	0.7	0.7	---	0.1	0.1
Total of identified metabolites	9.4	16.2	25.6	21.9	12.5	34.4
Not definable radioactivity in the extracts 1)	9.4	12.7	22.1	9.2	13.4	22.6
Polar fractions 2)	13.6	15.1	28.7	13.0	7.6	20.6
Nonextractable radioactivity	0.4	10.2	10.6	0.5	11.3	11.8
Total excretion (0-72h)	32.8	54.2	87.0	44.6	44.8	89.4

TABLE 4 (Cont'd)

[1)]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[2)]The polar fractions consist of a) aqueous phase of urine remaining after XAD2 treatment; b) aqueous phase remaining after enzyme hydrolysis and extraction at pH 7 of XAD2-extracted metabolites; or c) aqueous phase of feces remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 21, CBI p. 52.

TABLE 5. Composition of the Total Excreted Radioactivity (0-72 hr) After Administration of a Single Intravenous Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	urine	faeces	total	urine	faeces	total
a.i.	0.2	2.1	2.3	0.5	0.8	1.3
1/7	0.3	0.1	0.4	3.9	2.2	6.1
2	2.5	<0.1	2.5	3.4	<0.1	3.4
3	0.1	0.5	0.6	0.2	0.4	0.6
4	0.5	---	0.5	0.5	---	0.5
5	0.2	0.4	0.6	0.6	0.3	0.9
6	0.1	0.2	0.3	0.5	0.3	0.8
8	---	---	---	---	---	---
9	0.1	0.3	0.4	0.2	---	0.2
10	0.1	0.1	0.2	0.3	0.1	0.4
11	0.2	1.6	1.8	0.2	0.5	0.7
12	0.3	---	0.3	0.3	---	0.3
13	0.9	---	0.9	2.3	---	2.3
14	1.0	1.0	2.0	2.4	0.6	3.0
15	---	---	---	---	<0.1	<0.1
16	1.0	2.5	3.5	1.0	1.4	2.4
17	0.6	0.2	0.8	4.7	<0.1	4.7
18	0.3	0.6	0.9	0.7	0.3	1.0
19	0.2	0.2	0.4	0.4	<0.1	0.4
20	---	---	---	---	<0.1	<0.1
21	0.5	0.2	0.7	0.7	0.2	0.9
22	---	0.2	0.2	---	0.2	0.2
Total of identified metabolites	9.1	10.2	19.3	22.8	7.3	30.1
Not definable radioactivity in the extracts 1)	7.5	11.5	19.0	11.1	6.5	17.6
Polar fractions 2)	12.5	11.5	24.0	12.0	5.2	17.2
Nonextractable radioactivity	0.3	20.8	21.1	0.3	14.7	15.0
Total excretion (0-72h)	29.4	54.0	83.4	46.2	33.7	79.9

TABLE 5 (Cont'd)

[1)]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[2)]The polar fractions consist of a) aqueous phase of urine remaining after XAD2 treatment; b) aqueous phase remaining after enzyme hydrolysis and extraction at pH 7 of XAD2-extracted metabolites; or c) aqueous phase of feces remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 22, CBI p. 53.

TABLE 6. Composition of the Total Excreted Radioactivity (0–72 hr) After Administration of a Single Oral High Dose of 1000 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	urine	faeces	total	urine	faeces	total
a.i.	---	1.2	1.2	0.2	1.4	1.6
1/7	5.7	0.6	6.3	5.3	0.5	5.8
2	4.8	<0.1	4.8	6.4	<0.1	6.4
3	0.3	0.2	0.5	0.2	0.2	0.4
4	---	---	---	1.1	---	1.1
5	7.3	0.3	7.6	6.6	0.2	6.8
6	---	0.3	0.3	---	0.3	0.3
8	---	---	---	0.2	---	0.2
9	---	---	---	<0.1	---	<0.1
10	---	0.2	0.2	0.2	0.1	0.3
11	0.4	0.1	0.5	0.3	0.3	0.6
12	0.4	---	0.4	---	---	---
13	0.5	0.3	0.8	1.3	0.1	1.4
14	2.7	0.2	2.9	4.2	0.1	4.3
15	---	<0.1	<0.1	---	<0.1	<0.1
16	2.7	2.0	4.7	1.6	1.1	2.7
17	2.1	0.1	2.2	4.0	<0.1	4.0
18	0.9	0.3	1.2	0.9	0.1	1.0
19	0.5	0.5	1.0	0.6	<0.1	0.6
20	---	<0.1	<0.1	---	<0.1	<0.1
21	0.2	0.3	0.5	0.7	0.2	0.9
22	---	1.0	1.0	---	1.0	1.0
Total of identified metabolites	28.5	7.6	36.1	33.8	5.6	39.4
Not definable radioactivity in the extracts 1)	16.1	8.7	24.8	11.7	6.3	18.0
Polar fractions 2)	15.0	6.9	21.9	14.7	7.3	22.0
Nonextractable radioactivity	0.1	5.7	5.8	0.1	5.1	5.2
Total excretion (0-72h)	59.7	28.9	88.6	60.3	24.3	84.6

TABLE 6 (Cont'd)

[1)]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[2)]The polar fractions consist of a) aqueous phase of urine remaining after XAD2 treatment; b) aqueous phase remaining after enzyme hydrolysis and extraction at pH 7 of XAD2-extracted metabolites; or c) aqueous phase of feces remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 23, CBI p. 54.

TABLE 7. Composition of the Total Excreted Radioactivity (0-72 hr) After Administration of Multiple Oral Low Doses Followed by a Single Oral Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	urine	faeces	total	urine	faeces	total
a.i.	---	1.4	1.4	0.1	1.1	1.2
1/7	0.4	2.9	3.3	2.6	4.6	7.2
2	3.6	<0.1	3.6	9.8	<0.1	9.8
3	0.1	0.5	0.6	0.1	0.3	0.4
4	---	---	---	---	---	---
5	0.3	0.5	0.8	1.1	0.3	1.4
6	---	0.3	0.3	<0.1	0.6	0.6
8	---	---	---	<0.1	---	<0.1
9	---	---	---	---	---	---
10	---	---	---	<0.1	0.2	0.2
11	0.1	0.1	0.2	0.2	---	0.2
12	0.4	---	0.4	0.7	---	0.7
13	0.2	---	0.2	2.2	1.1	3.3
14	0.8	2.0	2.8	2.4	0.9	3.3
15	---	0.1	0.1	0.1	---	0.1
16	1.3	3.8	5.1	2.0	1.3	3.3
17	0.4	0.2	0.6	3.1	0.2	3.3
18	0.6	0.5	1.1	0.2	0.3	0.5
19	0.3	0.8	1.1	1.1	0.3	1.4
20	---	---	---	---	---	---
21	---	---	---	0.3	---	0.3
22	---	---	---	---	---	---
Total of identified metabolites	8.5	13.1	21.6	26.0	11.2	37.2
Not definable radioactivity in the extracts 1)	11.0	18.6	29.6	12.6	9.8	22.4
Polar fractions 2)	12.4	14.6	27.0	11.2	6.6	17.8
Nonextractable radioactivity	0.4	12.1	12.5	0.4	9.5	9.9
Total excretion (0-72h)	32.3	58.4	90.7	50.2	37.1	87.3

TABLE 7 (Cont'd)

[1)]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[2)]The polar fractions consist of a) aqueous phase of urine remaining after XAD2 treatment; b) aqueous phase remaining after enzyme hydrolysis and extraction at pH 7 of XAD2-extracted metabolites; or c) aqueous phase of feces remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 24, CBI p. 55.

Approximately 90 to 98% of the urinary radioactivity (29.6 to 59.2% of the ^{14}C dose) and 59 to 82% of the fecal radioactivity (19.2 to 47.1% of the dose) were recovered following the initial methanol extraction. The lowest excreta recoveries (59 and 65%) were for the feces of iv-dosed animals. Between 18 and 41% of the fecal ^{14}C (5.1 to 19.1% of the ^{14}C dose) was nonextractable following treatment with methanol; 1.7 to 12.5% of the fecal radioactivity (0.5 to 4.2% of the ^{14}C dose) remained bound following chemical hydrolysis and subsequent extractions with dichloromethane. Table 8 gives extraction recoveries for orally dosed rats.

