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Table 4 Tissue concentration of intact drug and its metabolite (Ro 40-7591) after a single oral administration of ¹⁴C-Ro 40-7592 to female rats at a dose of 20 mg/kg

		LIVER			KIDNEY		
		0.5hr	2hr	7hr	0.5hr	2hr	7hr
Ro 40-7592	µg eq./g tissue	1.51 ± 0.28	0.63 ± 0.09	N.D.	1.55 ± 0.73	0.21 ± 0.08	N.D.
	% of ¹⁴ C-radioactivity	7.71 ± 1.32	4.04 ± 0.19	N.D.	9.96 ± 5.49	1.58 ± 0.34	N.D.
Ro 40-7591	µg eq./g tissue	0.10 ± 0.00	0.07 ± 0.02	N.D.	0.05 ± 0.02	N.D.	N.D.
	% of ¹⁴ C-radioactivity	0.55 ± 0.10	0.49 ± 0.12	N.D.	0.35 ± 0.12	N.D.	N.D.
Total	% of ¹⁴ C-radioactivity	8.26 ± 1.39	4.54 ± 0.27	N.D.	10.34 ± 5.62	1.58 ± 0.34	N.D.

		SMALL INTESTINE			BRAIN		
		0.5hr	2hr	7hr	0.5hr	2hr	7hr
Ro 40-7592	µg eq./g tissue	1.81 ± 0.33	1.71 ± 0.36	0.06 ± 0.02	0.31 ± 0.20	N.D.	N.D.
	% of ¹⁴ C-radioactivity	5.66 ± 0.94	2.42 ± 0.69	0.99 ± 0.52	108.39 ± 74.34	N.D.	N.D.
Ro 40-7591	µg eq./g tissue	0.06 ± 0.01	0.15 ± 0.05	N.D.	N.D.	N.D.	N.D.
	% of ¹⁴ C-radioactivity	0.20 ± 0.04	0.23 ± 0.06	N.D.	N.D.	N.D.	N.D.
Total	% of ¹⁴ C-radioactivity	5.86 ± 0.94	2.64 ± 0.75	0.99 ± 0.52	108.39 ± 74.34	N.D.	N.D.

		BLOOD		
		0.5hr	2hr	7hr
Ro 40-7592	µg eq./g tissue	7.94 ± 3.52	4.83 ± 1.09	0.13 ± 0.05
	% of ¹⁴ C-radioactivity	63.79 ± 12.85	66.68 ± 5.90	21.97 ± 4.89
Ro 40-7591	µg eq./g tissue	0.33 ± 0.08	0.63 ± 0.17	0.11 ± 0.02
	% of ¹⁴ C-radioactivity	3.15 ± 0.79	8.87 ± 1.71	19.87 ± 2.67
Total	% of ¹⁴ C-radioactivity	67.20 ± 13.44	75.54 ± 4.58	41.84 ± 5.03

N.D.: Not detected
Each value represents the mean ± S.E. (n=4).

Table 5 Ex vivo binding of radioactive substances to plasma protein after a single oral administration of ¹⁴C-Ro 40-7592 to rats at a dose of 20 mg/kg

Time (hr)	Protein binding (%)			
	MALE		Female	
0.5	99.61 ± 0.04	0.04	99.74 ± 0.04	0.04
2	99.74 ± 0.08	0.08	99.44 ± 0.29	0.29
7	97.17 ± 1.00	1.00	96.07 ± 1.05	1.05
24	N.D.		N.D.	

N.D.: Not detected
Each value represents the mean ± S.E. (n=4).

Table 6 Ex vivo distribution in blood cells of ¹⁴C-radioactivity after a single oral administration of ¹⁴C-Ro 40-7592 to rats at a dose of 20 mg/kg

Time (hr)	Distribution in blood cells (%)			
	MALE		Female	
0.5	4.50 ± 0.63	0.63	4.14 ± 1.42	1.42
2	1.82 ± 0.72	0.72	13.81 ± 3.33	3.33
7	2.80 ± 1.86	1.86	12.18 ± 1.29	1.29
24	77.64 ± 1.01	1.01	N.D.	

N.D.: Not detected
Each value represents the mean ± S.E. (n=4).

D.5. [¹⁴C]-Tolcapone Distribution: Whole-Body Autoradiography (WBAR) in male and female albino rats after oral administration
Research Report #: B-165,037 Volume: 80

Summary:

The distribution of a single oral dose of 20 mg/kg [¹⁴C]-tolcapone was evaluated by WBAR in albino rats at 0.5, 5, 24 and 72 hrs (1 M, 1 F per time point). Plasma, urine and feces were analyzed by LSC.

Results were consistent with other distribution studies. Absorption was rapid as the highest blood and tissue levels were detected at 0.5 hrs. Distribution was limited to organs of absorption (gut) and elimination (liver, kidney, bile ducts), and lungs. Levels were reduced slightly by 5 hrs. By 72 hrs postdose, radioactivity was detected only in gut mucosa and contents, kidney, thyroid and white fat. In animals that were retained to 72 hrs, elimination was nearly quantitative as reflected by high percentages of radioactivity in urine and feces (84-93%), and low levels of radioactivity in the carcasses.

Blood:plasma ratios of radioactivity in terminal samples were approximately 0.6 in all animals.

D.6. [¹⁴C]-Tolcapone Distribution: Whole-Body Autoradiography (WBAR) in pregnant pigmented rats after oral administration
Research Report #: B-165,031 Volume: 80

Summary:

[¹⁴C]-Tolcapone was administered by gavage to pregnant pigmented rats as either a single dose on day 15 of gestation, or once daily on days 13-15 of gestation. The intended dose was 50 mg/kg; because of an inadequate supply of material, the dose was reduced to 40 mg/kg on days 14 and 15. One rat was used at each time point (total of 4 rats for the study); the rat used at 72 hrs was not pregnant. Distribution was evaluated at 1 hr after the single dose, or at 24, 48, and 72 hr after the last multiple dose. One side of the animal was analyzed by WBAR. Liver, kidney, muscle and brain were dissected from the remaining side and analyzed by LSC.

The results were generally consistent with those of previous distribution studies. In dams, absorption was rapid (high blood levels at 1 hr), and distribution was limited. High levels were present in the g.i. tract and bile ducts, and tissue levels approached blood levels only in the liver, kidney, and lung. Placental transfer was also limited, as only low fetal levels were detected. The drug was not significantly retained at 24-48 hrs after multiple dosing; intermediate levels of radioactivity were present in gut, and low levels remained in liver, kidney, bile ducts, and uterus.

Tolcapone was not retained in melanin-containing organs.

D.7. Placental transfer of [¹⁴C]-tolcapone into rat fetuses after oral administration

Research Report #: J-146,479

Volume: 82

Summary:

The placental transfer of [¹⁴C]-tolcapone was studied at 0.5 and 24 hr following oral administration of 20 mg/kg to rats on days 13 and 19 of gestation. Three to four dams were used at each time point, and five fetuses per dam were examined.

On day 13, the amount of radioactivity in fetuses (whole tissue) was less than 3% of maternal blood concentrations. At day 19, relative concentrations of radiolabel were higher (approximately 10% of maternal blood concentration) than those on day 13, suggesting an increase in distribution to the fetus. However, expressed as a fraction of the radioactivity administered, the percent of dose reaching the fetus is quite low (0.04).

Table 1 Tissue concentrations of radioactivity after a single oral administration of ¹⁴C-Ro 40-7592 to 13th-day or 19th-day pregnant rats (dose : 20 mg/kg)

Tissue	Concentration of radioactivity (µg eq/g or ml)			
	13th-day of pregnancy		19th-day of pregnancy	
	0.5 hr	24 hr	0.5 hr	24 hr
Blood	20.73 ± 8.59	0.06 ± 0.02	13.78 ± 7.06	0.09 ± 0.01
Brain	0.42 ± 0.14	N.S.	0.34 ± 0.13	N.S.
Liver	33.50 ± 11.12	0.33 ± 0.08	20.93 ± 10.25	0.64 ± 0.12
S. Intestine	50.74 ± 26.7	1.27 ± 0.53	21.93 ± 10.35	3.14 ± 0.56
Kidney	26.74 ± 8.60	0.63 ± 0.17	15.36 ± 5.74	1.01 ± 0.26
Placenta	4.59 ± 1.42	0.09 ± 0.03	3.22 ± 1.41	0.22 ± 0.08
Amniotic fluid	0.40 ± 0.10	N.M.	0.16 ± 0.08	0.79 ± 0.24
Fetus (whole)	0.44 ± 0.14	N.M.	N.S.	N.S.
Fetus (head)	N.S.	N.S.	1.13 ± 0.47	0.20 ± 0.07
Fetus (body)	N.S.	N.S.	1.31 ± 0.55	0.77 ± 0.21

Fetus	Distribution of radioactivity (% of dose/one body)			
	0.001 ± 0.000	N.M.	0.04 ± 0.02	0.03 ± 0.01

Each value represents the mean ± S.D. of 3 or 4 rats.

N.S. : No sample

N.M. : Not measurable

D.8. *In vitro* binding of tolcapone human, rat and dog plasma proteins

Research Report #: B-158,757

Volume: 80

Summary:

The binding of tolcapone to proteins in human, rat, and dog plasma, and potential for displacement by other compounds, was determined by equilibrium dialysis of ¹⁴C-tolcapone (up to 300 µg/ml) at 37°C (pH 7.4). Blood:plasma partitioning was also assessed.

Tolcapone binds strongly (>99.9%) to plasma proteins of all test species. Binding was saturable and dependent on albumin concentration. The primary binding sites were the warfarin and diazepam sites of albumin. Tolcapone was competitively displaced by both drugs, and also by salicylic acid, ibuprofen, and phenylbutazone. Tolcapone binding was non-competitively inhibited by fatty acids.

Only trace amounts of tolcapone are taken up into red cells.

Because of the high binding of tolcapone to plasma proteins, reductions in protein binding due to displacement by other highly bound drugs or in conditions of reduced plasma protein concentrations would be expected to cause large increases in the free drug fraction. The relative importance of this consideration is reduced because tolcapone is rapidly cleared from plasma.

D.9. Plasma Protein Binding: *In vitro* interaction with digitoxin, phenytoin, tolbutamide and warfarin in human plasma.

Research Report #: B-158,764

Volume: 83

Summary:

The displacement of tolcapone (10-50 µg/ml) from human plasma protein binding sites by other highly-bound radiolabeled drugs was studied *in vitro* by equilibrium dialysis at pH 7.4, 37°C. The highest test concentration of tolcapone is twice that seen in a human subject receiving the maximal oral dose of 800 mg, t.i.d. The test concentrations of the other compounds were in the therapeutic to toxic range.

Tolcapone only slightly displaced phenytoin (< 5% increase in free fraction) and tolbutamide (<12 % increase in free fraction) from plasma protein binding sites. Although tolcapone and warfarin were suggested to bind to the same binding site on domain I of human serum albumin, tolcapone did not increase the free fraction of warfarin (Sponsor Tables 1-4).

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Table 1

In vitro interaction of tolcapone on the binding of digitoxin in human plasma ¹⁾

digitoxin total drug ²⁾ (ng/ml)	control			with tolcapone 50 µg/ml				
	% free apparent	SD	% free ³⁾ unchanged	n ⁴⁾	% free apparent	SD	% free ³⁾ unchanged	n
9.0	4.33	0.08	n.d.	3	4.39	0.03	n.d.	3
27.0	4.29	0.06	2.91	4	4.37	0.07	2.78	4

Table 2

In vitro interaction of tolcapone on the binding of phenytoin in human plasma ¹⁾

phenytoin total drug ²⁾ (µg/ml)	control		with tolcapone 10 µg/ml		with tolcapone 50 µg/ml	
	% free	SD	% free	SD	% free	SD
4.0	15.24	0.12	15.35	0.07	15.94	0.03
7.9	15.57	0.08	15.79	0.10	16.14	0.01
11.8	15.69	0.08	15.80	0.15	16.27	0.05
15.6	15.79	0.13	15.97	0.13	16.33	0.05
38.7	16.56	0.21	16.65	0.02	17.13	0.11

Table 3

In vitro interaction of tolcapone on the binding of tolbutamide in human plasma ¹⁾

tolbutamide total drug ²⁾ (µg/ml)	control			with tolcapone 10 µg/ml			with tolcapone 50 µg/ml		
	% free	SD	n	% free	SD	n	% free	SD	n
24.5	BLC	-	3	n.d.	-	-	BLC	-	3
47.6	2.60	0.07	3	2.72	0.05	2	2.90	0.20	3
96.1	3.16	0.06	3	3.33	0.14	2	3.54	0.18	s 3
192	4.80	0.09	3	n.d.	-	-	5.54	0.16	s 3

Table 4

In vitro interaction of tolcapone on the binding of warfarin in human plasma ¹⁾

warfarin total drug ²⁾ (µg/ml)	control			with tolcapone 50 µg/ml		
	% free apparent	SD	% free ³⁾ unchanged	% free apparent	SD	% free ³⁾ unchanged
0.5	1.56	0.01		1.57	0.01	
0.9	1.56	0.01		1.57	0.01	
1.8	1.58	0.02		1.59	0.01	
3.6	1.58	0.01		1.59	0.01	
7.2	1.59	0.01	1.17	1.60	0.01	1.19
MEAN ± SD	1.58	0.01		1.59	0.02	

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D.10. *In vitro* metabolism of tolcapone by rat, dog and human liver microsomes
Research Report #: B-163,950

Volume:

Summary:

Three potential metabolic pathways for tolcapone were evaluated *in vitro* with rat, dog and human liver microsomes. The cytochrome P450 pathways involved in metabolism were identified through the use of specific inhibitors or inducers of the pathways:

Results:

1. Oxidation of the 4'-methyl group to the alcohol (Ro 47-1868):

In human microsomes, this pathway is putatively mediated by cytochrome 3A4 (inhibited by midazolam). In rat microsomes, cytochromes 3A1 (induced by erythromycin) and 2B (induced by phenobarbital) appear to be involved.

Kinetic parameters were derived:

	<u>K_m</u>	<u>V_{max}</u>
human	7 μ M	287 pmol/min.mg protein
rat	9 μ M	2600 "
dog - high affinity	0.8 μ M	44.5 "
low affinity	56 μ M	110 "

These data suggest that although the affinity of rat and human cytochromes for tolcapone are similar, the capacity of the rat to mediate this reaction is much greater. In contrast, the dog has a relatively low capacity for this pathway.

2. Nitro Reduction:

This pathway was evaluated (reported) only with rat liver microsomes. The amounts of tolcapone present in incubates under reducing conditions declined, and three novel peaks appeared. However, none of the peaks were identified.

3. Glucuronidation

This reaction was characterized with human liver microsomes (not purified enzyme). The kinetic parameters were:

$$\begin{aligned}K_m &= 75 \mu\text{M} \\V_{\text{max}} &= 1560 \text{ pmol/min.mg protein}\end{aligned}$$

Compared to the oxidative pathway, the conjugation reaction occurs at a higher rate despite the apparently lower affinity of the UDPGA glucuronyltransferase for tolcapone.

D.11. Plasma levels of tolcapone glucuronide in rats and dogs during oral toxicology studies
 Research Report #: B-161,420 Volume: 79

Summary:

The plasma levels of tolcapone glucuronide (Ro 61-1448), a significant plasma metabolite, were determined following oral administration of tolcapone to rats (20 and 200 mg/kg, p.o.) and dogs (10 and 80 mg/kg, p.o.) alone and in combination with L-DOPA. In rats, sampling was conducted on days 0 and 70 of the 13-week toxicity study at 1-24 hrs after drug treatment in one rat/sex/dose. In dogs, sampling was conducted on days 0 and 90 of the 13-week toxicity study at 1-24 hrs after drug treatment in one dog/sex/dose.

Increases in the level of metabolite were slightly less than dose-proportional. Analysis of plasma concentration-time profiles suggested that approximately 10% of the oral dose is converted to the glucuronide. Peak levels of metabolite were achieved at 1-3 hrs postdose in rats (Sponsor Table 3) and 2-7 hrs postdose in dogs (Sponsor Table 4). The larger tmax values tended to occur at higher doses, probably a result of slower absorption. No gender differences were evident. The coadministration of L-DOPA did not affect glucuronide formation.