Urine and feces contained both free and conjugated metabolites. For all experiments, the amount of free extractable metabolites (at pH 7 and pH 2) in the urine accounted for 14 to 20% of the ^{14}C dose given to males and 22 to 33% of that administered to females (Appendix Tables B1 to B4). The amount of conjugated metabolites (including both glucuronides and sulfates) recovered from the urine was dose and treatment dependent. Animals given either a single or repeated low dose (i.e., 10 mg/kg) of SAN 582H eliminated about 3% (males) and 10% (females) of the radioactive dose in the urine as conjugates within 72 hours after compound administration (Tables 4 and 7). High-dose males and females excreted approximately 23 to 24% of the ^{14}C dose in the urine as conjugates (Table 6). The primary metabolites recovered from the enzyme-treated urine of these animals were M5 (which represented about 6 to 7% of the ^{14}C dose), M1/M7 (about 5%), M2 (about 3%), M14 (approximately 2 to 4%), and M16 (about 1 to 2%). M17, a mercapturic acid metabolite, accounted for approximately 2 to 4% of the radioactive dose. Chemical hydrolysis of enzyme-treated urine samples released only 1 to 2% of the administered radiolabel, and of the ^{14}C in the remaining aqueous phase (7 to 9% of the dose), approximately 7 and 30% consisted of methylated and acetylated metabolites, respectively. Up to 16% of the ^{14}C dose, as extracted from the urine, was not identified, and an additional 11 to 15% consisted of polar fractions that were not further characterized. Trace amounts of the ^{14}C dose (<0.5%) were not extractable.

In contrast with urinary metabolite profiles, the amount of free extractable metabolites in feces (pH 7 and pH 2) was higher in males (16 to 30% of the ^{14}C dose) than in females (10 to 23.5%) and was lower for high-dose males and females (15.6 and 10.2%, respectively) when compared with other

TABLE 8. Distribution of Radioactivity in the Urine and Feces (0-72 Hr) of Rats Given Oral Doses of [^{14}C]SAN 582H[a]

	Percent of ^{14}C Dose Administered as:					
	10 mg/kg (single)[b]		1000 mg/kg (single)[b]		10 mg/kg (repeated)[c]	
Fraction	Males	Females	Males	Females	Males	Females
Extractable						
Identified						
Urine	9.4[d]	21.9	28.5	33.8	8.5	26.0
Feces	16.2	12.5	7.6	5.6	13.1	11.2
Total	25.6	34.4	36.1	39.4	21.6	37.2
Not identified						
Urine	9.4	9.2	16.1	11.7	11.0	12.6
Feces	12.7	13.4	8.7	6.3	18.6	9.8
Total	22.1	22.6	24.8	18.0	29.6	22.4
Polar Fractions						
Urine	13.6	13.0	15.0	14.7	12.4	11.2
Feces	15.1	7.6	6.9	7.3	14.6	6.6
Total	28.7	20.6	21.9	22.0	27.0	17.8
Total Extractable						
Urine	32.4	44.1	59.6	60.2	31.9	49.8
Feces	44.0	33.5	23.2	19.2	46.3	27.6
Total	76.4	77.6	82.8	79.4	78.2	77.4
Nonextractable						
Urine	0.4	0.5	0.1	0.1	0.4	0.4
Feces	10.2	11.3	5.7	5.1	12.1	9.5
Total	10.6	11.8	5.8	5.2	12.5	9.9
Total Excretion						
Urine	32.8	44.6	59.7	60.3	32.3	50.2
Feces	54.2	44.8	28.9	24.3	58.4	37.1
Total	87.0	89.4	88.6	84.6	90.7	87.3

[a]Prepared by the reviewers.

[b]Each animal received a single oral dose of 10 or 1000 mg [^{14}C]SAN 582H/kg.

[c]Each animal received 10 mg unlabeled SAN 582H/kg/day for 14 days followed by a single oral dose of 10 mg [^{14}C]SAN 582H/kg on day 15.

[d]Each value is the mean of six rats.

Source: CBI Tables 21, 23, and 24, CBI pp. 52, 54, and 55.

animals (20 to 30%, males; 13 to 23.5%, females) (Appendix Tables B5 to B8). Between 6 and 19% of the extractable fecal radioactivity was not identified; an additional 5 to 15% was "polar" material. The amount of nonextractable radioactivity in the feces accounted for 5 to 21% of the ^{14}C dose for all animals and was lowest (<6%) in high-dose rats. Chemical hydrolysis of the remaining aqueous phase and of the remaining fecal material yielded between 4 and 10% of the administered radioactivity. Excretion of conjugated metabolites in the feces was minimal for all groups, accounting for <2.5% of the ^{14}C dose administered. Major fecal metabolites included M13, which represented about 1.5 to 2% of the ^{14}C given to low-dose rats; M16, which accounted for 2 to 4% of that administered to all animals; and M1/M7, which accounted for approximately 2 to 5% of the radioactivity given to low- and repeated-dose rats. Most other fecal metabolites represented ≤1% of the administered radiolabel.

Between 11 and 16% of the ^{14}C dose was initially extracted from the bile (CBI p. 32); an additional 32 to 36% was conjugated, and approximately half of this consisted of glucuronide and/or sulfate conjugates of SAN 582H (Appendix Table B9). A large amount of the radioactivity in the bile (i.e., 48 to 51% of the ^{14}C dose) was "polar" material, and 6 to 11% was extractable but not identified. Only a small fraction of the administered radioactivity (<1.5%) was not extractable.

Between 1 and 2% of the test material was excreted unchanged, primarily in the feces (Tables 4 to 7).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Single low, single high, and repeated low oral doses of SAN 582H were readily absorbed and eliminated by male and female rats. Elimination of radioactivity was essentially complete within 3 days after dosing with ^{14}C-labeled test material, and more than 90% of the ^{14}C dose was excreted in the urine and feces or urine, feces, and bile within 7 days. The total amount of the radiolabel eliminated was independent of dose and sex. However, orally dosed females excreted more of the radioactive dose in the urine (47 to 63%) and less in the feces (26 to 48%) than males (31 to 62% and 30 to 62%, respectively. In addition, urinary levels of ^{14}C were much higher for high-dose rats (62 to 63% of the total dose) than for all other groups (31 to 53%), indicating possible saturation of biliary excretion in high-dose animals (see additional discussion below). Recovery of 75 to 82% of a single oral dose of 10 mg

[^{14}C]SAN 582H/kg in the bile demonstrated that the test material and its metabolites were excreted primarily via the liver.

SAN 582H was readily and extensively metabolized by all animals. In addition to small amounts (≤2% of the ^{14}C dose) of unchanged parent compound, 22 metabolites combined accounted for approximately 40% of the radioactivity administered. An additional 20% consisted of several extractable but unidentified metabolites, and the remaining 20% was described as a mixture of very polar plus conjugated metabolites that were not further characterized.

The major metabolic pathways for SAN 582H involve glutathione conjugation followed by (1) gamma-glutamyltransferase transpeptidation and N-acetylation of the resulting cysteinyl derivative to form mercapturic acid (metabolite M17) (Figure 1) or (2) hydrolysis to the mercaptan, which is further metabolized to the intermediate PL 36-88 by S-methylation; to metabolite M21 by cyclization and hydroxylation; or to M22 by dimerization (Figure 2). The S-methylated intermediate PL 36-88 appears to undergo O-demethylation to form the hydroxylated metabolite M1; PL 36-88 may also be oxidized to the sulfoxide M13 (Figure 3). Further metabolism of M13 involves oxidation of the 2-methyl group on the thiophene ring to form the hydroxymethyl metabolite M16; O-demethylation to M2 followed by oxidation to the hydroxy-sulfone metabolite M14 and subsequent oxidation to the carboxylic acid M19; or oxidation to the sulfone M10, which may, in turn, undergo O-demethylation to M14 and subsequent oxidation to M19 (Figure 3). The study author suggested that the S-methyl group on metabolite M1 is oxidized to the sulfoxide M2, which is then oxidized to metabolites M14 and M19 as described above; the methylethyl moiety on M1 is oxidized to carboxylic acid (Figure 3).

The minor metabolic pathways of SAN 582H involve direct breakdown of the parent compound. The predominant reactions include (1) demethylation to metabolite M7 (Figure 4) and (2) oxidation of the 2- or 4-methyl group on the thiophene ring to produce metabolite M5 and the proposed intermediate PL 77-88, respectively (Figure 5). Cyclization of metabolite M7 results in the formation of M9, which, in turn, undergoes hydroxylation to produce M15 (Figure 4); dehydration of the newly hydroxylated ring of M15 gives M8 (Figure 4). Cyclization of PL 77-88 and M5 results in metabolites M20 and M6, respectively (Figure 5).

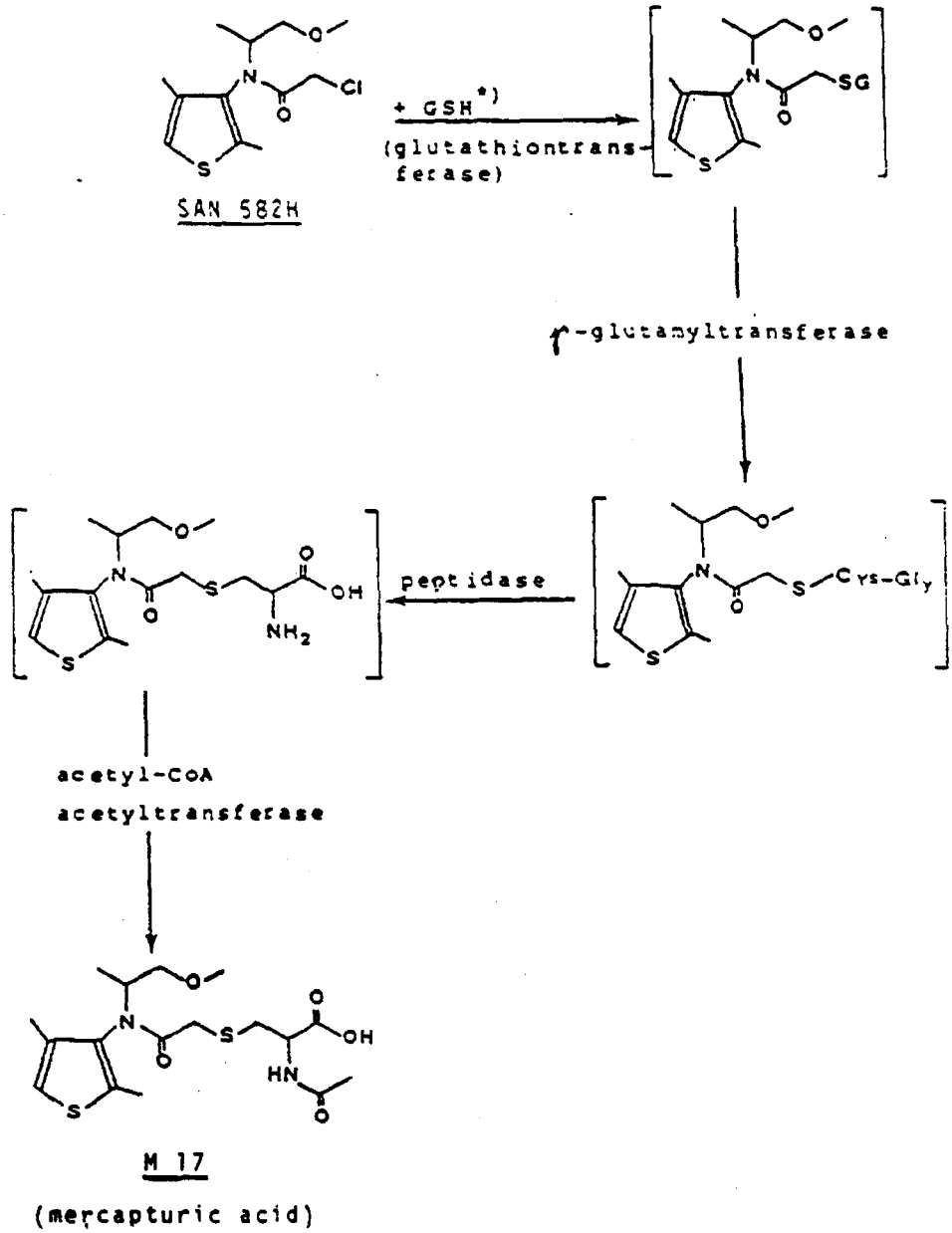
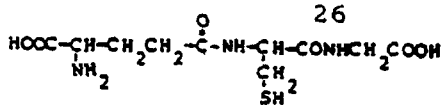


FIGURE 1. Proposed major metabolic pathway for the formation of the mercapturic acid metabolite M17 from SAN 582H in rats.

Source: CBI Figure 1a, CBI p. 57.

Compounds shown in brackets were not isolated in these studies but are the most logical intermediates in the pathways defined.

*) GSH= glutathione:



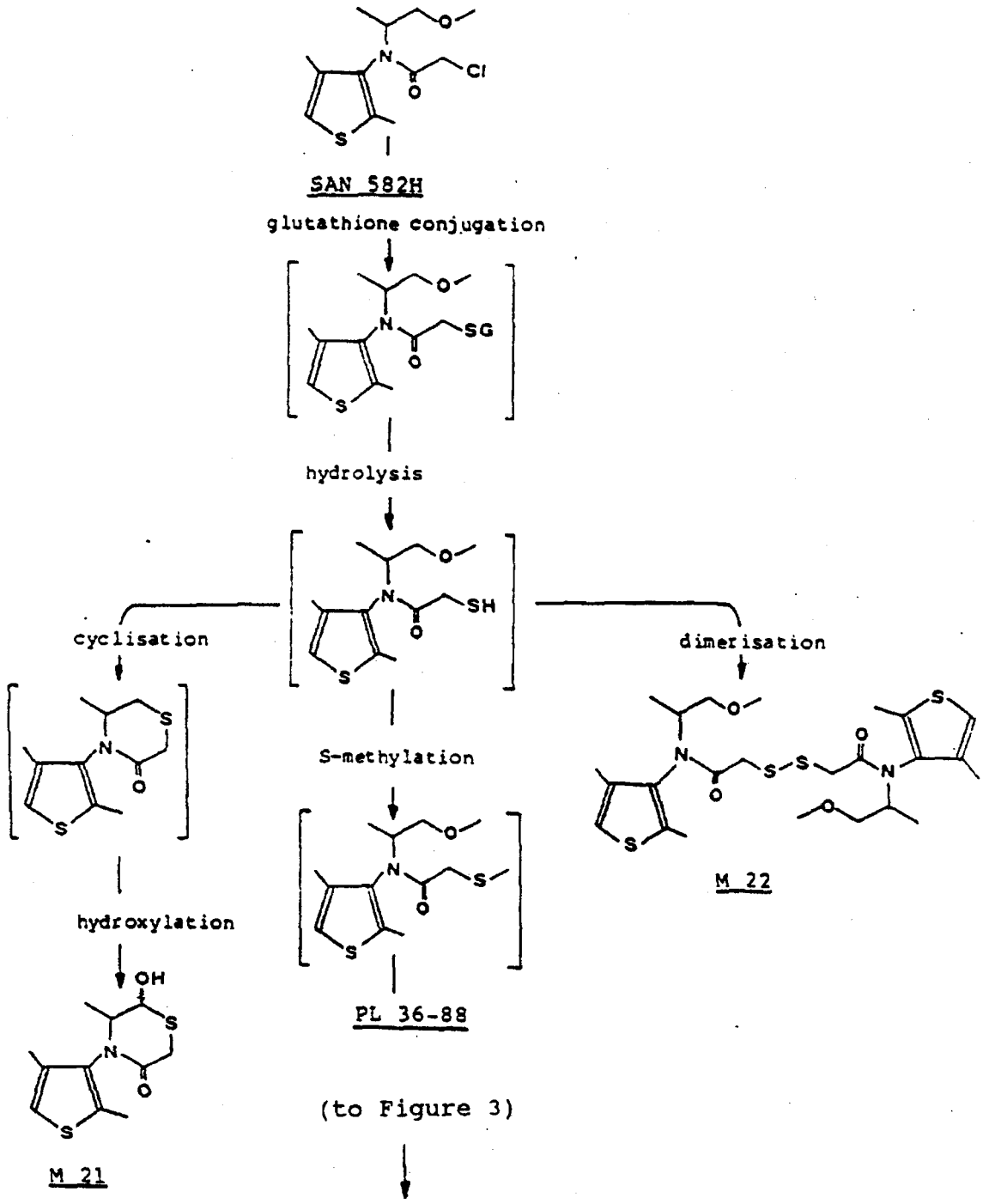


FIGURE 2. Proposed major metabolic pathway for SAN 582H in rats (continued in Figure 3).

Source: CBI Figure 1b, CBI p. 58.

Compounds shown in brackets were not isolated in these studies but are the most logical intermediates in the pathways defined.