Table 3 Maximum plasma concentrations (µg/ml) and AUC (h·µg/ml) of Ro 61-1448 and maximum concentration ratio of Ro 40-7592/61-1448 in rats

Dosage group	Day	Male rats				Female rats		
		Ro 61-1448		Ratio 40-7592/ 61-1448	Ro 61-1448		Ratio 40-7592/ 61-1448	
		Cmax	AUC*		Cmax	AUC*		
A	0	2.7	6.2	2.7	3.0	4.1	2.9	
	70	3.3	15	2.6	4.6	8.4	2.5	
B	0	29.2	108	1.8	29.1	82	2.6	
	70	26.5	79	2.1	34.1	88	2.6	
C	0	4.4	11	2.5	3.8	7.7	5.2	
	70	2.5	8.2	2.8	3.7	8.7	3.9	
D	0	23.8	86	2.5	22.5	64	3.0	
	70	14.7	59	2.4	26.7	60	3.7	

A: 20 mg/kg/day Ro 40-7592

B: 200 mg/kg/day Ro 40-7592

C: 20 mg/kg/day Ro 40-7592, 80 mg/kg/day L-Dopa, 20 mg/kg/day benserazide

D: 200 mg/kg/day Ro 40-7592, 80 mg/kg/day L-Dopa, 20 mg/kg/day benserazide

*: AUC, from 0 to 5 hours

Table 4 Maximum plasma concentrations (µg/ml) and AUC (h·µg/ml) of Ro 61-1448 and maximum concentration ratio of Ro 40-7592 / 61-1448 in dogs

Dosage group	Day	Male dogs			Female dogs		
		Ro 61-1448		Ratio 40-7592/ 61-1448	Ro 61-1448		Ratio 40-7592/ 61-1448
		Cmax	AUC		Cmax	AUC	
A	0	4.24	14 (7)	4.40	5.92	28 (7)	4.95
	90	5.11	20 (7)	4.70	6.23	17 (7)	4.47
B	0	6.24	25 (7)	4.05	18.5	57 (7)	2.88
			52 (24)			215 (24)	
		30.6	75 (7)	3.49		89 (7)	
		356 (24)		323 (24)			
C	0	1.06	4.0 (7)	6.35	1.34	3.5 (5)	4.85
	90	2.00	9.1 (7)	6.95	3.22	12 (7)	6.84
D	0	3.32	15 (7)	5.89	9.61	33 (7)	7.98
	90	30.3	100 (7)	3.63	7.61	58 (24)	4.79
			128 (24)			33 (7)	
						101 (24)	

A: 10 mg/kg/day Ro 40-7592

B: 80 mg/kg/day Ro 40-7592

C: 10 mg/kg/day Ro 40-7592, 80 mg/kg/day L-Dopa, 20 mg/kg/day benserazide

D: 80 mg/kg/day Ro 40-7592, 80 mg/kg/day L-Dopa, 20 mg/kg/day benserazide

D.12. Plasma levels of tolcapone glucuronide, tolcapone and 3-O-methyltolcapone after intravenous administration of tolcapone glucuronide to rats

Research Report #: B-161,421

Volume: 79

Summary:

This study was to determine the fate of tolcapone glucuronide following intravenous administration of 5 mg/kg to 3 male rats.

Peak levels of the glucuronide (26-27 $\mu\text{g/ml}$) declined rapidly with an elimination half-life of 0.5-0.6 hrs. Plasma clearance was 6-9 ml/min/kg. Like the parent compound, the Vd of the glucuronide metabolite was low (0.1-0.2 l/kg).

No free tolcapone was detected in plasma, but some 3-O-methyltolcapone was found (0.13-0.21 $\mu\text{g/ml}$). This suggests that any of the low level tolcapone formed by hydrolysis of the glucuronide is rapidly converted to the 3-O-methyl metabolite.

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D.13. Plasma metabolites of tolcapone after oral administration to humans, dogs and rats

Research Report #: B-161,433

Volume: 79

Summary:

Plasma metabolites of [¹⁴C]-tolcapone were determined by HPLC with radiometric detection at 0.5-24 hrs after the administration of a single oral dose to humans (200 mg; n=6M), dogs (5 mg/kg; n=2M) and rats (5 mg/kg; n=4M).

In all three species, unchanged parent was the largest radioactive fraction. In humans and dogs, the 3-O-glucuronide was the most abundant metabolite. In rats, the primary alcohol formed by oxidation of the methyl group was the major metabolite present. Oxidation appears to occur to a limited extent in humans, but not in dogs. The absence of this pathway in dogs raises an issue of interpretation of the toxicology data since metabolic activation may be involved in the renal toxicity of tolcapone.

The distribution of the major metabolites is summarized in the Table. The peak identification, nomenclature, and quantitation are from a subsequent study update (Report # 161,439):

Structure	Description	% dose of radioactivity (Peak Identification)		
		Human (2 hr)	Rat (0.5 hr)	Dog (2 hr)

Human:

At 2 hrs post-treatment, two major and four minor drug-related peaks appeared, five of which were tentatively identified:

- HP1: the primary alcohol (oxidation product), Ro 47-1868
- HP2: the glucuronide conjugate, Ro 61-1448
- HP3: the acid metabolite (secondary oxidation product), Ro 47-1669
- HP4: tolcapone
- HP5: 3-O-methyltolcapone

The chromatograms of samples collected at 2, 4, and 12 hrs demonstrate that the parent compound generates the largest peak the early time points (Sponsor Figure 3). At 2 hrs, tolcapone accounts for approximately 60% of radioactivity in plasma, and the glucuronide accounts for about 19%. By 12 hrs, the 3-O-methyl metabolite peak is the largest. Peaks corresponding to the alcohol metabolite suggest that the oxidative pathway is relatively minor *in vivo*, although the secondary oxidation product is detectable up to 12 hrs after administration. Also at 12 hrs, a peak eluting shortly after the glucuronide becomes evident. This was tentatively identified as the N-acetylamino reduction product, Ro 48-2485.

Rat:

Plasma metabolites in the rat were determined only at 0.5 hr post-treatment. Four major and four minor peaks were evident (Sponsor Figure 7):

- RP1: alcohol metabolite, Ro 47-1868
- RP2: glucuronide conjugate
- RP3: N-acetylamino metabolite, Ro 48-2485
- RP4: acid metabolite, 47-1669
- RP5: tolcapone
- RP6: 3-O-methyltolcapone, Ro 40-7591

The parent compound is the predominant peak and accounts for approximately 50% of radioactivity in plasma. The primary alcohol (14.1%), 3-O-methyl metabolite (10.4%), and glucuronide conjugate (7.6%) were also present in measurable amounts.

Dog:

At 2 hrs post-treatment, two major and one minor drug-related peaks were tentatively identified (Sponsor Figure 5):

- DP2: the glucuronide conjugate, Ro 61-1448
- DP4: tolcapone
- DP5: 3-O-methyltolcapone, Ro 40-7591

At 2 hrs, tolcapone accounts for approximately 48% of radioactivity in plasma, and the glucuronide accounts for about 25%. At 4 hrs, the glucuronide is the major peak, and tolcapone and 3-O-methyltolcapone were present in similar amounts. At 24 hrs, the 3-O-methyl metabolite peak is the largest. Peaks corresponding to the alcohol and acid oxidative metabolites were virtually undetectable.

HUMAN PLASMA CHROMATOGRAMS

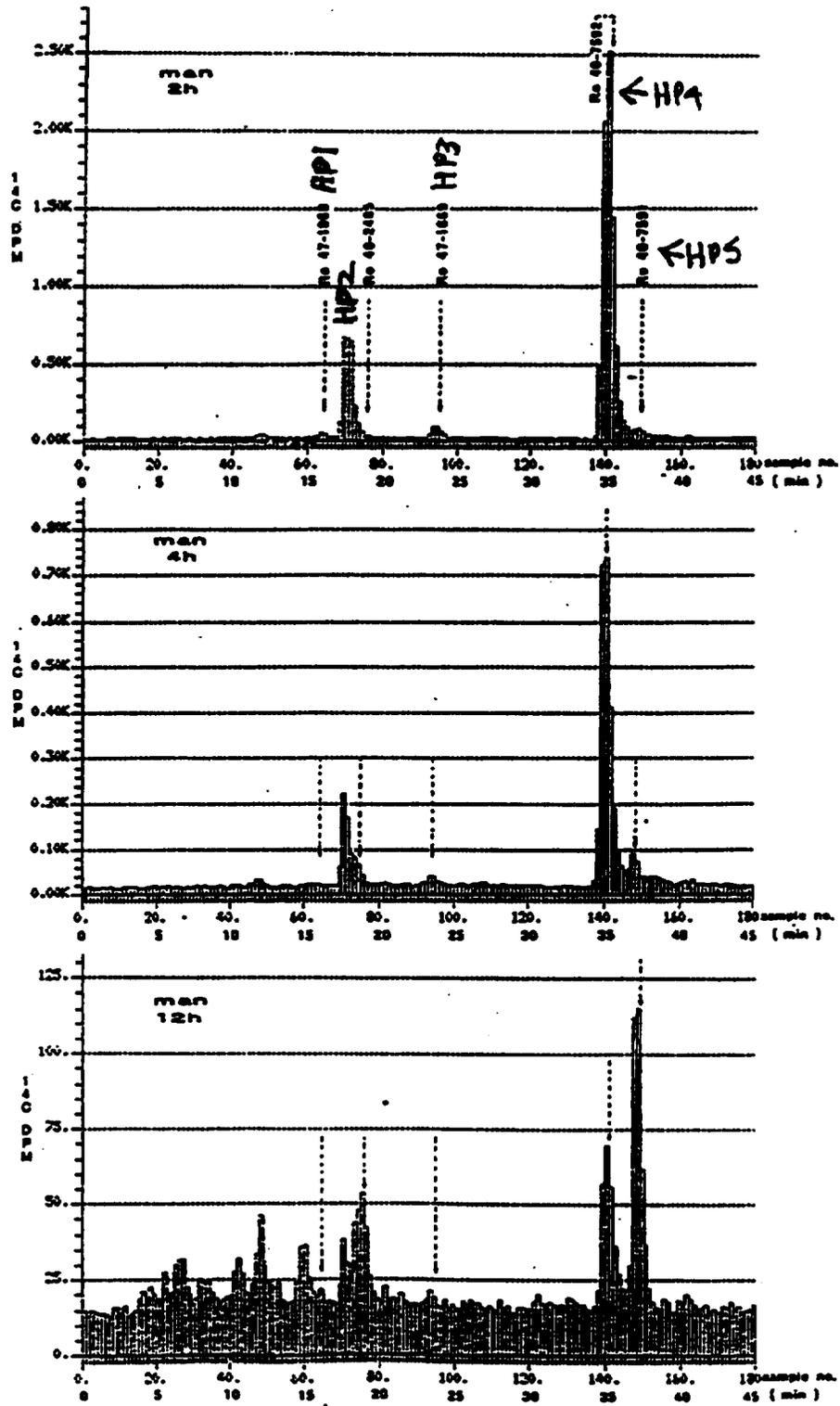
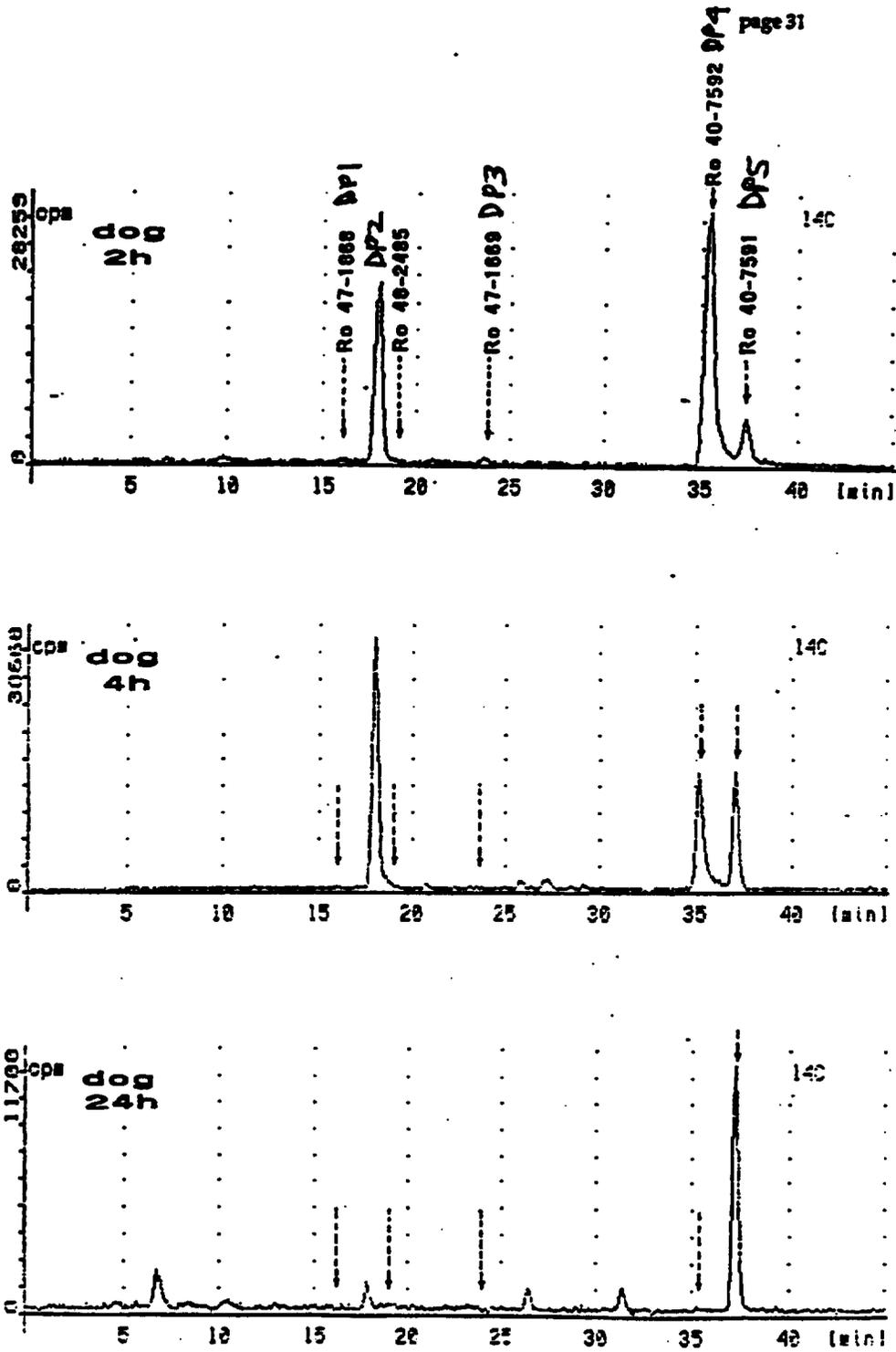


Figure 3

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DOG PLASMA CHROMATOGRAMS



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Figure 5

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HUMAN, DOG & RAT PLASMA CHROMATOGRAMS

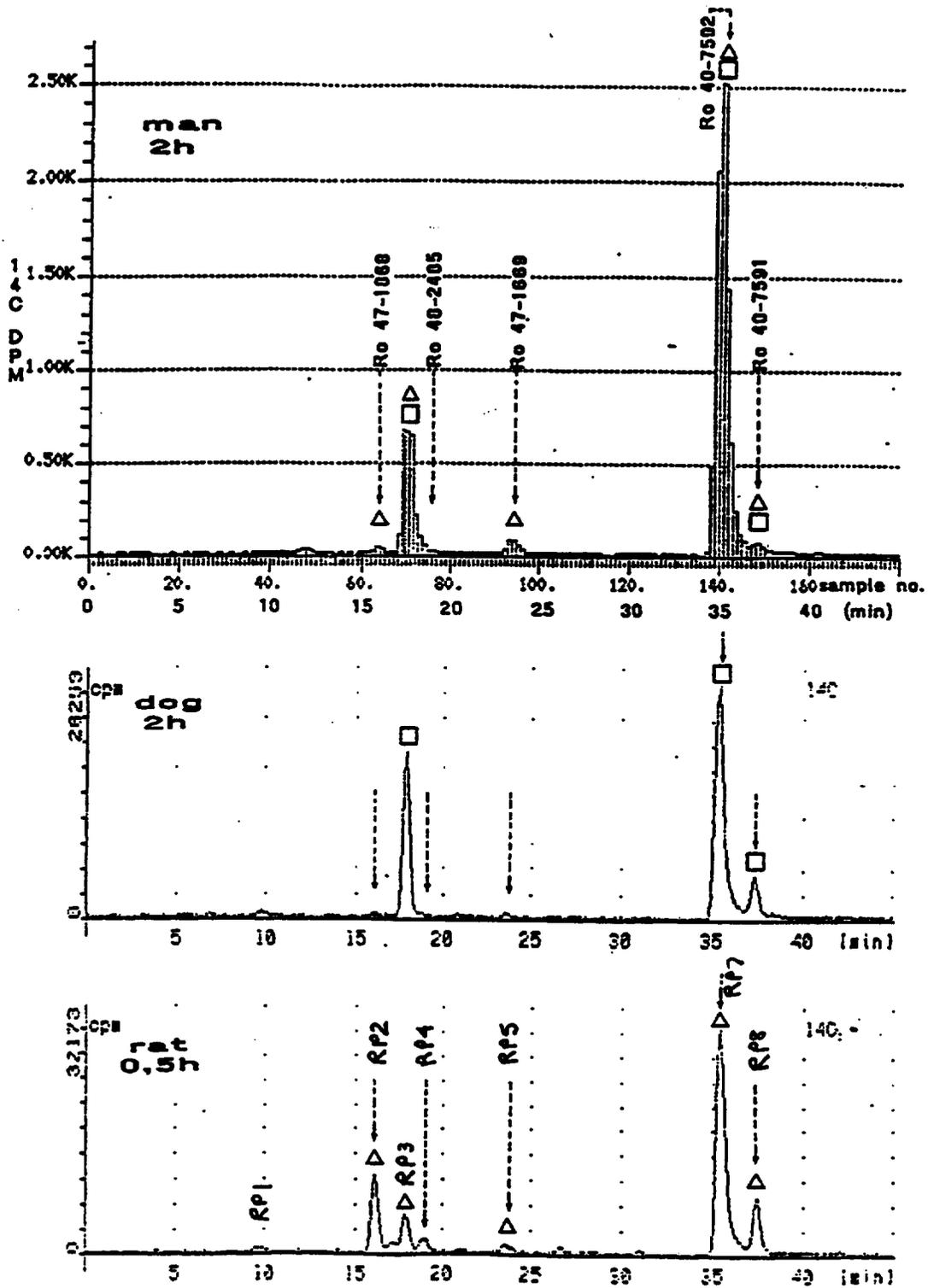


Figure 7

Summary:

Urinary metabolites of [¹⁴C]-tolcapone were determined by HPLC with radiometric detection at 0-72 hrs after the administration of a single oral dose to humans (200 mg; n=6M), dogs (5 mg/kg; n=2M) and rats (5 mg/kg; n=4M).

The relative amounts of urinary radioactivity recovered in 0-72 hrs were as follows:

	Urinary Radioactivity Recovery (0-72 hr)	
	% dose	% total urinary excretion
rat	32.4	98.9
dog	14.3	97.9
human	49.8	87.9

The number and amounts of identified metabolites in urine, expressed as percent of total radioactivity in urine and as percent of dose, were as follows:

	# metabolites	% urinary label	% dose
rat	11	75	24
dog	6	63	9
human	5	50	25

The quantitative distribution (Sponsor Tables 3, 4 & 6), structures (Sponsor Figures 3, 5 & 7), and chromatographic patterns (Sponsor Figures 2, 4 & 6) of urinary metabolites are shown in the following pages. The sponsor has a statement that parent drug is excreted unchanged in minor (rat) or trace (human, dog) amounts. The tabled data do not reflect this. Apparently, unidentified metabolites constitutes a large fraction of urinary radioactivity.

The major metabolites were tentatively identified as conjugates. Humans formed mainly glucuronide conjugates, whereas rat and dog formed mainly sulfate conjugates. For phase I reactions, oxidation is the primary route of metabolism in rat, and reduction is the primary route in humans and dogs.

The sponsor suggests that renal metabolism, specifically sulfoconjugation of the primary alcohol, is involved in the renal toxicity of tolcapone in rats. This hypothesis is based on the finding that the primary alcohol is a major plasma metabolite in rats, and only low/trace amounts are present in humans and dogs.

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- a) designations refer to Figure 3
- b) values refer to radioactivity in column charts
- c) values refer to radioactivity in urine, taking into account the total recovery of radioactivity during work-up and HPLC, i.e. 91.0 %
- d) values refer to radioactivity administered, taking into account the total recovery of radioactivity in 0-72 hours p.a. urine, i.e. 68.8 %

Tab. 3

Quantitative distribution of radioactivity in the HPLC-chromatograms of the metabolites in human urine, as shown in Figure 3

Integration intervals ^{a)}	Drug related peak or reference compound ^{b)}	Radioactivity		
		% of dose ^{b)}	% of urine ^{c)}	% of dose ^{d)}
A		21.5	30.1	8.1
B		3.0	2.7	1.4
C	No 01-0402	25.4	14.0	7.8
D		9.8	6.9	4.4
E	HU1	0.2	0.4	0.3
F	HU2	0.2	0.7	0.4
G	No 01-1000	28.0	25.4	12.7
H	No 01-1000	1.3	1.8	0.8
I		2.6	2.1	2.8
J		2.6	2.3	1.4
K	No 01-1000	2.4	2.2	1.3
L		0.3	0.5	0.3
M	No 01-0702	1.5	1.4	0.7
N		2.5	1.5	0.7
O	HU3	1.5	1.2	0.5
P	No 01-0702	0.9	0.8	0.4
Q	No 01-0701	0.9	0.8	0.4
R	No 01-1000	0.5	0.5	0.2
S	No 01-0702	0.5	0.5	0.2
T		1.8	0.9	0.5
U		0.0	0.0	0.0
A-U		100.0	91.0	68.8

alcohol
methyl
acid
sulfonic acid group

Tab. 4

Quantitative distribution of radioactivity in the HPLC-chromatograms of the metabolites in rat urine, as shown in Figure 4

RAT

- a) designations refer to Figure 4
- b) values refer to radioactivity in column charts
- c) values refer to radioactivity in urine, taking into account the total recovery of radioactivity during work-up and HPLC, i.e. 89.9 %
- d) values refer to radioactivity administered, taking into account the total recovery of radioactivity in 0-72 hours p.a. urine, i.e. 82.4 %

Integration intervals ^{a)}	Drug related peak or reference compound ^{b)}	Radioactivity		
		% of dose ^{b)}	% of urine ^{c)}	% of dose ^{d)}
A		1.3	1.3	0.4
B	HU1	4.5	2.8	1.9
C	No 01-0402	4.4	4.8	1.9
D		1.5	1.5	0.4
E		0.6	0.5	0.2
F	No 01-1000	0.7	0.6	0.2
G	HU2	2.1	1.9	0.6
H	No 01-1000	9.9	6.9	3.6
I	HU3	22.4	20.2	10.2
J		4.5	3.9	1.5
K	No 01-1000	16.6	16.8	8.4
L	HU4	2.5	2.2	0.7
M	HU7	1.6	0.9	0.4
N		1.8	0.9	0.4
O	HU5	2.2	2.0	0.6
Q	No 01-0701	0.9	0.8	0.3
R	HU6, HU9	4.3	3.9	1.5
S	No 01-0702	0.6	0.5	0.2
T		2.5	2.1	0.8
U	HU11	1.0	2.7	1.0
V		0.1	0.1	0.0
A-V		100.0	89.9	82.4

sulfonic acid group

Tab. 5

Quantitative distribution of radioactivity in the HPLC-chromatograms of the metabolites in dog urine, as shown in Figure 5

DOG

- a) designations refer to Figure 5
- b) values refer to radioactivity in column charts
- c) values refer to radioactivity in urine, taking into account the total recovery of radioactivity during work-up and HPLC, i.e. 97.9 %
- d) values refer to radioactivity administered, taking into account the total recovery of radioactivity in 0-72 hours p.a. urine, i.e. 84.5 %

Integration intervals ^{a)}	Drug related peak or reference compound ^{b)}	Radioactivity		
		% of dose ^{b)}	% of urine ^{c)}	% of dose ^{d)}
A		8.3	8.1	0.7
B		3.9	3.8	0.5
C	No 01-0402	12.1	10.7	1.5
D		3.3	3.2	0.5
E		2.2	2.1	0.3
F	DU1	6.9	6.7	0.8
G	DU2	0.7	0.4	0.1
H	No 01-0402	1.1	1.1	0.1
I	DU3	2.6	2.6	0.3
J	No 01-0402	0.5	0.5	0.1
K	DU4	2.5	2.4	0.3
L	DU5	4.3	4.4	0.5
M		1.3	1.3	0.2
N		1.3	1.3	0.2
O	DU6	24.0	24.4	3.0
P	No 01-0702	1.9	1.9	0.2
Q	No 01-0701	0.9	0.9	0.1
R	No 01-1000	2.1	1.9	0.2
S	No 01-0702	1.9	1.9	0.2
T		0.6	0.6	0.1
U		0.0	0.0	0.0
A-U		100.0	97.9	84.5

TEST REPORT

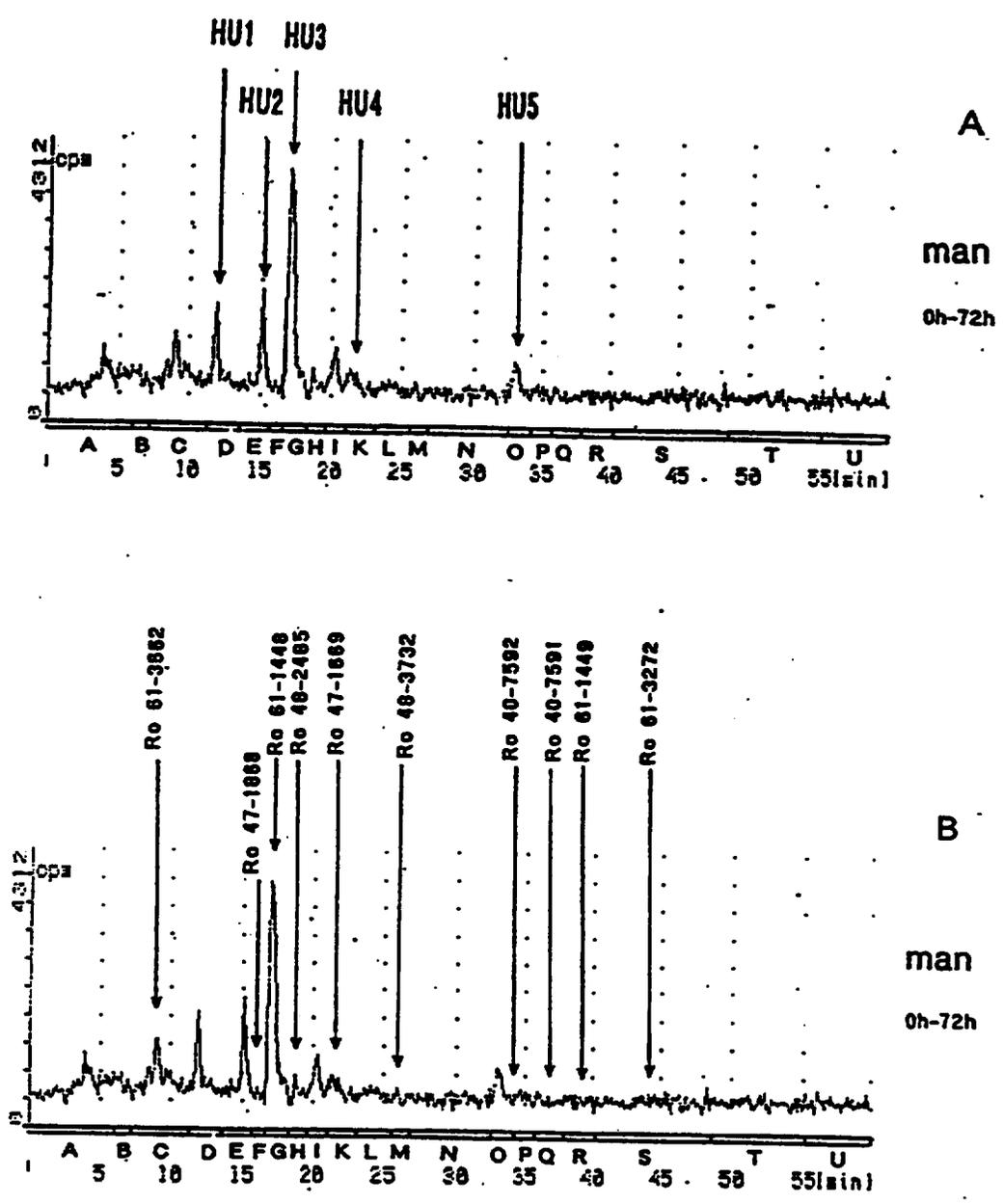


Figure 2

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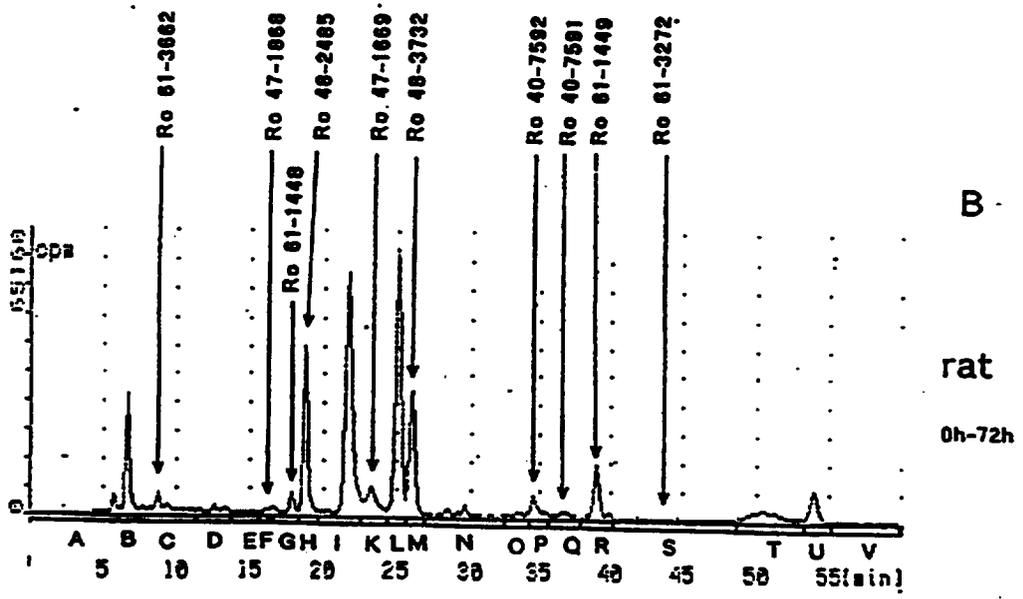
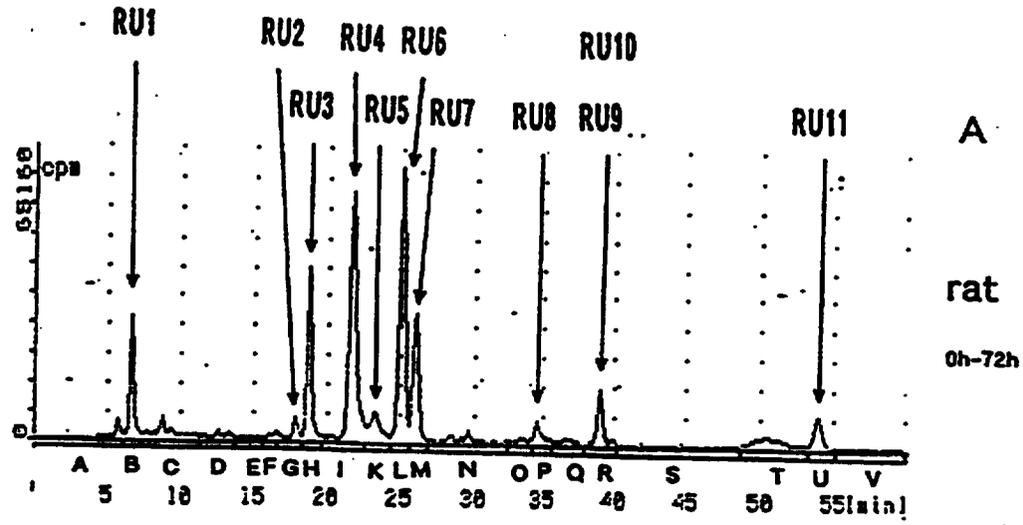