(from Figure 2)

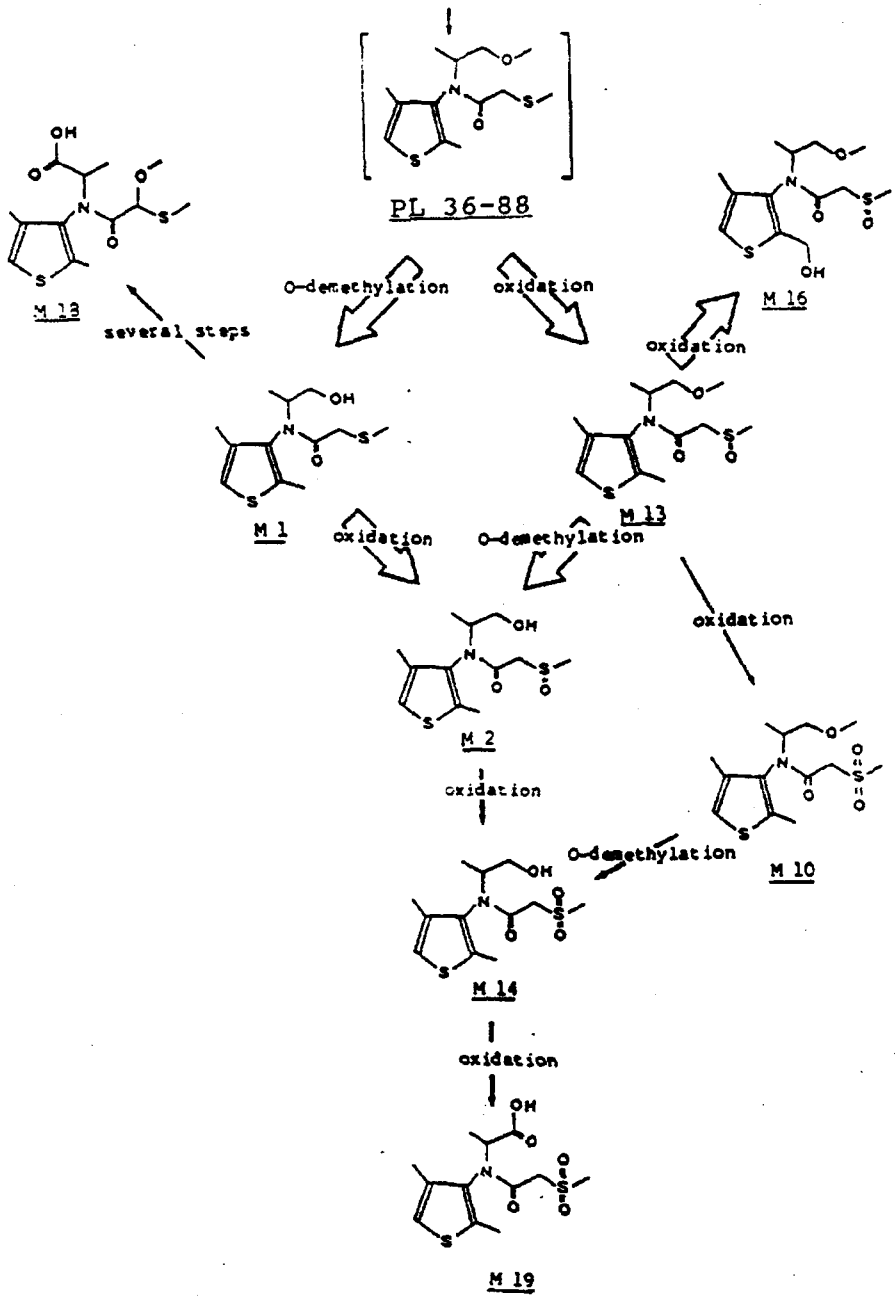


FIGURE 3. Proposed major metabolic pathway for SAN 582H in rats (continued from Figure 2).

Source: CBI Figure 1c, CBI p. 59.

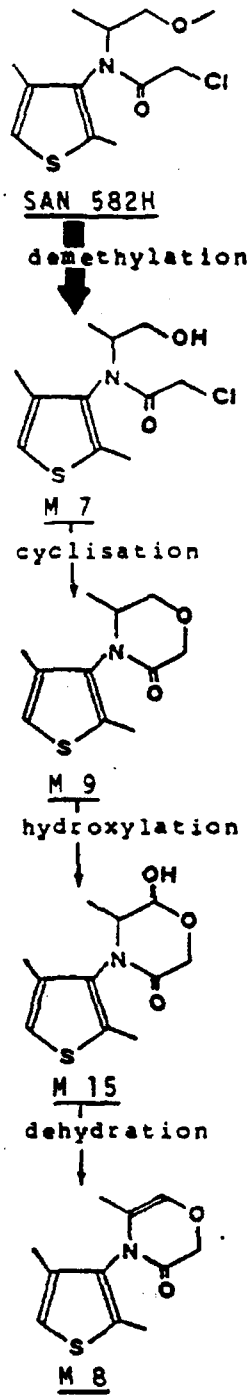


FIGURE 4. Proposed minor metabolic pathway for SAN 582H in rats.

Source: CBI Figure 1d, CBI p. 60.

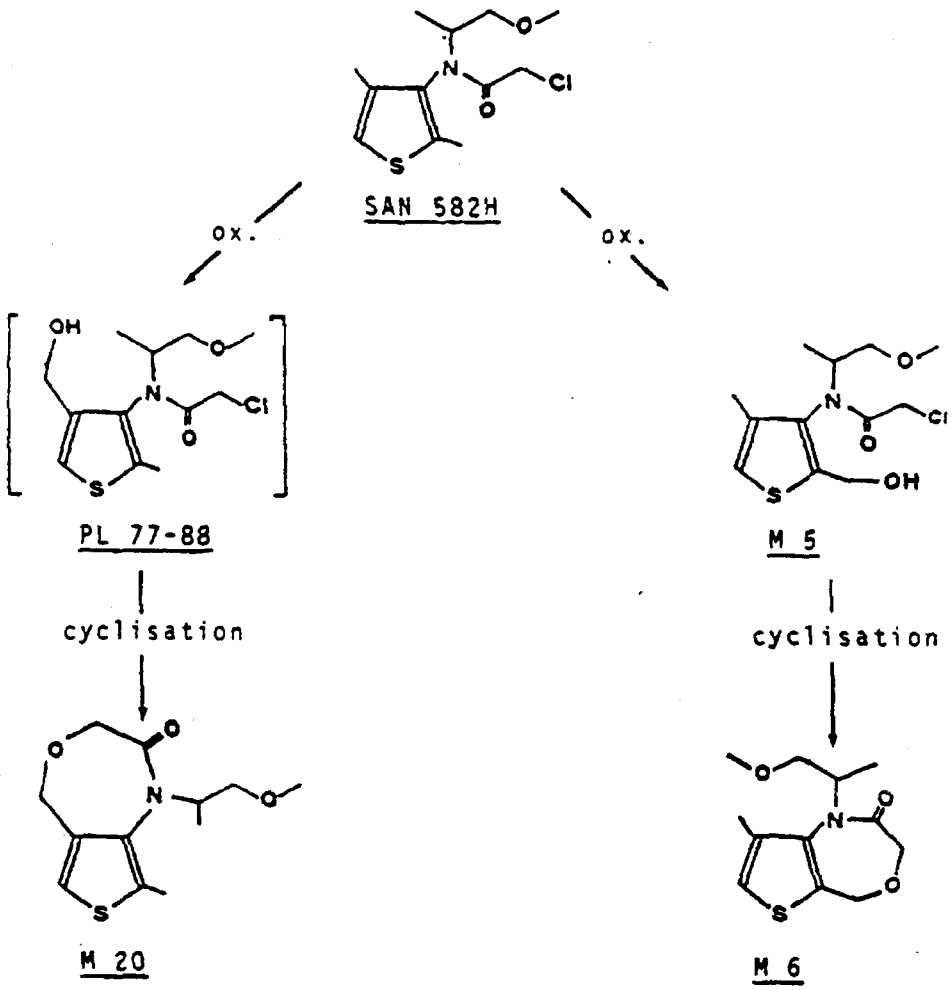


FIGURE 5. Proposed minor metabolic pathway for SAN 582H in rats.

Source: CBI Figure 1e, CBI p. 61.

Compounds shown in brackets were not isolated in these studies but are the most logical intermediates in the pathways defined.

Additional minor pathways for the metabolism of SAN 582H involve hydrolysis, i.e., replacement of the chlorine by a hydroxyl group to produce M11; direct oxidation of the sulfur in the thiophene ring to produce the unstable sulfoxide, metabolite M4; and reductive dechlorination to M3 and subsequent demethylation to M12 (Figure 6).

The study author concluded that, in general, the metabolic pathways for SAN 582H were independent of sex and dose. However, glutathione conjugation appeared to be significantly higher in low-dose animals than in high-dose rats: the ratio of the amounts of the identified metabolites formed via glutathione conjugation versus by direct transformation of the parent compound was 2:1 for the high-dose group and 4:1 for low-dose animals. The lower ratio for the high-dose group was attributed to possible saturation of glutathione conjugation at the 1,000-mg/kg dose level.

B. A quality assurance statement, signed and dated November 16, 1989, and a statement of compliance with Good Laboratory Practices (GLPs), signed and dated November 13, 1989, were included in the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

This study provides supplementary information on the metabolism of SAN 582H in male and female rats for all treatment regimens required by EPA (Guideline 85-1): single low, single high, and repeated low oral dosing. Although the study author reported that all major metabolites were identified, between 61 and 81% of the ^{14}C dose was not identified or characterized, and data supporting the statement that this fraction of the dose consisted of primarily minor polar and/or conjugated metabolites were not presented. The absence of these data and the inability to characterize up to 30% of the radioactive dose that was extractable may indicate inadequate methodology. The report would be strengthened and the conclusions better supported through the inclusion of complete chromatographic results.

Orally administered [^{14}C]SAN 582H is readily absorbed and extensively metabolized by rats. Elimination of radioactivity was essentially complete within 3 days after dosing, and the primary route of elimination, based on bile duct cannulation studies, was via the liver. As noted by the

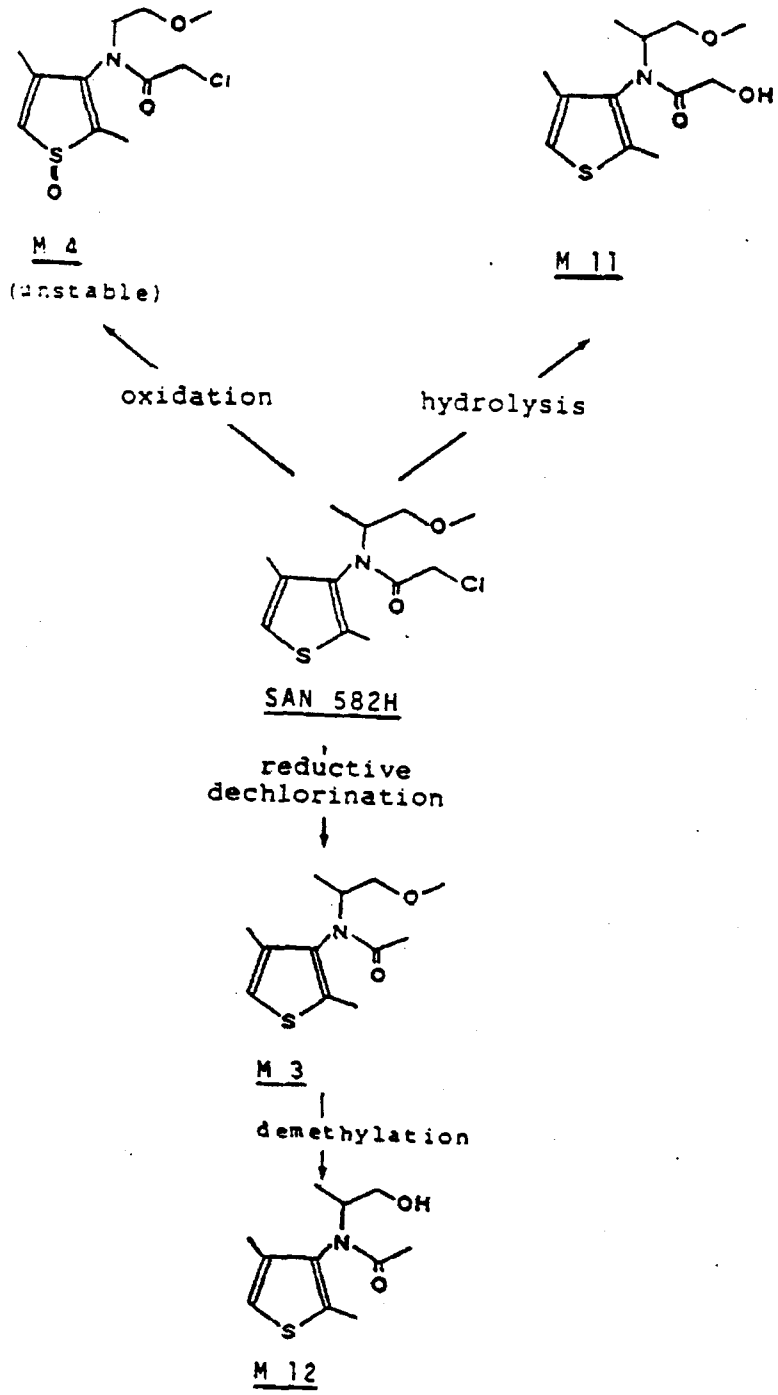


FIGURE 6. Proposed minor metabolic pathways for SAN 852H in rats.

Source: CBI Figure 1f, CBI p. 62.

study author, the total amount of radiolabel eliminated was independent of dose level, dosing regimen, and sex. However, orally dosed females tended to eliminate more of the ^{14}C administered in the urine (47 to 63%) and less in the feces (26 to 48%) than males (31 to 62% and 30 to 63%, respectively). In addition, although urinary elimination of radioactivity by high-dose rats was somewhat slow during the first 24 hours postdosing, these animals eliminated more of the total dose (i.e., 62 to 63%) in the urine than all other groups (31 to 53%) within 72 hours after compound administration. These data suggest that biliary excretion of SAN 582H and its metabolites may have been saturated in females and following administration of the high dose (1000 mg/kg) to both males and females.

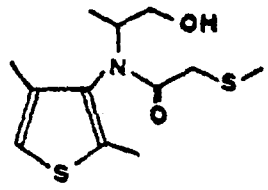
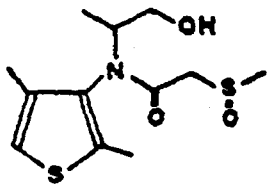
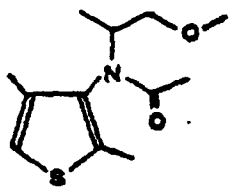
On the basis of the metabolites identified, the data presented support the conclusions of the study author that SAN 582H is extensively and essentially completely metabolized, and that the primary metabolic pathway for SAN 582H involves glutathione conjugation followed by breakdown of the glutathione conjugate via the mercapturic acid pathway or hydrolysis of the thio bond of the mercaptan (which is then methylated and oxidized to the methylsulfoxide and methylsulfone). Overall, this study was classified as supplementary.

Items 15 and 16--see footnote 1.

APPENDIX A

Reference Standards
for SAN 582H Metabolites
(CBI Table 9, CBI pp. 33-40)

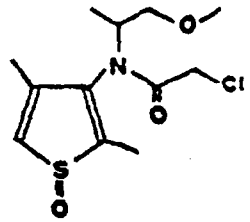
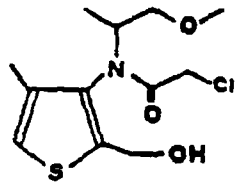
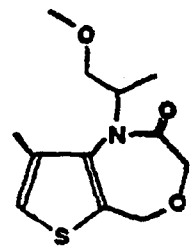
Table 9 : STRUCTURES OF THE METABOLITES OF SAN 582 H IN THE RAT

metabolite no.	structure chemical name	empirical formul molecular weight
M 1 *	 N-(2,4-dimethyl-3-thienyl)-N-(2-hydroxy-1-methylethyl)-2-(methyl-thio)-acetamide	$C_{12}H_{19}NO_2S_2$ 273
M 2 *	 N-(2,4-dimethyl-3-thienyl)-N-(2-hydroxy-1-methylethyl)-2-(methyl-sulfinyl)-acetamide	$C_{12}H_{19}NO_3S_2$ 289
M 3 *	 N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide	$C_{12}H_{19}NO_2S$ 241

* Synthesized reference standards available

Table 9 : STRUCTURES OF THE METABOLITES OF SAN 582 H IN THE RAT

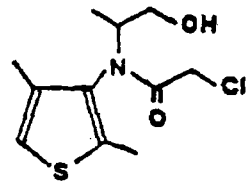
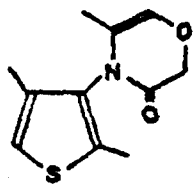
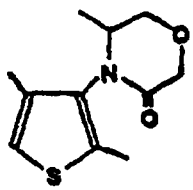
(cont.)