Figure 6

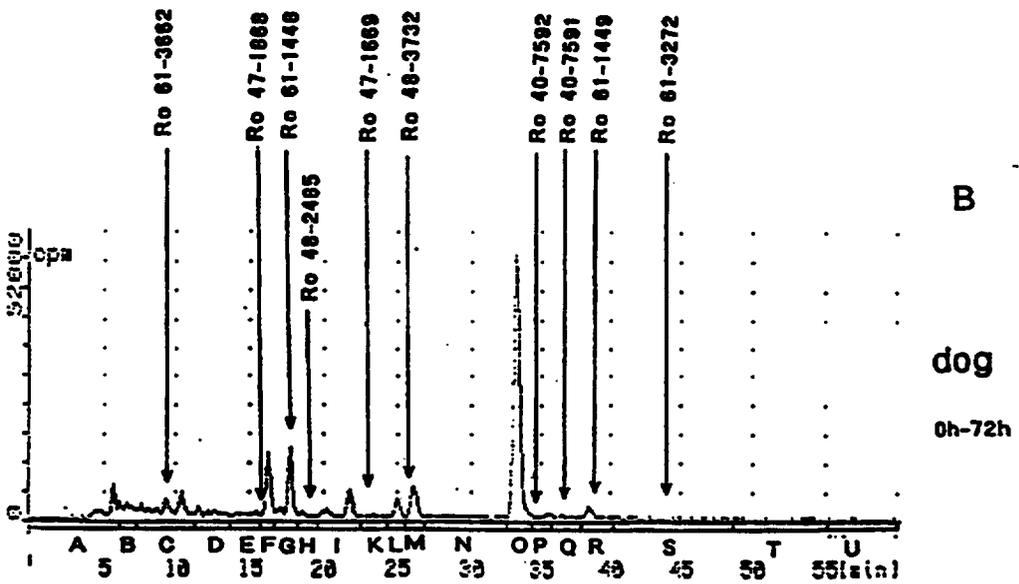
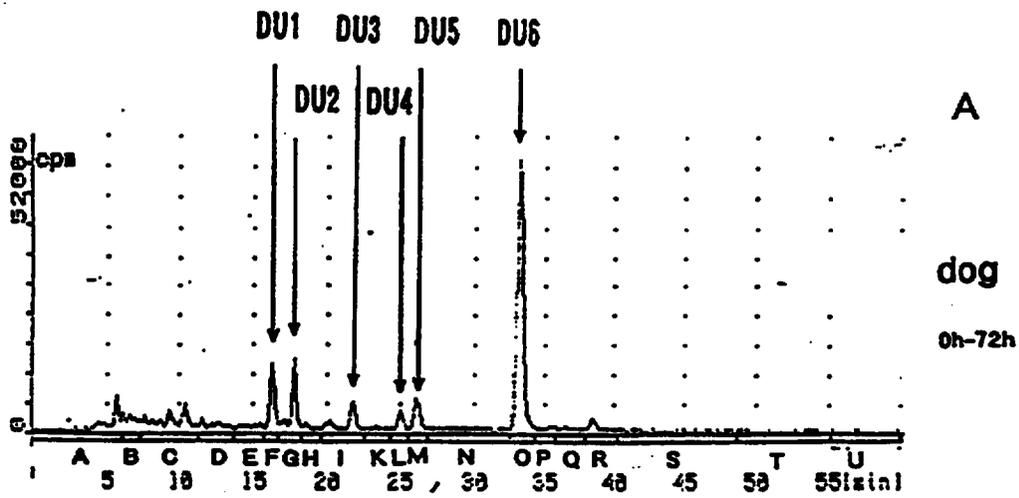


Figure 4

D.15. Urinary metabolites of tolcapone in rat and mouse after oral and i.v. treatment
Research Report #: B-163,961 Volume: 81

Summary:

After a single oral or intravenous dose of 5 mg/kg [¹⁴C]-tolcapone to mice (n=6) or rats (n=2-3), approximately 20% of radiolabel was excreted in urine in 48 hrs. In both species, the predominant urinary metabolites were Phase II conjugates. The major phase I reactions were reduction in the mouse, and oxidation in the rat.

At 0-24 hr, the predominant urinary metabolite in the mouse by either route was tentatively identified as a conjugate of the N-acetylamino metabolite (elutes at 4.9 min; possibly equivalent to RU1). The second most prominent metabolite coelutes with the 3-O-glucuronide conjugate (Ro 61-1448). In the rat, the main metabolites at 0-24 hrs were sulfate conjugates of the primary alcohol that elute at 8.6 min (RU4) and 10.4 min (RU6). RU4 was tentatively identified as the sulfate conjugate of the 4'-primary alcohol metabolite (Ro 47 1868), and RU6 as the 3-O-methylated derivative of RU4. The third major metabolite in rat urine was Ro 48-3732, the 3-O-methyl conjugate of the carboxylic acid derivative of tolcapone (Ro 47-1669). The putative sulfoconjugates of the primary alcohol, RU4 and RU6, were detected in smaller quantities in mouse urine than rat urine.

At 24-48 hrs, the main identified metabolite in both species was the N-acetylamino derivative (Ro 48-2485). The large peak that eluted at 4.9 min is putatively RU1, a conjugate (unknown) of the N-acetylamino metabolite. In mice, the conjugated metabolite exceeded the unconjugated metabolite; the reverse was observed in the rat.

The primary alcohol metabolite (Ro 47-1868) was not identified in the text description of results or in the table, but discernible peaks that coeluted with standard were evident in chromatograms of mouse extracts (elution time = 13.6 min). These were not evident in chromatograms of rat extracts, possibly because the metabolite was quickly conjugated with sulfate or oxidized to the carboxylic acid.

Since the sponsor suspected that a rat-specific metabolic process may underlie the renal toxicity (and tumorigenicity) of tolcapone, they conducted a species comparison of renal sulfoconjugation of the alcohol metabolite using S9 fractions from rat and mouse kidney. This study demonstrated that the renal sulfoconjugation by mouse S9 exceeded that of rat S9. Based on this, the sponsor concludes that the disparity in sulfoconjugation *in vivo* is because the reductive pathway predominates in the mouse, so the mouse kidney is less exposed to the alcohol than the rat kidney. However, the *in vivo* data suggests that free alcohol is present in mouse urine even between 24-48 hrs (if the fraction that elutes at 13.6 min is indeed the alcohol), suggesting that the *in vitro* model does not precisely reflect the *in vivo* renal handling of tolcapone.

A better assessment of sulfoconjugation in the two species may be obtained by comparing the amounts of putative sulfoconjugated urinary metabolites in mice and rats. The raw data are shown in sponsor's Table 2. For comparisons to the preceding study of urinary metabolites in rat, dog, and human, the data are expressed as percent of dose in the following Table: — —

TEST POSSIBLE

"Structure" (tentative ID)	Peak Label	Species	% Dose	
			Oral	I.V.

These data suggest that sulfoconjugation in the mouse is a minor process in vivo. These data, combined with the absence of renal toxicity of tolcapone in the mouse carcinogenicity study, are consistent with the sponsor's hypothesis that renal sulfoconjugation is involved in the toxic mechanism. However, it appears that the amount of sulfoconjugation in the mouse is lower than that in humans. Thus, humans may be more susceptible than mice to the renal toxic effects of tolcapone.

Sponsor's Tables

Table 2: Distribution of radioactivity of metabolites in rat urine

Drug-related peak or reference compound	Radioactivity		Radioactivity
	% of urinary fraction 0-24h after po application	% of urinary fraction 24-48h after po application	% of urinary fraction 0-24h after iv application
3.9 min	2.6		2.2
4.9 min	6.8	26.5	
7.0 min	5.5	17.3	4.1
8.6 min <i>RU9</i>	30.3		50.0
10.4 min <i>RU6</i>	20.1		16.9
11.9 min	2.3	8.9	2.3
Re 61-1448	5.3		2.7
Re 48-2485	7.1	40.7	2.1
17.0 min	0.9		
18.0 min	2.5		3.4
Re 47-1669	2.0		7.0
Re 48-3732	13.0		9.2
29.8 min	0.1		
Re 40-7992	1.4		

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Table 3: Distribution of radioactivity of metabolites in mouse urine

Drug-related peak or reference compound	Radioactivity		Radioactivity
	% of urinary fraction 0-24h after po application	% of urinary fraction 24-48h after po application	% of urinary fraction 0-24h after iv application
3.9 min	0.3		3.9
4.9 min	22.8	48.1	27.9
7.0 min	6.4		3.2
✓ 8.6 min <i>?RU9</i>	4.3		4.6
✓ 10.4 min <i>?RU6</i>	8.8		
11.9 min	6.7	15.6	5.2
✓ 13.6 min	9.2	7.1	6.5 ← ? alcord
Re 61-1448	18.8		24.9
Re 48-2485	6.2	29.1	10.4
✓ Re 47-1669	0.6		2.1
✓ Re 48-3732	4.2		2.1
Re 40-7992	2.8		2.1

D.16. Biliary metabolites in rats after oral [¹⁴C]-tolcapone
 Research Report #: B-161,438

Volume: 82

Summary:

The excretion of biliary and urinary radioactivity was studied over 48 hrs in two bile duct-cannulated rats following the oral administration of 5.2 mg/kg [¹⁴C]-tolcapone.

The excretion of radioactivity by the two routes was as follows:

	bile	urine	total
0-24 hr	58	21	79
0-48 hr	61	22	83

The predominant metabolite (RB3) appears to be the 3-O-glucuronide conjugate (Ro 61-1448). The next largest fractions were RB1, tentatively identified as a conjugate of the N-acetylamino metabolite (Ro 48-2485), and RB4, which appeared to be the 4-O-glucuronide conjugate (incorrectly labeled in the table). A number of additional peaks were evident, but relatively minor (Sponsor Table 2, Figure 3):

Table 2

Integration Interval *	Drug related peak or reference compound *	Radioactivity	
		% of bile *	% of dose *
A		1.1	0.09
B	RB1	9.2	0.73
C		1.9	0.15
D	RB2	4.6	0.37
E		4.9	0.39
F		7.4	0.59
G		2.6	0.21
H		1.1	0.09
I		1.2	0.10
J	RB3	27.6	2.20
K	RB4	7.4	0.59
L	"	0.4	0.03
M	RB5	0.3	0.02
N		1.9	0.15
O		0.4	0.03
P		0.9	0.07
Q	RB6	2.9	0.23
R		1.7	0.14
S	RB7	4.7	0.37
T		0.8	0.06
U		0.4	0.03
V		0.7	0.06
W		0.5	0.04
X		1.7	0.14
Y	RB8	4.9	0.39
Z		0.0	0.0
A-Z		100.0	8.77

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The observed trend that reduced compounds were a greater fraction of identified biliary metabolites is in contrast to the plasma and urinary metabolism profile in rats where oxidized products were more abundant. Other trends were that conjugated products exceeded unconjugated products, and glucuronides exceeded sulfates. These trends are tempered by the fact that 30% of biliary metabolites were not even tentatively identified.

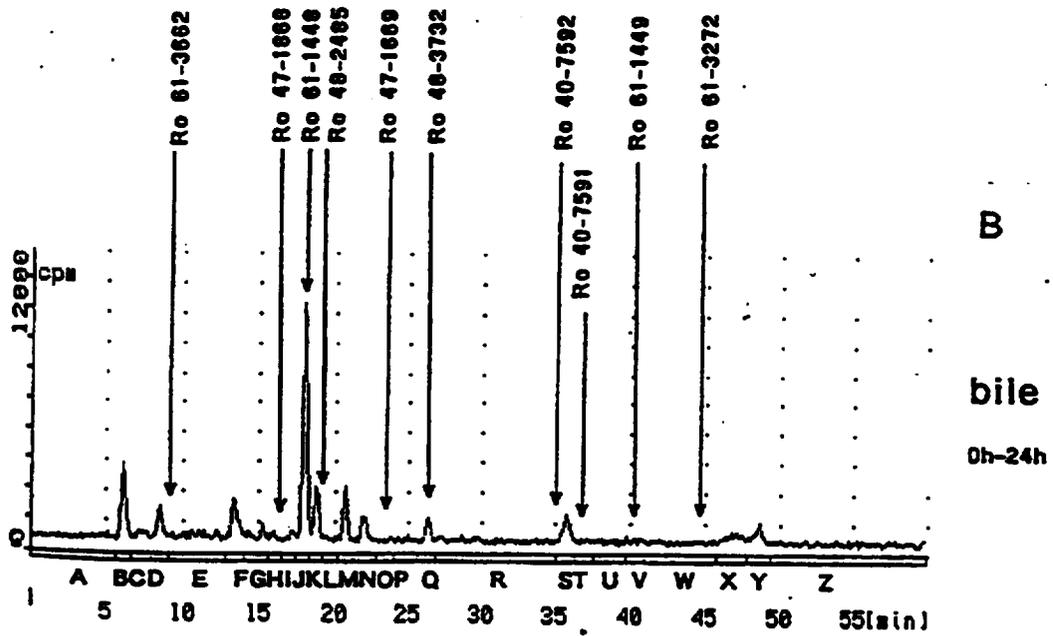
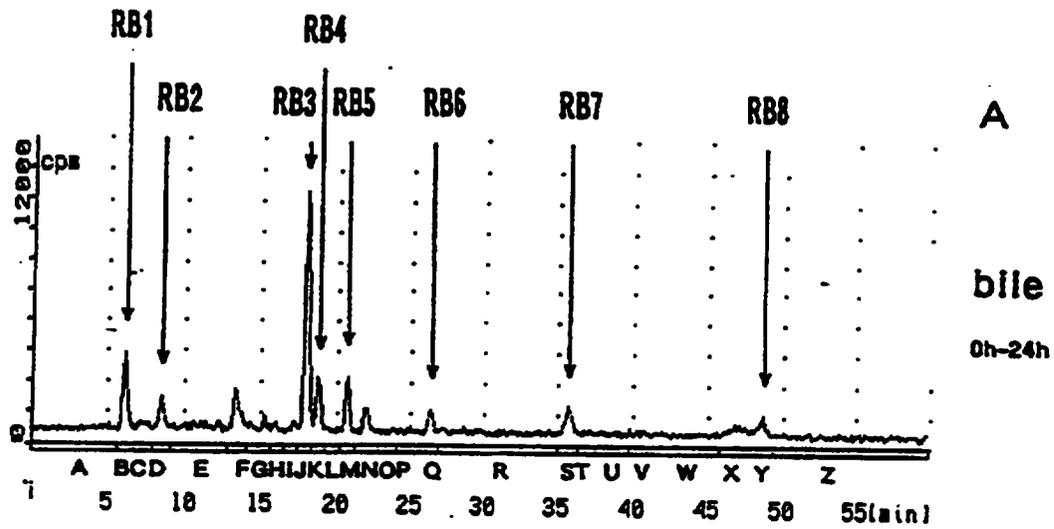


Figure 3

D.17. Biliary metabolites in dogs after oral [¹⁴C]-tolcapone
 Research Report #: B-164,029

Volume: 82

Summary:

The biliary and urinary excretions of radiolabeled metabolites were studied in a bile duct-cannulated dog following oral administration of 4 mg/kg [¹⁴C]-tolcapone.