metabolite no.	structure chemical name	empirical formul molecular weight
M 4	 2-Chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide-S-oxide	$C_{12}H_{18}NO_3SCl$ 291
M 5	 2-Chloro-N-(2-hydroxymethyl-4-methyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide	$C_{12}H_{18}NO_3SCl$ 291
M 6	 1,5-Dihydro-1-(2-methoxy-1-methylethyl)-8-methyl-thieno[2,3-f][4,1]oxazepin-2(3H)-one	$C_{12}H_{17}NO_3S$ 255

* Synthesized reference standards available

Table 9 : STRUCTURES OF THE METABOLITES OF SAN 582 H IN THE RAT

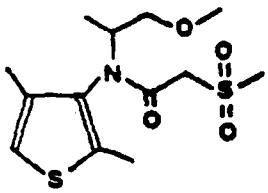
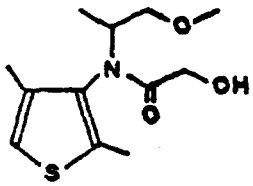
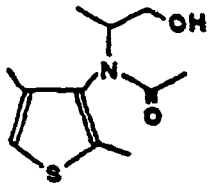
(cont.)

metabolite no.	structure chemical name	empirical formu molecular weigh
M 7 *	 2-Chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-hydroxy-1-methylethyl)-acetamide	$C_{11}H_{16}NO_2ClS$ 261
M 8 *	 3,4-Dihydro-4-(2,4-dimethyl-3-thienyl)-5-methyl-2H-1,4-oxazin-3-one	$C_{11}H_{13}NO_2S$ 223
M 9 *	 4-(2,4-dimethyl-3-thienyl)-5-methyl-3-morpholinone	$C_{11}H_{15}NO_2S$ 225

* Synthesized reference standards available

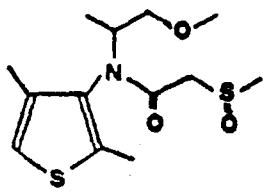
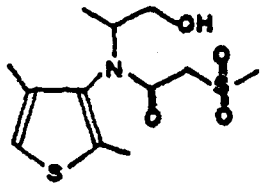
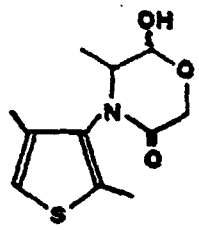
Table 9 : STRUCTURES OF THE METABOLITES OF SAN 582 H IN THE RAT

(cont.)

metabolite no.	structure chemical name	empirical formu molecular weigh
M 10 *	 N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-2-(methylsulfonyl)-acetamide	$C_{13}H_{21}NO_4S_2$ 319
M 11 *	 N-(2,4-dimethyl-3-thienyl)-2-hydroxy-N-(2-methoxy-1-methylethyl)-acetamide	$C_{12}H_{19}NO_3S$ 257
M 12 *	 N-(2,4-dimethyl-3-thienyl)-N-(2-hydroxy-1-methylethyl)-acetamide	$C_{11}H_{17}NO_2S$ 227

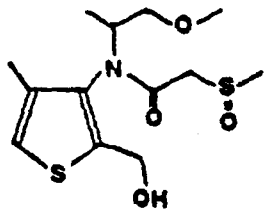
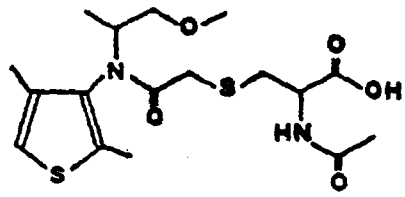
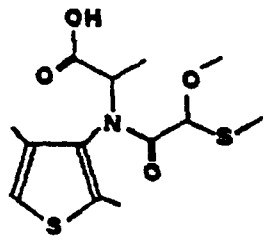
* Synthesized reference standards available

Table 9 : STRUCTURES OF THE METABOLITES OF SAN 582 H IN THE RAT (cont.)

metabolite no.	structure chemical name	empirical formula molecular weight
M 13 *	 N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-2-(methylsulfinyl)-acetamide	$C_{13}H_{21}NO_3S_2$ 303
M 14 *	 N-(2,4-dimethyl-3-thienyl)-N-(2-hydroxy-1-methylethyl)-2-(methylsulfonyl)-acetamide	$C_{12}H_{19}NO_4S_2$ 305
M 15 *	 4-(2,4-dimethyl-3-thienyl)-6-hydroxy-5-methyl-3-morpholinone	$C_{11}H_{15}NO_3S$ 241

* Synthesized reference standards available

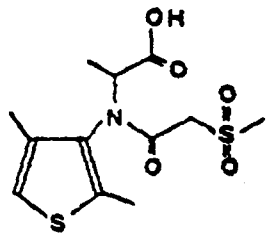
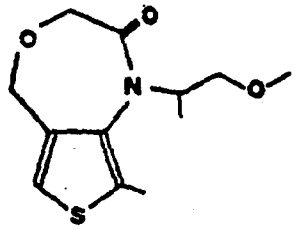
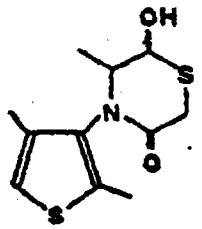
Table 9 : STRUCTURES OF THE METABOLITES OF SAN 582 H IN THE RAT (cont.)

metabolite no.	structure chemical name	empirical formul. molecular weight
M 16	 N-(2-hydroxymethyl-4-methyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-2-(methylsulfinyl)-acetamide	$C_{13}H_{21}NO_4S_2$ 319
M 17 *	 N-Acetyl-S-[2-[N'-(2,4-dimethyl-3-thienyl)-N'-(2-methoxy-1-methylethyl)amino]-2-oxoethyl]-cysteine	$C_{17}H_{26}N_2O_5S_2$ 402
M 18 *	 N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-2-methylthio-acetyl)-alanine	$C_{13}H_{19}NO_4S_2$ 317

* Synthesized reference standards available

Table 9 : STRUCTURES OF THE METABOLITES OF SAN 582 H IN THE RA

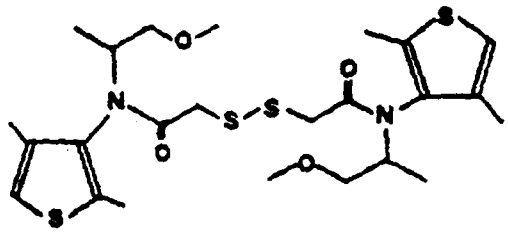
(cont.)

metabolite no.	structure chemical name	empirical formu molecular weig
M 19 *	 N-(2,4-dimethyl-3-thienyl)-N-[(methylsulfonyl)acetyl]-alanine	$C_{12}H_{17}NO_5S_2$ 319
M 20 *	 1,5-Dihydro-1-(2-methoxy-1-methylethyl)-8-methyl-thieno[3,4-f][4,1]oxazepin-2(3H)-one	$C_{12}H_{17}NO_3S$ 255
M 21 *	 4-(2,4-dimethyl-3-thienyl)-6-hydroxy-5-methyl-3-thiomorpholinone	$C_{11}H_{15}NO_2S_2$ 257

* Synthesized reference standards available

Table 9 : STRUCTURES OF THE METABOLITES OF SAN 582 H IN THE RAT

(cont.)

metabolite no.	structure chemical name	empirical formul molecular weight
M 22 *	 2,2'-Dithiobis[N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide]	$C_{24}H_{36}N_2O_4S_4$ 544

* Synthesized reference standards available

APPENDIX B

Distribution of Free and Conjugated
Metabolites in the
Urine, Feces, and Bile

(CBI Tables 12-20, CBI pp. 43-51)

TABLE B1. Composition of the Radioactivity in Urine (0-72 hr) After Administration of a Single Oral Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	0.2	---	0.2	0.6	---	0.6
1/7	0.2	0.2	0.4	0.5	1.1	1.6
2	2.4	0.9	3.3	3.6	2.8	6.4
3	0.2	---	0.2	0.1	---	0.1
4	0.2	---	0.2	---	---	---
5	0.1	0.1	0.2	0.3	0.8	1.1
6	0.1	---	0.1	0.3	---	0.3
8	---	---	---	---	---	---
9	<0.1	---	<0.1	0.1	---	0.1
10	<0.1	---	<0.1	0.2	---	0.2
11	---	<0.1	<0.1	<0.1	0.2	0.2
12	0.3	0.1	0.4	0.2	0.2	0.4
13	1.0	---	1.0	2.8	---	2.8
14	0.4	0.6	1.0	0.6	1.6	2.2
15	---	---	---	---	---	---
16	0.7	0.2	0.9	0.9	0.3	1.2
17	0.4	*2)	0.4	3.3	*2)	3.3
18	0.4	*	0.4	0.6	*	0.6
19	0.5	*	0.5	0.3	*	0.3
20	---	---	---	---	---	---
21	0.2	---	0.2	0.5	<0.1	0.5
22	---	---	---	---	---	---
Total of identified metabolites	7.3	2.1	9.4	14.9	7.0	21.9
Not definable radioactivity in the extracts 3)	7.8	1.6	9.4	7.0	2.2	9.2
Polar fractions 4)			13.6			13.0
Nonextractable radioactivity			0.4			0.5
Total excretion (0-3days)			32.8			44.6

TABLE B1 (Cont'd)

[1]After enzymatic hydrolysis with ß-glucuronidase and arylsulfatase.