The time-course of radiolabel excretion is in Sponsor Table 1:

Table 1

Excretion of total radioactivity in bile, urine and feces of a bile duct cannulated male dog after oral administration of 4 mg/kg ¹⁴C-Ro 40-7592/002.

time (h p.a.)	radioactivity (% of dose)			
	bile	urine	feces	total
0-6	39.8	3.6	-	43.4
6-24	8.5	6.5	18.4	33.4
24-48	0.7	2.8	3.0	6.5
total	49.0	12.9	21.4	83.3

Radiolabel appeared in two main fractions from samples collected between 0-24 hr. Unchanged parent compound accounted for 45.7% of radiolabel in bile, and the free amine reduction product accounted for 10.4% of radiolabel in bile. The third largest fraction (F) was not identified (Sponsor Table 2).

Table 2

Quantitative distribution of the radioactivity in the HPLC-radiochromatogram of the metabolites in dog bile (0-24 h p.a.), as shown in Figure 2.

Integration interval ^a	reference compounds ^b	% of radioactivity in eluate ^c	% of radioactivity in bile ^d	% of dose ^e
A	Ro 61-3662	11.8	10.4	5.0
B		2.6	2.3	1.1
C		2.3	2.0	1.0
D	Ro 47-1868	2.4	2.1	1.0
E	Ro 48-2485	3.7	3.3	1.6
F		8.3	7.3	3.5
G	Ro 47-1449	2.2	1.9	0.9
H	Ro 48-3732	2.8	2.5	1.2
I	Ro 40-7592	51.9	45.7	22.0
J	Ro 40-7591	6.4	5.6	2.7
K		3.2	2.8	1.3
L	Ro 61-1449	2.5	2.0	1.0
total		99.9	87.9	42.2

The presence of free amine, which appears in trace amounts in rat and human urine because of subsequent conjugation, in dog bile reflects the lack N-acetyltransferase in this species.

D.18. Drug Interaction Studies: In vitro metabolism studies

Research Report #: B-163,954

Volume: 83

Summary:

The potential for metabolic interactions between tolcapone and several drugs that interact with cytochrome P₄₅₀ was studied *in vitro* with human liver microsomes. The specific metabolic routes assessed were formation of the alcohol (Ro 47-1868) and formation of the glucuronide (Ro 61-1448).

a. Oxidation of tolcapone to the alcohol Ro 47-1868

The results of studies on the inhibition of microsomal formation of the alcohol from tolcapone (80 μM) by other P₄₅₀ substrates ($\leq 200 \mu\text{M}$) were:

<u>Compound</u>	<u>Isozyme</u>	<u>Results</u>	<u>Ki (μM)</u>
midazolam	3A	inhibition	7.4
narigenin	3A	inhibition	24
phenytoin	2C9	little/no inhibition	170
tolbutamide	2C9	slight inhibition	1228

These results suggest that 3A is more important in this oxidation. The clinical relevance of this to humans is questionable since the *in vivo* studies suggest that oxidation is a relatively minor pathway (compared to glucuronidation). [The sponsor cites a manuscript suggesting that oxidation accounts for approximately one-third of tolcapone metabolism in humans, which is larger than that projected from the *in vivo* studies.]

b. Inhibition of glucuronide formation from tolcapone (20 μM):

<u>Compound</u>		<u>Results</u>	<u>Ki (μM)</u>
desipramine	($\leq 50 \mu\text{M}$)	slight inhibition	126
naproxen	($\leq 100\mu\text{M}$)	slight inhibition	134

It is unlikely that the interaction with desipramine is clinically relevant because of its low therapeutic plasma levels (0.4 μM). However, therapeutic plasma levels of naproxen are 195-390 μM , which raises the possibility of competition with tolcapone for this enzyme.

c. Inhibition of P₄₅₀ metabolism by tolcapone

Substrate	Isozyme	Result	Substrate Km (μM)	Tolcapone Ki (μM)
midazolam	3A	inhibition	4.1	49
terfenadine	3A	inhibition	2.9	42
cyclosporine	3A	neg. inhibition	7.3	1194
coumarin	2A6	inhibition	2.2	21
caffeine	1A2	inhibition	300	30
diclofenac	2C9	inhibition	16.6	7.4
tolbutamide	2C9	inhibition	80	25
naproxen	2C9	inhibition	5	28
S-mephenytoin	2C19	no inhibition		
desipramine	2D6	no inhibition		

Tolcapone has the potential to inhibit the metabolism of 2C9 substrates, diclofenac and tolbutamide; an effect on naproxen metabolism is unlikely. Inhibition of the 3A substrates and coumarin is also unlikely since the Ki of tolcapone is larger than the substrate Km.

The sponsor has provided a relative comparison of tolcapone's effect on 3A and 2C9 metabolism with other compounds:

Table VI: Ki values of in vitro competition of various drugs with midazolam hydroxylation (from [15])

drug	Ki (nM)	drug	Ki (nM)
itraconazole	0.1	SKP 525A	110
itraconazole	1	quinidine	120
clonitazone	<1	erythromycin	148
cyclosporine	2	chloroquine	150
methylergometrine	5	primaquine	160
nifedipine	5	azithromycin	170
dihydroergotamine	7	troleandomycin	180
ergotamine	7	mefloquine	250
nicardipine	8	quinine	250
levomepromazine	10	clarithromycin	330
amlodipine	15	caffeine	480
Ro 41-3096	15	cimetidine	550
bromocriptine	40	theophylline	>1000
venetranil	100		

Table VII: Ki values of in vitro competition of various drugs with diclofenac hydroxylation (from [21 and 22]). Substrates of CYP 2C9 are depicted in bold.

drug	affinity (nM)
sulfaphenazole	0.2
diclofenac	6
meftamic acid	7
amlodipine	8
itraconazole	20
fluconazole	22
phenytoin	29
ibuprofen	30
piroxicam	40
piroxicam	40
tenoxicam	40
indomethacin	40
sulfadiazine	47
tolbutamide	85
sulfamethoxazole	114
acetylsalicylic acid	200

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D.19. Effect on hepatic metabolism *in vivo*

Research Report #: J-146,484

Volume: 83

Summary:

The effect of daily administration of tolcapone (20, 100 300 mg/kg/day for seven days) on liver metabolic capacity was determined in rats.

Slight variations observed were an increase in relative liver weight, decrease in microsomal protein content, decrease in aminopyrine N-demethylase activity, and increase in 7-ethoxycoumarin O-demethylase activity.

Compound Dosage(mg/kg/day)	Ro 40-7592				phenobarbital 80
	20	100	300	withdrawal	
relative liver weight (%)	98.4	111.6*	106.1	105.5	120.6**
microsomal protein (mg/g liver)	77.5*	82.0	88.6	121.9	127.4*
cytochrome P-450 (nmol/mg protein)	86.3	99.8	82.6	100.9	137.7*
aniline hydroxylase (nmol/min per mg protein)	90.8	101.6	102.8	-	128.5
aminopyrine N-demethylase (nmol/min per mg protein)	78.7	93.1	70.9*	83.6*	177.9**
7-ethoxycoumarin O-demethylase (nmol/min per mg protein)	118.2	116.4	138.5*	77.1	86.5

More significant effect on testosterone hydrolases were noted. These reductions reflect inhibition of cyp 2C11 in rats, which may correspond to 2C9 in humans.

Compound Dosage(mg/kg/day)	Ro 40-7592				phenobarbital 80
	20	100	300	withdrawal	
testosterone 2 α -hydroxylase (μ g/min per mg protein)	82.5	76.0	44.4**	37.4**	17.3**
testosterone 2 β -hydroxylase (μ g/min per mg protein)	100.5	77.9	81.3	-	213.4**
testosterone 6 β -hydroxylase (μ g/min per mg protein)	92.1	79.5	78.2	-	190.6*
testosterone 7 α -hydroxylase (μ g/min per mg protein)	103.7	97.4	86.4	-	196.2
testosterone 16 α -hydroxylase (μ g/min per mg protein)	89.7	75.8	39.2*	16.7**	70.9
testosterone 16 β -hydroxylase (nmol/min per mg protein)	100.0	100.0	100.0	-	..*
Ro 47-1868 formation (μ g/min per mg protein)	80.7*	111.5	105.5	-	144.8*

No self-inductive effects of tolcapone were evident.

D.20. Excretion balance and blood levels after i.v. and oral [¹⁴C]-tolcapone in rats

Research Report #: B-156,750 (GLP study)

Volume: 80

Summary:

The excretion balance of [¹⁴C]-tolcapone was determined in male and female pigmented (piebald) rats following single intravenous (3 mg/kg) or oral (5 mg/kg) administrations. Total radioactivity was determined in blood, urine, and feces at 24-96 hrs postdose. The experimental design was such that 2 animals/sex were sacrificed at each time point for blood collection. Thus, a total of 8 animals/sex were used for the oral study, and 6 animals/sex were used in the i.v. study.

Unchanged drug was quantitated in the 24 hr sample. Balances were as follows:

5 mg/kg oral [¹⁴C]-tolcapone

		Urine	Feces
0-24 hr	M	30	37
	F	13	58
24-48 hr	M	1.9	17
	F	1.4	24
48-72 hr	M	0.4	2.4
	F	0.3	3.2
72-96 hr	M	0.2	0.2
	F	0.1	1.2

3 mg/kg i.v. [¹⁴C]-tolcapone

		Urine	Feces
0-24 hr	M	50	43
	F	21	68
24-48 hr	M	3.6	1.4
	F	1.8	5.0
48-72 hr	M	0.4	0.2
	F	0.4	0.7

After oral administration, the major fraction of radioactivity was eliminated in the feces by 72 hrs. After intravenous administration, the urinary and fecal excretion fractions were relatively similar in males, but in females the fecal fraction was predominant. The existence of a true gender difference with respect to elimination pathways is difficult to determine because of the limited number of animals (n = 2-8 per time point). In general, it appears that the biliary route of excretion is the more important route.

The amount of unchanged drug in the 24 hr blood samples was less than 1.5%. The approximate amount of unchanged drug in 24 hr urine samples (i.e., eluting *near* the time of authentic tolcapone) was 2-4%.

D.21. Excretion balance of [¹⁴C]-tolcapone in dogs after i.v. and oral administration

Research Report #: B-161,431 (GLP study)

Volume: 80

Summary:

The excretion balance of [¹⁴C]-tolcapone was determined in two male beagles for 0-120 hrs following single intravenous (2.5 mg/kg) or oral (5 mg/kg) administrations. Total radioactivity was determined in urine and feces, and total radioactivity, parent compound, and 3-O-methyltolcapone were determined in plasma.

Excretion balances were as follows:

Table 1

Excretion of the total radioactivity in urine and feces of 2 male dogs following a single i.v. dose of ca. 2.5 mg [¹⁴C]-tolcapone per kg body weight in a glycerol 7% physiological NaCl (0.4, v-v) solution of 20 mg/ml. The results are expressed in % of the administered dose.

Time after administration in hours	Dog Basil		Dog Fina	
	urine	feces	urine	feces
0-4	0.78		7.71	
4-24	4.79	22.14	8.57	61.59
24-48	2.72	8.73	1.85	11.11
48-72	0.30	7.89	0.14	2.39
72-96	0.09	2.79	0.01	0.20
96-120	0.05	0.24	0.04	0.05
0-120 cage wash	0.67		0.26	
0-120 plasma *	0.50		0.34	
Total	20.77		92.97	

* proportion of the radioactive dose removed by collection of plasma samples

Table 2

Excretion of the total radioactivity in urine and feces of 2 male dogs following a single p.o. dose of ca. 5.0 mg [¹⁴C]-tolcapone per kg body weight in a glycerol 7% physiological NaCl-water (0.04, v-v-v) solution of 10 mg/ml. The results are expressed in % of the administered dose.

Time after administration in hours	Dog Basil		Dog Fina	
	urine	feces	urine	feces
0-4	1.20		0.94	
4-24	0.39	9.46	9.48	48.85
24-48	2.09	23.29	2.67	21.85
48-72	3.52	7.86	0.17	0.69
72-96	0.41	28.84	0.05	0.06
96-120	0.18	0.48	0.03	0.04
0-120 cage wash	1.94		1.37	
0-120 plasma *	0.39		0.34	
Total	92.81		92.82	

* proportion of the radioactive dose removed by collection of plasma samples

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By either route, the major fraction of radioactivity was eliminated in the feces. Less than 20% was eliminated in the urine.

In plasma samples, the largest fraction of radioactivity between 0.5-2 hr was parent compound. The parent compound could not be detected after 6 hrs post-treatment. Between 4-30 hrs post-treatment, the 3-O-methyl metabolite was the largest radioactive fraction identified.

D.22. Excretion into rat milk after oral administration of [¹⁴C]-tolcapone

Research Report #: J-146,480

Summary:

Excretion of [¹⁴C]-tolcapone and [¹⁴C]-3-O-methyltolcapone into milk was studied in lactating rats (n=5; 14 days post-partum) following an oral dose of 20 mg/kg.

As shown in the table, milk levels of tolcapone (or the metabolite) exceeded blood levels at every time point. Parent compound was by far the most significant component in milk. The milk: blood ratio for the parent compound increased with time, as milk levels increased and decreased more slowly than blood levels.

		0.5 hr		1 hr		2 hr		4 hr		7 hr	
		µg/ml	m:b	µg/ml	m:b	µg/ml	m:b	µg/ml	m:b	µg/ml	m:b
TOL	milk	37.0	4.7	42.9	5.5	32.1	10.3	18.8	15.7	5.7	57
	blood	7.8		7.8		3.1		1.2		0.1	
3-O-meTol	milk	1.0	2.0	1.0	2.5	0.9	3.0	0.5	1.7	0.2	1.0
	blood	0.5		0.4		0.3		0.3		0.2	

SUMMARY

PHARMACOLOGY

Tolcapone inhibits the activity of both central and peripheral catechol-O-methyl transferase, the enzyme that catalyzes the methylation of catechols at the 3-hydroxyl position. By inhibiting the 3-O-methylation of L-DOPA, a primary route of L-DOPA inactivation, tolcapone increases plasma exposures to L-DOPA and thereby improves its pharmacokinetic profile and antiparkinsonian activity.

The COMT-inhibitory activity of tolcapone was demonstrated in several *in vitro*, *in vivo* and *ex vivo* experiments. Potent inhibitory effects of tolcapone are achieved at nanomolar concentrations *in vitro*, and in the 10-30 mg/kg range following oral administration (inhibition of central COMT activity in rats). Distribution studies have suggested that a only small fraction of tolcapone (<1% of plasma levels) is present in brain. Thus, the magnitude of central COMT inhibition is reflective of the drug's high potency. COMT inhibition by tolcapone is reversible. The pharmacological activity of tolcapone appears selective as the drug has no substantial activity on other enzymes important in neurotransmitter metabolism, monoamine neurotransmitter uptake, or at various neurotransmitter binding sites.

In studies on the effect of tolcapone on L-DOPA pharmacokinetics, a single oral administration of 30 mg/kg caused an approximate four-fold increase in plasma L-DOPA exposure in rats. Plasma levels of 3-O-methyl-DOPA were correspondingly reduced. Similarly, an approximate four-fold increase in striatal L-DOPA exposure, and a virtual abolition of striatal 3-O-methyl-DOPA formation, was observed following tolcapone. In macaques, an oral dose of 10 mg/kg tolcapone caused a 3-fold increase in the AUC for L-DOPA.

The potential efficacy of tolcapone in combination with L-DOPA was evaluated in standard neurotoxin-induced lesion models of Parkinson's disease. In albino rats unilaterally-lesioned by injection 6-hydroxydopamine into the substantia nigra, tolcapone (30 mg/kg, i.p.) prolonged the duration of action of L-DOPA/benserazide on rotation from 2 hrs to 4-4.5 hr. Peak effects were similar in the presence and absence of tolcapone. In two female rhesus monkeys lesioned by unilateral intracarotid infusion of MPTP, tolcapone (10 mg/kg, i.p., or 30 mg/kg, p.o.) increased by 50% the number of rotations/session in response to L-DOPA, and prolonged the duration of action of L-DOPA. Oral doses lower than 15 mg/kg tolcapone did not potentiate the effect of L-DOPA.

Thus, tolcapone has a selective mechanism of action as an inhibitor of COMT, and has demonstrated some efficacy in animal models for improving the therapeutic effects of L-DOPA.

SAFETY PHARMACOLOGY

In assays of potential CNS-related activities, oral tolcapone was devoid of anti- and pro-convulsant activity, analgesic activity, hypnotic effects, or effects on body temperature in mice. Some minor EEG changes were observed in cats after doses of 1-10 mg/kg, i.v. Tolcapone did not demonstrate a propensity for inducing dependence, withdrawal, or self-administration.

In studies on cardiovascular/respiratory effects of tolcapone, slight decreases in heart were seen in conscious monkeys and dogs with 10 or 30 mg/kg, p.o. No additive effect of Sinemet was seen in the dog

study. In anesthetized beagles, dose-dependent decreases in systolic and diastolic pressure and femoral blood flow, and a significant increase in respiration were evident following 10 mg/kg. The only notable ECG change was a slight shortening of the QRS complex. In spontaneously hypertensive rats, oral doses of 10, 30 and 100 mg/kg tolcapone did not alter systolic pressure or heart rate at 1, 3 or 6 hours post-dose.

In vivo and *in vitro* studies on GI function revealed minimal inhibitory effects of tolcapone on motility and contractility.

Urine output and sodium excretion were significantly reduced in female rats by 100 mg/kg oral tolcapone at 0-5 hrs post-dose.

TOXICOLOGY

Acute Toxicology

Studies were conducted in mice, rats and dogs. Animals were observed for up to 14 days following treatment. The lowest lethal oral doses in rats and mice 500 and 400 mg/kg, respectively, and high rates of lethality occurred at 1000 and 800 mg/kg (data from the tolcapone-only animals in the combination studies). By the intraperitoneal route, doses of 80 and 71 mg/kg caused high rates of lethality in rats and mice, respectively. Sinemet did not markedly enhance the lethality of tolcapone. Prominent signs of toxicity were ataxia, hypomotility, respiratory depression, and loss of righting reflex. In dogs, oral doses of 100 mg/kg or greater caused emesis, and diarrhea/mucoid feces occurred at higher doses. No fatalities resulted from doses up to 300 mg/kg. Reddening of the gastrointestinal mucosa was observed in both dogs at necropsy.

Repeat-Dose Combination Toxicology Studies of Tolcapone and Sinemet

Tolcapone will be used as an adjunct to L-DOPA preparations in the treatment of Parkinson's disease. Therefore, the sponsor has conducted subchronic, reproductive and genetic toxicology studies of the combination. In some instances, both Sinemet (L-DOPA + carbidopa) and Madopar (L-DOPA + benserazide) were evaluated by the sponsor. Only the studies with Sinemet were reviewed. These studies were generally designed to use a fixed dose of Sinemet, usually a 4:1 ratio of L-DOPA to carbidopa, and vary the dose of tolcapone.

No clear instances of potentiation of a toxicity by L-DOPA were identified in these studies. The toxicities that were identified were generally clearly attributable to one of the two components.

Rat: Tolcapone (20 and 200 mg/kg/day) was orally administered alone and in combination with Sinemet (100 mg/kg/day; 80 mg/kg L-DOPA/20 mg/kg carbidopa) to Sprague-Dawley rats for 13 weeks. Clinical signs of hypomotility, salivation, and lacrimation were observed in the groups that received Sinemet. Only slight effects on body weight were seen in high-dose tolcapone (HT), low-dose tolcapone with Sinemet (LT/S), and high-dose tolcapone with Sinemet (HT/S) males.

The degree of kidney toxicity in this study was relatively modest. Relative kidney weights were increased in LT/S and HT/S males (22%), in HT/S females (9.5%), and in Sinemet-only males (16%). The prevalence and severity of the renal histopathological findings (degeneration, pigment deposits and

vacuolation of the PTE in 1-2 animals per group; a nephroblastoma in an HT male) were far lower than those seen in the 1- and 2-year rat studies. However, since no renal changes were evident in the 6-month rat study (which was not reviewed), the findings in this 13-week study suggest that the onset of renal toxicity may be earlier than expected (based on the absence of findings in the 6-month study). Because of the low incidence and distribution of renal findings in all treatment groups, it is not clear that Sinemet markedly enhanced the renal toxicity of tolcapone.

Additional histopathologies were identified in the forestomach epithelium (hyperkeratosis, thickening, and one case of necrosis) of HT and HT/S males, in submandibular gland (high incidences of acinar cell hypertrophy in the combination groups and a Sinemet-only male, but not in any tolcapone-only animals), and isolated cases of hepatocyte necrosis.

Toxicokinetic data demonstrated dose proportional increases in tolcapone, and that the drug does not accumulate. Exposures (AUC_{0-24}) in HDM were 4.5 times the expected therapeutic exposures in humans (AUCs were not calculated in female rats). The relative increases in L-DOPA due to tolcapone coadministration were rather small (1.3-fold) at both dosage levels of tolcapone.

Dog: Tolcapone (10 and 80 mg/kg/day) was orally administered alone and in combination with Sinemet (100 mg/kg/day; 80 mg/kg L-DOPA/20 mg/kg carbidopa) to beagle dogs for 13 weeks. The major clinical sign was emesis in the Sinemet only and combination group males and females, and in the HT males. The Sinemet dosage was reduced to 50 mg/kg on day 42 to reduce this problem. No remarkable changes in hematology, clinical chemistry, urinalysis, or ECG were observed. Notable histopathological findings were prostate atrophy/reduced spermatogenesis in males receiving the combinations and Sinemet only, and pigment deposits in submandibular glands of animals from the combination and Sinemet only groups. Thus, no remarkable changes were unique to the tolcapone-only groups. A similar profile was observed in the combination study with Madopar (not reviewed). Toxicokinetic data were confounded by emesis. Thus, increases in L-DOPA exposures due to tolcapone generally small and not clearly dose-related.

Monkey: Tolcapone (10, 40 and 150 mg/kg/day) was orally administered alone and in combination with Sinemet (250 mg/kg/day; 4:1, L-DOPA:carbidopa) to cynomolgus monkeys for 13 weeks. Because of excessive hyperactivity in monkeys treated with Sinemet, the dose was reduced in steps to 100 mg/kg by day 9. One HT/S female was sacrificed on day 3 due to self-mutilation. Aside from the clinical signs, which also included occasional emesis, no remarkable toxicological findings were noted. Some LDH elevations (relative to control values) were noted. Toxicokinetic data suggested that exposures to the high dose of tolcapone produced plasma levels that were only slightly higher (1.5 times) than expected human therapeutic exposures. Tolcapone did not markedly increase exposures to L-DOPA, possibly because of decreased absorption of Sinemet (or an effect of emesis). L-DOPA exposures were 10-50 fold higher than those of humans receiving Sinemet (25/100) and tolcapone.

Chronic toxicology

The effects of chronic administration of tolcapone were assessed in one-year rat and one-year dog studies. The test dosage ranges and conditions were appropriate. In the rat study, the kidney was identified as a target organ. Similar renal pathologies were not observed in the dog, where the highest dose tested was one-third of the high dose in the rat study. Higher doses were not tested in the dog

because of emesis.

Rat: Tolcapone was administered in the diet to Sprague-Dawley rats for 52 weeks at doses of 20, 150 and 450 mg/kg. Administration of the highest test dose resulted caused significant reductions in body weight and food intake. Six HDM were anemic at week 52; two of these animals had renal tumors (1 nephroblastoma, 1 adenocarcinoma). The only other abnormal findings in the tumor animals were slightly elevated ASAT in the nephroblastoma animal, and an increase in round epithelial cells in urine of the adenocarcinoma animal. High incidences of histopathological abnormalities were in the kidney (degeneration of proximal tubule epithelium, atypical nuclei) and forestomach (epithelial hyperplasia) of both sexes. No clinical chemistry changes (i.e., BUN, creatinine) were evident in animals displaying renal histopathologies. An increase in lipid droplets in the adrenal zona fasciculata occurred in six HDM.

Plasma levels were determined at only 1-2 time points post-dose, so relative exposure comparisons based on AUC could not be determined. Thus, the following exposure comparisons were derived from plasma levels obtained near the peak intake of food (and drug) in rats (at 0600) and the estimated steady-state levels in humans receiving 200 tolcapone mg, t.i.d. (6.4-7.6 µg/ml):

LD rats:	below human exposures
MD rats:	1.2 - 2.1 times greater than human exposures
HD rats:	5.1-- 8.7 "

Toxicokinetic analyses also indicated that tolcapone concentrations increased dose-proportionally, and tolcapone levels tended to be higher in females compared to males. The 3-O-methyl metabolite was not detectable in most samples.

To further assess the significance of the renal findings, the sponsor assayed for high proliferation rates in PTE cells using proliferating cell nuclear antigen (PCNA). An increased incidence in the number PCNA-positive cells relative to all cells and relative to cells with atypical nuclei was apparent to the reviewer. However, the sponsor concluded that the number of "PCNA-positive cells among atypical nuclei did not increase clearly"; thus, the observations of atypical nuclei were not considered indicative of carcinogenic potential. In the opinion of the reviewer, the relationship between cell proliferation and atypical nuclei appears strong. In view of the occurrence of renal tumors in the 2-year rat study, along with high incidences of karyomegaly and tubular hyperplasia, the sponsor's dismissal of the possible relationship between cell proliferation, atypical nuclei and carcinogenicity seems unfounded. Karyomegaly and tubular hyperplasia are considered (by some) as early events in renal carcinogenesis (Bannasch and Zerban, Pathology of Neoplasia and Preneoplasia in Rodents, 1994). The high incidence of PTE cells with atypical nuclei in this study tends to suggest that this is an intermediate stage of the toxic response to tolcapone, and that tubule hyperplasia, karyocytomegaly, and renal tumors observed in the two-year study represent the later stages.

Dog: Tolcapone was administered orally at doses of 0, 10, 40 and 150 (2 x 75) mg/kg/day to beagle dogs (4 or 5/sex/dose) for 12 months (the high dose was increased to 180 mg/kg/day for the second half of the study). One dog/sex in the control and HD groups were allowed to recover for 8 weeks.

Vomiting and diarrhea were encountered frequently at the mid and high doses. Decreases in mean

erythrocyte parameters (RBCs, Hb, PCV, MCV, MCH), but only minor, reversible individual variations, were seen in high-dose males at 9 and 12 months. Statistically significant increases in relative weights of kidney (22%) and liver (24%) in HDM were not accompanied by any histopathological changes. Increases in plasma exposure to tolcapone were approximately dose proportional, and no sex differences were apparent. Relative to plasma exposures in humans receiving the projected maintenance dose of 200 mg, t.i.d. ($AUC_{0-24} = 80 \mu\text{g}\cdot\text{hr}/\text{ml}$), tolcapone exposures in dogs were:

LD: below human exposures
MD: 1.0 - 2.0 times the human exposures
HD: 4.5 - 8.2 "

Toxicological findings in 6-month (hyperemia of the stomach mucosa in HD animals) and one month studies (epithelial cells in urine in MD and HD animals) were not evident in this study, despite the use of similar dosage levels.

Reproductive Toxicology

The toxic potential of tolcapone on reproductive parameters was evaluated in Segment I-III studies in rats and a Segment II study in rabbits. The combination of tolcapone and Sinemet was evaluated in Segment II studies in rats and rabbits. All studies were acceptable with respect to dosage selection and conditions. The plasma exposures achieved in rabbits were relatively low, but dosing was limited by maternal toxicity. The rat is usually not an appropriate model for Segment II studies of compounds that elevate dopaminergic activity and thereby inhibit the prolactin-dependent stage of implantation. However, tolcapone did not cause as dramatic an effect on associated parameters (i.e., low implantations, high early resorptions) as that produced by direct dopamine agonists. Hence, the number of pups evaluable for the teratogenicity portion of the study was acceptable, and the drug appears to be devoid of this property. Six of 36 dams (including 4 of 9 that died during gestation) treated with the high (300 mg/kg) dose of tolcapone in the rat Segment II study had resorptions, and stillborns and pup loss were observed at the mid (150 mg/kg) and high dose levels. Since the dose-relationship for the findings was not strong and the incidences were marginal, these suggestions of embryotoxicity/fetotoxicity of tolcapone in rats are considered equivocal. In rabbits, tolcapone displayed abortive potential at 100 and 400 mg/kg. The number of evaluable pups from HD does was 40% lower than other groups, but probably adequate for teratogenic evaluations. In the rat Segment I study, no significant impairment of reproductive performance and fertility was observed. In the rat Segment III study, pup development (learning performance in 1 of 2 assays) was impaired by the high dose of 250 mg/kg, and the loss of an HD litter resulted in a reduced "lactation index" (# of pups surviving at day 23/# of liveborn pups). In the combination Segment II studies, maternotoxic and fetotoxic effects of the combination appear attributable to L-DOPA.

As recommended by the sponsor, the human use of tolcapone in combination with L-DOPA is sufficient to warrant Pregnancy category C classification. The abortive potential of tolcapone in rabbits should be added to the label. The additional findings from rat Segment II and III studies that are suggestive, but equivocal evidence for embryotoxic, fetotoxic and developmental impairments by tolcapone are being considered by Dr. Ed Fisher for possible inclusion in the label.

Segment I. Rat: Tolcapone (30, 100 and 300 mg/kg/day) was administered by gavage to males rats (SD-S1c) for 63 days prior to mating, and to females from 14 days prior to mating through gestation (sacrifice on day 21 for delivery of pups by Caesarean section), or weaning (21 days). No clearly drug-related effects on fertility, pup skeletal, visceral and external anomalies, litter or developmental parameters were identified. A slightly increased percentage of litters with early deaths per total C-section litters in MD and HD groups was not statistically significant. Five of 17 HD dams died during the weaning period of unknown causes. The pregnancy rate of F₁ females from the HD dams was reduced to 73%, but this was not statistically significant. Thus, tolcapone was maternally toxic at doses of 300 mg/kg/day, but did not adversely affect fertility, reproductive parameters, or pup development in this study.

Segment II. Rat: Tolcapone (50, 150 and 300 mg/kg/day, p.o.) was administered on days 6-15 of gestation (n = 36 mated females per dose). The most notable toxicity was maternal deaths (nine HD dams from the rearing group) between days 11-13 of gestation; no cause of death and no adverse necropsy findings were identified. All 9 dams had implantations, and 4 had resorptions. No drug-related skeletal, visceral or external anomalies were evident in pups from the C-section groups. In the rearing groups, two HD dams had complete resorptions of litters with a low number of implantations, and two LD dams had complete resorptions. Two MD dams delivered several stillborn (7 and 5; historical control range = 0.6 - 4.2), and two had pup loss during lactation (5 and 4 pups each). Pup developmental was not impaired. The stillborns, resorptions, and pup loss at the MD and HD are considered equivocal evidence for the embryotoxic and fetotoxic potential of tolcapone at 150 and 300 mg/kg/day.

Segment II. Rabbit: Tolcapone (25, 100, 400 mg/kg/day) was administered by gavage to pregnant Swiss hare rabbits from day 6-18 of gestation. The main drug-related finding in rabbits was induction of abortions between days 20 - 29 in 2/18 MD and 6/18 HD does. No drug-related external, skeletal or visceral anomalies were identified. Findings from the HD group must be considered equivocal because of the low number of evaluable pups.

Segment III. Rat: Tolcapone (40, 100 and 250 mg/kg/day) was administered by gavage from day 15 of gestation to day 22 of lactation. The high dosage was reduced to 150 mg/kg/day after 6-8 days of treatment due to a high rate of maternal mortality; 13 dams died between days 19-22 of gestation. Litter size was reduced, and the number of resorptions increased at the HD level, but these effects were not statistically significant. A large loss of HD pups from one litter during lactation resulted in a decreased lactation index. Developmental impairments in pups from HD dams were noted in performance on one of two learning tests, and a slight (non-significant) decrease pup weights was evident at study termination. No drug-related abnormalities were apparent in pups sacrificed after weaning.