[2]Values not determined.

[3]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[4]The polar fractions consist of a) aqueous phase of urine or bile remaining after XAD2 treatment and b) aqueous phase remaining after enzyme hydrolysis and extraction at pH 7 of XAD2-extracted metabolites.

Source: CBI Table 12, CBI p. 43.

TABLE B2. Composition of the Radioactivity in Urine (0-72 hr) After Administration of a Single Intravenous Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	0.2	---	0.2	0.5	---	0.5
1/7	0.3	---	0.3	3.9	---	3.9
2	2.3	0.2	2.5	3.2	0.2	3.4
3	0.1	---	0.1	0.2	---	0.2
4	0.5	---	0.5	0.5	---	0.5
5	0.2	---	0.2	0.6	<0.1	0.6
6	0.1	---	0.1	0.5	---	0.5
8	---	---	---	---	---	---
9	0.1	---	0.1	0.2	---	0.2
10	0.1	---	0.1	0.3	---	0.3
11	0.2	---	0.2	0.2	---	0.2
12	0.3	---	0.3	0.3	---	0.3
13	0.9	---	0.9	2.3	---	2.3
14	1.0	---	1.0	2.4	---	2.4
15	---	---	---	---	---	---
16	1.0	<0.1	1.0	1.0	<0.1	1.0
17	0.6	*2)	0.6	4.7	*2)	4.7
18	0.3	*	0.3	0.7	*	0.7
19	0.2	*	0.2	0.4	*	0.4
20	---	---	---	---	---	---
21	0.5	---	0.5	0.7	---	0.7
22	---	---	---	---	---	---
Total of identified metabolites	8.9	0.2	9.1	22.6	0.2	22.8
Not definable radioactivity in the extracts 3)	6.8	0.7	7.5	10.4	0.7	11.1
Polar fractions 4)			12.5			12.0
Nonextractable radioactivity			0.3			0.3
Total excretion (0-3 days)			29.4			46.2

TABLE B2 (Cont'd)

[1]After enzymatic hydrolysis with ß-glucuronidase and arylsulfatase.

[2]Values not determined.

[3]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[4]The polar fractions consist of a) aqueous phase of urine or bile remaining after XAD2 treatment and b) aqueous phase remaining after enzyme hydrolysis and extraction at pH 7 of XAD2-extracted metabolites.

Source: CBI Table 13, CBI p. 44.

TABLE B3. Composition of the Radioactivity in Urine (0-72 hr) After Administration of a Single Oral High Dose of 1000 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	---	---	---	0.2	---	0.2
1/7	0.7	5.0	5.7	0.2	5.1	5.3
2	1.4	3.4	4.8	3.2	3.2	6.4
3	0.3	---	0.3	0.2	---	0.2
4	---	---	---	1.1	---	1.1
5	0.7	6.6	7.3	0.3	6.3	6.6
6	---	---	---	---	---	---
8	---	---	---	0.2	---	0.2
9	---	---	---	<0.1	---	<0.1
10	---	---	---	0.2	---	0.2
11	---	0.4	0.4	---	0.3	0.3
12	0.4	---	0.4	---	---	---
13	0.5	---	0.5	1.3	---	1.3
14	0.4	2.3	2.7	0.5	3.7	4.2
15	---	---	---	---	---	---
16	0.9	1.8	2.7	0.6	1.0	1.6
17	2.1	*2)	2.1	4.0	*2)	4.0
18	0.9	*	0.9	0.9	*	0.9
19	0.5	*	0.5	0.6	*	0.6
20	---	---	---	---	---	---
21	0.2	---	0.2	0.3	0.4	0.7
22	---	---	---	---	---	---
Total of identified metabolites	9.0	19.5	28.5	13.8	20.0	33.8
Not definable radioactivity in the extracts 3)	11.2	4.9	16.1	8.4	3.3	11.7
Polar fractions 4)			15.0			14.7
Nonextractable radioactivity			0.1			0.1
Total excretion (0-3 days)			59.7			60.3

TABLE B3 (Cont'd)

[1)]After enzymatic hydrolysis with ß-glucuronidase and arylsulfatase.

[2)]Values not determined.

[3)]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[4)]The polar fractions consist of a) aqueous phase of urine or bile remaining after XAD2 treatment and b) aqueous phase remaining after enzyme hydrolysis and extraction at pH 7 of XAD2-extracted metabolites.

Source: CBI Table 14, CBI p. 45.

TABLE B4. Composition of the Radioactivity in Urine (0-72 hr) After Administration of Multiple Oral Low Doses Followed by a Single Oral Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	---	---	---	0.1	---	0.1
1/7	<0.1	0.4	0.4	0.6	2.0	2.6
2	2.7	0.9	3.6	6.1	3.7	9.8
3	0.1	---	0.1	0.1	---	0.1
4	---	---	---	---	---	---
5	0.1	0.2	0.3	0.4	0.7	1.1
6	---	---	---	---	<0.1	<0.1
8	---	---	---	---	<0.1	<0.1
9	---	---	---	---	---	---
10	---	---	---	<0.1	---	<0.1
11	---	0.1	0.1	---	0.2	0.2
12	0.2	0.2	0.4	0.3	0.4	0.7
13	0.2	---	0.2	2.2	---	2.2
14	0.1	0.7	0.8	0.8	1.6	2.4
15	---	---	---	---	0.1	0.1
16	0.9	0.4	1.3	1.5	0.5	2.0
17	0.4	*2)	0.4	3.1	*	3.1
18	0.6	*	0.6	0.2	*	0.2
19	0.3	*	0.3	1.1	*	1.1
20	---	---	---	---	---	---
21	---	---	---	0.3	---	0.3
22	---	---	---	---	---	---
Total of identified metabolites	5.3	2.9	8.5	16.8	9.2	26.0
Not definable radioactivity in the extracts 3)	8.7	2.3	11.0	9.6	3.0	12.6
Polar fractions 4)			12.4			11.2
Nonextractable radioactivity			0.4			0.4
Total excretion (0-3 days)			32.3			50.2

TABLE B4 (Cont'd)

[1)]After enzymatic hydrolysis with ß-glucuronidase and arylsulfatase.

[2)]Values not determined.

[3)]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[4)]The polar fractions consist of a) aqueous phase of urine or bile remaining after XAD2 treatment and b) aqueous phase remaining after enzyme hydrolysis and extraction at pH 7 of XAD2-extracted metabolites.

Source: CBI Table 15, CBI p. 46.

TABLE B5. Composition of the Radioactivity in Feces (0-72 hr) After Administration of a Single Oral Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	1.0	---	1.0	1.2	---	1.2
1/7	2.5	0.2	2.7	1.2	0.5	1.7
2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
3	0.6	---	0.6	0.3	---	0.3
4	---	---	---	---	---	---
5	0.4	---	0.4	0.3	---	0.3
6	0.7	0.1	0.8	0.2	0.1	0.3
8	0.2	---	0.2	---	---	---
9	---	---	---	---	---	---
10	0.7	---	0.7	0.2	---	0.2
11	0.3	---	0.3	0.2	---	0.2
12	---	---	---	---	---	---
13	1.5	---	1.5	1.8	---	1.8
14	1.4	---	1.4	1.7	---	1.7
15	---	0.1	0.1	---	---	---
16	2.8	0.1	2.9	3.0	0.2	3.2
17	0.9	*2)	0.9	0.2	*2)	0.2
18	0.5	*	0.5	0.4	*	0.4
19	0.5	*	0.5	0.3	*	0.3
20	0.5	---	0.5	0.3	---	0.3
21	0.4	0.1	0.5	0.2	0.1	0.3
22	0.7	---	0.7	0.1	---	0.1
Total of identified metabolites	15.6	0.6	16.2	11.6	0.9	12.5
Not definable radioactivity in the extracts 3)	11.7	1.0	12.7	11.9	1.5	13.4
Polar fractions 4)			15.1			7.6
Nonextractable radioactivity			10.2			11.3
Total excretion (0-3 days)			54.2			44.8

TABLE B5 (Cont'd)

[1)]After chemical hydrolysis with 1 N sodium hydroxide.