Combination Segment II Studies: The reproductive toxicities of tolcapone in combination with Sinemet were evaluated in Segment II studies. No treatment-related effects on reproductive parameters were evident in either rats (10, 30 or 50 mg/kg tolcapone + 150 mg/kg Sinemet) or rabbits (25 or 100 mg/kg tolcapone + 100 mg/kg Sinemet). Average fetal body weights were significantly reduced in all rat groups that received Sinemet (150 mg/kg), but not in the group that received tolcapone alone (50 mg/kg). Fetal body weights were also reduced in the rabbit Sinemet-only treatment group. The rat combination group exhibited possible delays in ossification, but no frank malformations or variations. A slightly (non-significant) increased incidence of malformed fetuses was observed in the HT/S rabbit group. The effects of the Sinemet combination were attributed to decreased maternal food consumption (skeletal

findings occurred only in groups with reduced maternal intake and reduced fetal body weights). The absence of significant findings in the groups that received the high dose of tolcapone alone suggests that the maternotoxic and fetotoxic effects of the combination in rats were due to L-DOPA.

Exposures to tolcapone in the high dose groups were 1.5-3.6 times the human exposure in rats, and less than one-half of the human exposure in rabbits.

Mutagenicity

The mutagenicity of tolcapone was evaluated in an extensive battery of assays including the Ames tests, the V79/HPRT gene mutation assay, unscheduled DNA synthesis assay, chromosomal analysis in cultured human lymphocytes, and an *in vivo* mouse micronucleus test. In addition, tolcapone was tested in combination with Sinemet in the Ames test, a mouse lymphoma/thymidine kinase (ML/TK) assay, and a mouse micronucleus test. The assays were designed and conducted in accordance with OECD guidelines, and generally appropriate concentrations or doses were used. Some experimental flaws were identified such as the use of only two analyzable concentrations in the chromosomal analysis study, and a failure to appropriately identify the high dosage level and analyze the recommended number of PCEs in the micronucleus study. The impact of the flaw in the chromosomal analysis study can probably be disregarded since cytotoxicity was achieved, and there were no indications of chromosomal damage at the test concentrations. The flaw in the micronucleus study can be disregarded since the subsequent combination assay included an evaluation of tolcapone alone. The appropriateness of using the mouse for the *in vivo* micronucleus study is questionable since tolcapone appears to cause rat-specific tumors in kidney and uterus. Although rats are not generally used for this study because they tend to scavenge the micronucleated erythrocytes at a higher capacity than the mouse, a species-specific metabolite obviously would not have been detected in the mouse. The UDS assay conformed to guidelines, but the findings are considered limited since a very low concentration range was used because of dose-limiting toxicity at $> 5 \mu\text{g/ml}$.

The major finding from the mutagenicity battery was in the ML/TK study of tolcapone in combination with Sinemet. The sponsor's analyses considered only total colony formation where only marginal increases in mutant frequency were noted. Based on these analyses the sponsor concluded that tolcapone produced an equivocal positive response at cytotoxic concentrations. However, inspection of the data broken down by colony size suggested more substantial increases in small colony formation by tolcapone than were revealed by analysis of total colonies. These changes occurred at concentrations which decreased cell viability, but not to a level that is expected to confound interpretation of the data. The reviewer's conclusions from these data were that tolcapone is weakly mutagenic in the absence of metabolic activation, and mutagenic in the presence metabolic activation in the ML/TK assay.

Although this appears to be an isolated finding when considered in the context of the entire mutagenicity battery, its significance should not be disregarded in light of tumor findings in rat kidney and uterus. While the histopathology evidence is consistent with a non-genotoxic mechanism of renal tumor formation by tolcapone (chronic epithelial degeneration in the proximal tubules followed by regenerative hyperplasia), the clearly dose-dependent and reproducible increases in small colony formation by tolcapone raises the possibility that genotoxic mechanisms may also be involved. The apparent enhancement of mutagenicity by inclusion of S9 is consistent with the involvement of a metabolite, but not the metabolite(s) speculated by sponsor (i.e., renal sulfoconjugates).

Carcinogenicity

The carcinogenic potential of tolcapone administered in the diet was evaluated in rats and mice. The mouse study was terminated at week 80 in females and week 95 in males because the mortality rate had reached 50% in one of the drug treatment groups. Doses in the mouse study (100, 300 and 800 mg/kg/day) were selected based on MTD (mortality) in a pilot study. The rat doses (50, 250, 450 mg/kg/day) were based on both MTD and exposure data from a pilot study.

The test dose range in the mouse study is considered appropriate, since toxicity was achieved and final mean body weights were not reduced by greater than 10%. However, the exposures in the mice were relatively low in comparison to humans; the AUC in HD mice were 2.4 - 6.0 times greater than the expected exposures in humans. Clear toxicities were also achieved in rats at the high dose, and the large reductions in body weights may have affected the formation of tumors in the high dose groups (22% reduction in HDM, 27% in HDF). Exposures in the high dose group were up to 15 and 30 times higher than human exposures in male and female rats, respectively.

Epithelial or squamous cell hyperplasia, and inflammation of the forestomach were consistent non-neoplastic findings in both species at the intermediate and high dose levels. Tumors of the forestomach were observed in rats (one HDM: squamous cell carcinoma; 2 HDM: squamous cell papilloma). The sponsor suggests a direct local irritant effect of the drug as a causative factor of the forestomach pathologies. Since humans lack a forestomach, these changes are of questionable relevance, although primate esophagus sometimes responds similarly to rodent forestomach (P. Greaves, Histopathology of Preclinical Toxicity Studies, 1990).

Other findings in mice were liver changes including hepatocellular hypertrophy (MDM, HDM, HDF), granulocytosis (MDM, HDM, MDF), single cell necrosis (HDM), Kupffer cell proliferation (MDF, HDF, HDM), and lymphoid cell infiltration (HDM). Most of these changes occurred at a relatively high rate in control animals, but the increased incidence in treated animals is considered drug-related. No pathogenic mechanism for these changes was established.

In rats, marked lesions occurred in the kidney, as was observed in the one-year study. Almost all rats from the high-dose group exhibited some type of renal lesion that was identified with the summarizing term "tubulopathy" (tubular cell degeneration, tubular single cell necrosis, tubular cell hyperplasia, and/or karyocytomegaly in the straight portion of the proximal tubules). Tubular hyperplasia with atypical nuclei was seen in 4-10% of MD and HD rats. Compared to the one-year rat study in which all high-dose females exhibited this lesion, the incidence of atypical nuclei in the 2-year study was much lower. One interpretation of this trend is that the appearance and formation of atypical nuclei is an intermediate stage prior to the stages of degeneration, necrosis and regenerative hyperplasia. Alternatively, the terms "atypical nuclei" and "karyocytomegaly" (nuclear replication without cell division) may have been used to describe the same histopathological finding, as the two studies were conducted at separate locations. Both terms convey the impression that the cells of the straight portion of the proximal tubule are reacting to cell injury with a heightened state of nuclear replication, the rate of which exceeds the cell's ability to divide. According to Dr. Hard, the sponsor's expert evaluator of the data, karyocytomegaly is not necessarily a precursor stage of renal tumor formation. Nonetheless, a small number of tumors were identified. In the two-year study, tubular cell carcinomas were diagnosed in 1 MDM, 3 HDM, and 1 HDF, and tubular cell adenomas were found in 2 MDF and 1 THDF. Renal

tumors were found in the one-year study rat study (one nephroblastoma, one adenocarcinoma), and in the 13-week combination study with Sinemet (one nephroblastoma) (NOTE: Renal adenomas, carcinomas, and adenocarcinomas are epithelial tumors; nephroblastoma is an embryonal tumor. Thus, these tumors are pathomorphologically distinct. The tumors of primary interest are the epithelial tumors). Since tolcapone caused a positive response in only one of several mutagenicity assays, the sponsor's conclusion that tolcapone is not a "direct-acting" carcinogen remains viable. Despite the absence of strong mutagenic effects of tolcapone in more than one assay and the assertion of the sponsor's expert, the question of whether the atypical nuclei and karyomegaly are themselves evidence of a genotoxic event needs to be clarified before dismissing direct genotoxic actions as a mechanism of carcinogenicity. Additional genotoxicity studies are warranted since the standard mutagenicity assays would probably not detect mutagenic activity of a renal-specific sulfoconjugate metabolite of tolcapone (see below). Irrespective of the distinction between direct and indirect mechanisms, the constellation and continuum of cellular and nuclear abnormalities observed in the one- and two-year rat studies is consistent with the conclusion that tolcapone is a renal carcinogen in rats.

The proposed initiating event, degeneration of proximal tubule epithelium, is speculated to result from "metabolic overload" and exhaustion. A viable scheme to support this mechanism has been presented. The rat kidney contains enzymes, particularly in the P3 segment, which catalyze sulfoconjugation of xenobiotics. As discussed in the Pharmacokinetics & Metabolism section of the review, tolcapone is metabolized (sulfoconjugated) and excreted by the rat kidney. However, this process does not appear to be entirely species-specific according to the reviewer's interpretation of the metabolism studies. Most relevant to this argument is the possibility that the sulfoconjugation process may be more prevalent in humans than in the dog or mouse. The data from these studies are limited, and the sponsor has not conclusively identified or quantitated the metabolites in question (see PK & Metabolism section and Summary). Thus, the sponsor will have to clarify the issue of sulfoconjugation in the various species to adequately assess the relevance of the renal toxicity of tolcapone in humans.

Papillary degeneration and hyaline casts, which were also seen in the 2-year rat study, were not considered part of the changes in the proximal tubules. However, they may have arisen because of the collection cell debris from the degenerating PTE cells.

Uterine adenocarcinomas were diagnosed in 2/110 control rats (1.8%), 3/54 LD rats (5.5%), and 3/53 MD rats (5.7%), and 8/57 HD rats (14.0%). The sponsor discounted these findings as within historical control. However, the historical control range from European Pathology Services (EPS), who conducted the histopathology for the study, indicates that the highest rate of uterine adenocarcinomas in 27 studies from 1985-1990 was 8%. Thus, the incidence rate and dose-relationship (positive trend test, $p < 0.005$) suggests that the tumors were drug-related. The fact that body weight in HDF was markedly suppressed, which may have reduced the appearance of tumors, amplifies the possibility that tolcapone induces uterine tumors. Endometrial hyperplasia tended to occur at a higher incidence in drug-treated animals, but a dose-relationship was not evident (highest incidence in MD females).

PHARMACOKINETICS AND METABOLISM

Single-Dose Pharmacokinetics

Similar pharmacokinetic characteristics were observed in rats and dogs. Tolcapone has a short elimination half-life (<2 hr), low volume of distribution (0.16-0.20 L/kg), low plasma clearance (rat: 8.6, dog 1.5-2.2 ml/min/kg), and good oral bioavailability (60-75%). First-pass metabolism partly accounts for the incomplete absorption. Increases in exposures (AUC) are dose-proportional. T_{max} tends to increase with dose.

Tolcapone is rapidly metabolized to 3-O-methyltolcapone. The pharmacokinetic properties of the metabolite are similar to those of the parent compound with the exception of the elimination half-life, which is somewhat longer for 3-O-methyltolcapone. This results in relatively comparable exposures for tolcapone and 3-O-methyltolcapone, despite the lower peak levels achieved by the metabolite.

Distribution

Tolcapone has a low volume of distribution, primarily due to high protein binding (>99%). Distribution is rapid, and peak tissue levels were generally detected at the first time point (0.5 hr). Highest tissue concentrations were in the organs of absorption (gut) and elimination (kidney, liver, bile ducts). Only trace amounts of radioactivity were detected in brain (<1% of plasma). Radioactivity was essentially eliminated by 48-72 hrs. No unexpected organ specific retentions were identified, including in melanin-containing structures (pigmented epithelium of retina, pigmented skin). Only limited placental transfer to the fetus (<0.1% of dose) was observed in pregnant rats.

Binding to plasma proteins is saturable and dependent on albumin concentration. The primary binding sites were the warfarin and diazepam sites of albumin. Tolcapone was competitively displaced by both drugs, and also by salicylic acid, ibuprofen, and phenylbutazone, and non-competitively displaced by fatty acids. Although tolcapone and warfarin were suggested to bind to the same binding site, tolcapone did not increase the free fraction of warfarin. Tolcapone only slightly displaced phenytoin (< 5% increase in free fraction) and tolbutamide (<12 % increase in free fraction) from plasma protein binding sites.

Only trace amounts of tolcapone are taken up into red cells.

Metabolism

The pattern of *in vitro* and *in vivo* metabolism was qualitatively similar in rat, dog, and man. The major routes are Phase II conjugations with glucuronide and sulfate, O-methylation, and N-acetylation. Limited phase I reactions are oxidation of the methyl group and nitro-reduction.

In vivo metabolism studies with radiolabeled tolcapone revealed quantitative differences in metabolic routes. The parent compound is the major radioactive fraction in plasma (human: 59%, dog: 48%, rat: 50%). The 3-O-glucuronide conjugate of tolcapone is the main plasma metabolite in humans and dogs, accounting for 19% and 25% of drug-related radioactivity, respectively. The 3-O-methyl metabolite is the next largest fraction in humans (2%) and dogs (8%). In rat, the primary alcohol

generated by oxidation of the methyl group (14%) is slightly more abundant than both 3-O-methyltolcapone (10%) and the glucuronide conjugate (8%). Coadministration of L-DOPA did not affect glucuronide formation in rats or dogs.

Little or no parent compound was detected in urine following [¹⁴C]-tolcapone administration, but apparently a significant fraction of urinary metabolites have not been identified. In humans, the largest urinary fraction was the 3-O-glucuronide (13% of dose) of tolcapone, and the next largest fractions were sulfate or glucuronide conjugates of metabolites. In dogs, the sulfate conjugate of the nitro-reduction product was the largest urinary fraction (6.3%). Unchanged tolcapone was the largest radiolabeled biliary fraction in dogs (22% of dose), but tolcapone was not detected in rat bile. The 3-O-glucuronide conjugate was the major biliary metabolite in rat.

In the rat, the primary alcohol and sulfate conjugates derived from this metabolite were the major urinary components. This sponsor suggests that these "rat-specific" metabolites may underlie the renal toxicity of tolcapone in this species. Inspection of the chromatographic data suggests that the sulfoconjugates may not be species-specific, although the sponsor has not clearly identified the sulfoconjugates in the human and dog urinary extracts. Peaks have been identified by the reviewer in dog and human extracts which may correspond to the sulfoconjugated metabolites. The specific metabolite suggested as a potential "toxic mediator" by the sponsor is the sulfate conjugate of the primary alcohol. The fraction of dose that this metabolite represents in the urine of the various species (according to the reviewer) is:

rat:	6.5 - 8.3 %
human:	2.5 %
dog:	0.8 %
mouse:	0.7 %

These data raise the possibility that the human may be more likely to metabolize tolcapone by the potentially toxic sulfoconjugation pathway than either the dog or mouse. While it is clear that the exposures achieved in the rat toxicology exceed those expected to be routinely achieved in humans, the question remains of whether cumulative drug effects will be manifested during long-term therapy with tolcapone in humans. Therefore, in the absence of more convincing evidence from the sponsor that the renal toxicity of tolcapone is indeed a species-specific effect, the potential for this toxicity should be clearly labeled.

Elimination

Fecal excretion is the primary route of [¹⁴C]-tolcapone elimination in rats (65-78%) and dogs (76-81%). In humans, the urinary fraction is slightly larger (57%) than the fecal fraction (40%). Elimination is 90-96% complete by 5 days post-treatment. [¹⁴C]-Tolcapone is extensively excreted into milk of lactating rats.

Metabolic Drug Interactions

Tolcapone exhibited the potential to inhibit the metabolism of 2C9 substrates (diglofenac and tolbutamide), but not that of substrates for the 3A, 2A6, 2C19, and 2D6 (desipramine) isozymes.

Repeated administration of tolcapone to rats caused only slight variations in microsomal content and enzyme activity.

Effect of Tolcapone on L-DOPA Exposures

Combination toxicology studies with tolcapone and Sinemet (also Madopar) were conducted in rats, dogs, and monkeys. In both rats and dogs, the lower dosage levels of tolcapone (rat:20-50 mg/kg; dog:10 mg/kg) caused a 1.5-2 fold increase in L-DOPA AUCs, without markedly affecting the peak levels of L-DOPA in animals that received 100 mg/kg Sinemet, p.o. Higher doses of tolcapone did not cause a corresponding increase in L-DOPA exposures. However, dose-related reductions in the 3-O-methyl metabolite of L-DOPA (3-O-methyl-DOPA) were observed following tolcapone. In cynomolgus monkeys, 10-150 mg/kg tolcapone did not appreciably increase either the peak levels or AUCs of L-DOPA (250 mg/kg Sinemet, p.o.), but almost completely abolished 3-O-methyl-DOPA formation.