[2)]Values not determined.

[3)]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[4)]The polar fractions consist of the aqueous phase remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 16, CBI p. 47.

TABLE B6. Composition of the Radioactivity in Feces (0-72 hr) After Administration of a Single Intravenous Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	1.9	0.2	2.1	0.8	---	0.8
1/7	0.1	---	0.1	2.1	0.1	2.2
2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
3	0.3	0.2	0.5	0.3	0.1	0.4
4	---	---	---	---	---	---
5	0.4	---	0.4	0.2	0.1	0.3
6	0.2	---	0.2	0.3	---	0.3
8	---	---	---	---	---	---
9	0.3	---	0.3	---	---	---
10	0.1	---	0.1	0.1	---	0.1
11	1.3	0.3	1.6	0.2	0.3	0.5
12	---	---	---	---	---	---
13	---	---	---	---	---	---
14	1.0	<0.1	1.0	0.6	---	0.6
15	---	---	---	---	<0.1	<0.1
16	2.3	0.2	2.5	1.3	0.1	1.4
17	0.2	*2)	0.2	<0.1	*2)	<0.1
18	0.6	*	0.6	0.3	*	0.3
19	0.2	*	0.2	<0.1	*	<0.1
20	---	---	---	---	<0.1	<0.1
21	0.2	---	0.2	0.2	---	0.2
22	0.2	---	0.2	0.2	---	0.2
Total of identified metabolites	9.3	0.9	10.2	6.6	0.7	7.3
Not definable radioactivity in the extracts 3)	10.3	1.2	11.5	6.0	0.5	6.5
Polar fractions 4)			11.5			5.2
Nonextractable radioactivity			20.8			14.7
Total excretion (0-3 days)			54.0			33.7

TABLE B6 (Cont'd)

[1]After chemical hydrolysis with 1 N sodium hydroxide.

[2]Values not determined.

[3]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[4]The polar fractions consist of the aqueous phase remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 17, CBI p. 48.

TABLE B7. Composition of the Radioactivity in the Feces (0-72 hr) After Administration of a Single Oral High Dose of 1000 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	1.2	<0.1	1.2	1.3	0.1	1.4
1/7	0.6	<0.1	0.6	0.4	0.1	0.5
2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
3	0.1	0.1	0.2	0.1	0.1	0.2
4	---	---	---	---	---	---
5	0.3	---	0.3	0.1	0.1	0.2
6	0.3	<0.1	0.3	0.3	<0.1	0.3
8	---	---	---	---	<0.1	<0.1
9	---	---	---	---	---	---
10	0.2	---	0.2	0.1	---	0.1
11	---	0.1	0.1	---	0.3	0.3
12	---	---	---	---	---	---
13	0.3	---	0.3	0.1	---	0.1
14	0.2	---	0.2	0.1	---	0.1
15	---	<0.1	<0.1	---	<0.1	<0.1
16	1.9	0.1	2.0	0.9	0.2	1.1
17	0.1	*2)	0.1	<0.1	*2)	<0.1
18	0.3	*	0.3	0.1	*	0.1
19	0.5	*	0.5	<0.1	*	<0.1
20	---	<0.1	<0.1	---	<0.1	<0.1
21	0.3	---	0.3	0.2	---	0.2
22	1.0	<0.1	1.0	1.0	<0.1	1.0
Total of identified metabolites	7.3	0.3	7.6	4.7	0.9	5.6
Not definable radioactivity in the extracts 3)	8.3	0.4	8.7	5.5	0.8	6.3
Polar fractions 4)			6.9			7.3
Nonextractable radioactivity			5.7			5.1
Total excretion (0-3 days)			28.9			24.3

TABLE B7 (Cont'd)

[1]After chemical hydrolysis with 1 N sodium hydroxide.

[2]Values not determined.

[3]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[4]The polar fractions consist of the aqueous phase remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 18, CBI p. 49.

TABLE B8. Composition of the Radioactivity in Feces (0-72 hr) After Administration of Multiple Oral Low Doses Followed by a Single Oral Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	1.4	---	1.4	1.1	---	1.1
1/7	2.9	---	2.9	4.6	---	4.6
2	<0.1	<0.1	<0.1	<0.1	---	<0.1
3	0.4	0.1	0.5	0.2	0.1	0.3
4	---	---	---	---	---	---
5	0.5	---	0.5	0.2	0.1	0.3
6	0.3	---	0.3	0.6	---	0.6
8	---	---	---	---	---	---
9	---	---	---	---	---	---
10	---	---	---	0.2	---	0.2
11	---	0.1	0.1	---	---	---
12	---	---	---	---	---	---
13	---	---	---	1.1	---	1.1
14	2.0	---	2.0	0.9	---	0.9
15	---	0.1	0.1	---	---	---
16	3.7	0.1	3.8	1.3	---	1.3
17	0.2	*2)	0.2	0.2	*2)	0.2
18	0.5	*	0.5	0.3	*	0.3
19	0.8	*	0.8	0.3	*	0.3
20	---	---	---	---	---	---
21	---	---	---	---	---	---
22	---	---	---	---	---	---
Total of identified metabolites	12.7	0.4	13.1	11.0	0.2	11.2
Not definable radioactivity in the extracts 3)	17.3	1.3	18.6	8.5	1.3	9.8
Polar fractions 4)			14.6			6.6
Nonextractable radioactivity			12.1			9.5
Total excretion (0-3 days)			58.4			37.1

TABLE B8 (Cont'd)

[1)]After chemical hydrolysis with 1 N sodium hydroxide.

[2)]Values not determined.

[3)]The not definable radioactivity in the extracts consists of materials that could not be characterized as definite compounds by chromatographic methods.

[4)]The polar fractions consist of the aqueous phase remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 19, CBI p. 50.

TABLE B9. Composition of the Radioactivity in Bile (0-72 hr) After Administration of a Single Oral Low Dose of 10 mg [^{14}C]SAN 582H/kg (As Percent of Administered Dose)

Metabolite No:	Male rats			Female rats		
	free	conj.1)	total	free	conj.1)	total
a.i.	0.1	---	0.1	<0.1	---	<1.0
1/7	0.6	4.7	5.3	0.3	4.5	4.8
2	<0.1	0.3	0.3	<0.1	0.7	0.7
3	---	---	---	---	---	---
4	1.1	0.7	1.8	0.8	0.5	1.3
5	0.3	5.9	6.2	0.1	2.0	2.1
6	---	---	---	---	---	---
8	0.1	0.5	0.6	<0.1	0.7	0.7
9	<0.1	---	<0.1	---	---	---
10	0.1	---	0.1	0.1	---	0.1
11	---	0.3	0.3	---	0.2	0.2
12	---	---	---	---	---	---
13	---	---	---	---	---	---
14	---	0.4	0.4	---	0.1	0.1
15	---	---	---	---	0.2	0.2
16	0.1	0.7	0.8	0.1	0.3	0.4
17	3.5	*2)	3.5	3.9	*2)	3.9
18	0.2	*	0.2	0.1	*	0.1
19	0.4	*	0.4	0.2	*	0.2
20	---	---	---	---	---	---
21	1.2	0.9	2.1	1.0	0.8	1.8
22	---	---	---	---	---	---
Total of identified metabolites	7.7	14.4	22.1	6.6	10.0	16.6
Not definable radioactivity in the extracts 3)	7.8	2.7	10.5	4.4	1.4	5.8
Polar fractions 4)			48.0			51.3
Nonextractable radioactivity			1.4			1.2
Total excretion (0-3 days)			82.0			74.9

TABLE B9 (Cont'd)

[1]After chemical hydrolysis with 1 N sodium hydroxide.

[2]Values not determined.

[3]The not definable radioactivity in the extracts consists of Materials that could not be characterized as definite compounds by chromatographic methods.

[4]The polar fractions consist of the aqueous phase remaining after chemical hydrolysis and extraction at pH 7.

Source: CBI Table 20, CBI p. 51.