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EVALUATION

The main issue identified in nonclinical studies that is pertinent to the human use and labeling of tolcapone were the renal histopathologies in the long-term (≥ 1 year) rat toxicology studies. Similar histopathologies were not identified in long-term mouse or dog studies, or in the 6-month rat study. A low incidence of renal changes were observed in the 13-week rat combination study. The comparative exposures and doses used in the major toxicology studies are shown in a table on the following page.

In the one-year rat study, degeneration of the proximal tubule epithelium was seen in 6/20 females treated with 450 mg/kg/day. Atypical nuclei in proximal tubule epithelial cells were found in 10/20 females treated with 150 mg/kg/day, and 10/20 males and 20/20 females treated with 450 mg/kg/day. In the two-year study, nearly all ($\geq 95\%$) rats treated with 450 mg/kg/day, and a large fraction of rats treated with 250 mg/kg/day (78% of males, 96% of females) exhibited tubulopathy (a summarizing term when either tubular cell degeneration, necrosis, hyperplasia, and/or karyocytomegaly were seen in the straight portion of the proximal tubule). Atypical nuclei were observed at a lower incidence rate in the two-year study; it is not clear whether this finding is a precursor stage of the subsequent findings, or if different terminologies were used by the study pathologists. Renal tumors were identified in both the one-year (1 HDM adenocarcinoma, 1 HDM nephroblastoma) and two-year studies (tubular cell carcinoma: 1/51 MDM, 3/52 HDM, 1/55 HDF; tubular cell adenoma: 2/50 MDF, 1/55 HDF). A nephroblastoma was also diagnosed in a male rat treated with 200 mg/kg/day for 13 weeks. The historical incidence database submitted by the sponsor (EPS) indicated that in carcinogenicity studies conducted between 1985-1990, the highest background incidence of tubular adenomas was 2% in 2 of 27 studies. Tubular carcinomas were not seen in any of the 27 studies (no data on nephroblastomas or adenocarcinomas were provided). Thus, these tumors rarely arise spontaneously. In view of the prevalent and severe non-neoplastic renal lesions, which clearly identify the rat kidney as a target of tolcapone toxicity, the tumors are considered drug-related.

With reference to the Comparative Exposure Table, exposures at the mid-dose (250 mg/kg/day) in the two-year study were 3.4-7.2 and 4.1-14.5 times higher in male and female rats, respectively, than the expected human exposures (AUC = 80 $\mu\text{g}\cdot\text{hr}/\text{ml}$). Exposures at 450 mg/kg were 6.3-14.2 (males) and 8.6-32 (females) times higher than the expected human exposures. A precise comparison of tolcapone exposures in the one-year study could not be made since plasma levels were determined at one or two time points in rats. These data suggest that a relatively narrow margin (as low as 3- to 4-fold) exists between exposures which cause significant renal toxicity in the rat and expected therapeutic exposures in humans. The comparative data also suggests that tolcapone exposures in dogs and mice were lower than rats, which limits the sponsor's contention that the renal toxicity of tolcapone is rat-specific.

The sponsor asserts that the mechanism of renal toxicity involves a rat-specific renal metabolic pathway. In contrast to humans and dogs whose primary route of tolcapone metabolism is via conjugation with glucuronide, the primary route of rat metabolism is oxidation of the 4'-methyl group to the primary alcohol. This metabolite may subsequently be conjugated with sulfate in the P3 segment of the proximal tubule, which contains a large amount of enzymes for xenobiotic metabolism. According to the sponsor's mechanism, the excessive amounts of substrate cause an "overload" and "exhaustion" of the cells in this region leading to degeneration and necrosis. A regenerative hyperplasia occurs in response to degeneration leading to the apparent derangements in nuclear replication and cell division (atypical nuclei, karyocytomegaly).

Comparative Exposures to Tolcapone Among Species

Species	Duration	Dose	C _{max} (µg/ml)	AUC ₀₋₂₄ (µg.hr.ml)	
Human	maintenance dose	200 mg, t.i.d.	6.4 - 7.6	80	
Rat	13 wk (w/ Sinemet)	20 mg/kg/day	M: F:	3.2 - 9.8 4.5 - 12.1	363 - 485
			M: F:	30.2 - 54.1 23.8 - 140.0	
	6 months ^a	500 "	M: F:	50 100	905 2095
			M: F:	1.25 1.56	
	12 months	20 "	M: F:	9.4 13.2	
			M: F:	38.7 55.6	
			M: F:	3.1 - 4.2 3.2 - 9.4	54.5 - 76.5 56.3 - 166
	24 months	50 "	M: F:	16.1 - 29.4 27.1 - 61.2	274 - 573 328 - 1161
			M: F:	28.0 - 58.9 51.3 - 182	503 - 1138 691 - 2563
			M: F:	2.4 - 13.2 3.9 - 11.6	6.6 - 33.5 ^b 7.6 - 33.9 ^b
Dog	13 wk (w/ Sinemet)	10 "	M: F:	6.0 - 35.3 8.1 - 54.4	16.5 - 113 ^b 34.4 - 201 ^b
			M: F:	8.5 - 14.5	20.8 - 39.7
	12 months	40 "	M: F:	28.3 - 44.9	80 - 159
			M: F:	42.4 - 94.2	345 - 655
			M: F:	2.8 - 3.0 3.4 - 5.4	42.7 - 55.9 68.0 - 97.8
Mouse	95 weeks (male) 80 weeks (female)	100 "	M: F:	4.4 - 9.7 6.2 - 7.5	101 - 180 102 - 157
			M: F:	11.9 - 21.3 24.3 - 26.1	189 - 397 345 - 483

a = not reviewed; b = AUC₀₋₇

The mechanism posited by the sponsor is viable, and consistent with the histopathology according to an expert evaluation (Gordon Hard, current affiliation is unknown). However, a review of the metabolism data suggests that the sulfoconjugation process may not be species-specific. A comparison and quantitation of (unidentified) peaks in chromatograms of human, dog and mouse urine that appear to coelute with the rat peaks identified as the sulfoconjugate of the alcohol, suggests that humans form approximately one-third as much of the sulfoconjugate as the rat. More importantly, humans form three times as much of the sulfoconjugate as the dog or the mouse. Therefore, if sulfoconjugation is involved in the renal toxicity of tolcapone, humans may be more susceptible than dogs or mice, but less susceptible than rats. It should be stressed that this conclusion is contingent upon the conclusive identification and quantitation of the sulfoconjugation pathway in the four species. The (lowest) cumulative margin between the amounts of metabolite that is necessary to induce renal toxicity and the amounts of sulfoconjugated metabolite that may be generated by humans is approximately 12 (4-fold difference in exposure at rat mid-dose x 3-fold difference in amounts of sulfoconjugate formed). While this margin is relatively large and conservative, human safety considerations should also include the expected long-term duration of tolcapone use and the potential for cumulative drug effects. The need for an appropriate labeling statement on the renal findings is increased by the absence of coincident changes in laboratory markers in rats (BUN, creatinine, urinalysis) that could identify the onset of renal pathologies.

An additional consideration with respect to the renal toxicity of tolcapone is the possible influence of coadministration of L-DOPA. The sponsor has conducted 13-week multiple-dose toxicology studies of tolcapone and L-DOPA preparations (Sinemet, Madopar) in rats and dogs. Although L-DOPA did not dramatically potentiate tolcapone toxicity in either species, a low incidence (1-2 animals per group) of renal changes were observed in rats in the 13-week study (degeneration, vacuolation, pigment deposits and hyaline droplets in proximal tubule epithelium; a nephroblastoma in a high-dose tolcapone male is likely incidental). No renal changes were observed in the 6-month rat study at doses up to 500 mg/kg/day, which raises the possibility that L-DOPA may reduce the onset time necessary for evoking the renal changes, even at the lower doses used in the 13-week study (200 mg/kg/day). Since L-DOPA and dopamine are also substrates for renal sulfoconjugation (Kienzl et al., *J. Neural Transm. Suppl.*, 32:471, 1990), an additional "overload" or "exhaustion" of the PTE cells would be expected with a consequent enhancement of the renal toxic effects of tolcapone. The distribution of the findings among treatment groups in the 13-week study (i.e., in both combination and tolcapone-only animals) is not consistent with the conclusion that Sinemet enhanced the toxic effects of tolcapone. A 6-month combination study may have provided a clearer indication of L-DOPA potentiation of tolcapone toxicity, although it is not clear whether a better indication of the human relevance for this interaction would be obtained. The fact that L-DOPA exposures in rats during the combination studies greatly exceeded human therapeutic exposures without markedly enhancing tolcapone toxicity over a short duration is reassuring in this regard.

A dose-related increased incidence of uterine adenocarcinomas was also observed in the two-year rat study. At the highest dose, the incidence ($8/57 = 14\%$) appears to exceed historical controls (highest rate in 27 EPS studies was 8%); the p value of the trend test was <0.0005 . The sponsor contends that the incidence rate is within historical control, and cites a literature review containing data on the spontaneous occurrence of uterine adenocarcinomas in 5 studies with Wistar European rats (see attached Table 4 from Brown and Leininger, in *Pathobiology of the Aging Rat*, ILSI Publishing, 1992). The maximal rate of spontaneous tumor formation in female rats between 24-26 months old was 12.7. The highest

spontaneous incidence rate (39) was seen in a study of rats older than 26 months of age, which renders that value inappropriate for comparison to the tolcapone study. In the opinion of the reviewer, the most appropriate comparisons are derived from the database of the testing laboratory EPS. On the basis of this historical value and the highly significant trend test, the uterine adenocarcinomas in this study are considered drug-related. These findings should be added to the labeling statement (they were not present in the sponsor's proposed label).

As with renal tumor formation, a direct genotoxic action is not the most likely mechanism of uterine tumor formation because of the single positive mutagenicity finding in the ML/TK assay. A non-neoplastic lesion, endometrial hyperplasia, was seen in both controls (10%) and treated animals (14-30%), but did not increase in a dose-related manner (highest incidence at the mid-dose). The sponsor suggests that estrogenic effects of tolcapone may contribute to these lesions, but no hormone analyses were conducted. Although dopamine agonists are generally estrogenic in rats, the degree to which tolcapone can stimulate hormone release in the absence of the L-DOPA must be experimentally determined. Thus, the relationship of tolcapone to estrogen release and endometrial hyperplasia is unclear. However, a plausible hypothesis analogous to that for renal tumor formation can be developed for the uterine tumors (i.e., a hyperplastic response to tissue injury by tolcapone).

As stated, indirect, non-genotoxic mechanisms for tumorigenicity are the most plausible because of the largely negative genotoxicity results. In the opinion of the reviewer, the mouse lymphoma/thymidine kinase assay suggests that tolcapone was mutagenic, particularly with respect to small colony formation, in the presence and absence of metabolic activation. This conclusion differs from that of the sponsor, who states in the proposed label that tolcapone is not genotoxic. The clearly reproducible and dose-related increases in small colony formation caused by tolcapone in the ML/TK assay, particularly in the presence of metabolic activation, should be included in the label. Since it is unlikely that standard mutagenicity assays would have detected the genotoxic actions of a rat kidney-specific sulfoconjugated metabolite of tolcapone, appropriately designed mutagenicity studies should be designed with the specific aim of characterizing genotoxicity by this mechanism. Studies could include an *in vitro* test for chromosomal or DNA damage in a rat kidney cells (lines or primary cultures). The possible involvement and species-specificity of renal metabolism could also be evaluated in a standard mutagenicity assay in which kidney metabolizing fractions are used. In these studies, the speculated intermediate/substrate of sulfoconjugation, the 4'-primary alcohol, should be evaluated in addition to tolcapone since it may not be derived by the renal metabolizing fraction.

The sponsor has appropriately recommended a Pregnancy category C classification for tolcapone. The basis for the recommendation was that tolcapone will be used as an adjunct to L-DOPA, which causes skeletal and visceral malformations in rabbits. The sponsor concluded that tolcapone by itself was not teratogenic and did not impair fertility or reproductive performance. However, tolcapone demonstrated an abortive potential in rabbits at 100 and 400 mg/kg. Embryotoxic and fetotoxic potential was evident in the Segment II rat study as resorptions (6 of 36 dams at 300 mg/kg), stillborns (2 dams at 150 mg/kg with 7 and 5 stillborns in their litters), and early pup loss (2 dams at 150 lost 5 and 4 pups, respectively). Developmental impairments (body weight development, learning performance) were seen in pups from dams treated with 250 mg/kg in the rat Segment III study. These findings suggestive of reproductive and developmental toxicities of tolcapone by itself should be added to the Pregnancy labeling.

Table 4. Laboratory data on the incidence of nonneoplastic and neoplastic lesions in Wistar European rats, according to age and reproductive status

Age interval (months)	3			6			10-15			18			24-26			>26														
Reproductive status	NP			NP			NP			NP			P			NP														
Endocrine profile	Normal cycle-estrus ^a 4 days			Normal cycle-estrus ^a 4 days			Constant estrus ^a			Persist. diestrus			Persist. diestrus			Persist. diestrus														
Hormone levels E (pg/ml)																														
Pr (ng/ml)																														
TA (pg/ml)																														
Uterine weight-(% b. wt)																														
Laboratory	F	G	H	F	G	H	F	G	H	F	G	H	L	F	N	H	L	N	G	H										
Lesions—nonneoplastic (%):																														
Cystic endometrial hyperplasia																														
Luminal dilation (hydrometra)	24.5						20						18.2			11.8			14.3											
Cystic or dilated glands	4.0						6.0						18.2						11.2											
Endometritis (PMNs)	6.1						4.0						3.9			5.9			2.0											
Stromal pigment							34.0						22.1																	
Epithelial metaplasia																5.9														
Decidual reaction (deciduoma)																														
Lesions—neoplastic (%):																														
Fibromatous polyp (stromal)													33.8 ^a			24.0			5.9			3.0								
Adenoma/adenomatous polyp													1.3			2.0			0.5			3.0								
Adenocarcinoma (carcinoma)													1.3			12.7			5.0			11.0			39.0			0.5		
Stromal sarcoma										0.5															4.1					
Leiomyoma																1.0														
Leiomyosarcoma																1.0									1.0					
Fibroma																														
Fibrosarcoma																														
Squamous cell carcinoma													3.0 ^b			3.0			4.0			4.1								
Carcinosarcoma																1.0									12.0					
Sarcoma, NOS																16.7									4.0					
Embryonal carcinoma																									1.0					
Hemangioma																4.0														
Mesenchymal tumors													3.0			4.0			18.0			5.0								

For definitions of abbreviations, footnotes, and key to laboratory data, see Table 2.

Abbreviations: P, parous; NP, nonparous; E, estradiol-17 β ; Pr, progesterone; TA, total androgens

Footnotes (see also Tables 3-5):

^a Based on 17 animals.^b Based on 20 animals.^c Values given are for diestrous animals at 10 months, considered by the author to be comparable to constant estrous animals.^d The laboratory noted a two- to fourfold variability in incidence between studies.^e Data are from rats at 240 days of age.^f Data from a single 90-day NTP study (10 animals).^g Organ wt. courtesy of Dr. L. Gordon, Merck Sharp and Dohme.^h Hormone values taken from Tang et al. (1984) Biol Reprod 31:399-413.ⁱ Organ wt. courtesy of Dr. M. McKenzie, Ortho Pharmaceutical.^j Organ wt. courtesy of Dr. T. Hara, Kyowa Hakko Kagaku.

Laboratory data key (see also Tables 3-5):

A—Data courtesy of Dr. K. Yoshimura

30 virgin, 1-20 weeks old

B—Data courtesy of Merck Sharp and Dohme, West Pt., PA

1820 virgin, 1-30 weeks old

352 virgin, 21-33 weeks old

359 virgin, 34-39 weeks old

2017 virgin, 60-200 weeks old

C—Data courtesy of National Toxicology Program, NIEHS—based on four studies

152 virgin, up to 110 weeks old

87 virgin, up to 136 weeks old

D—Data courtesy of Dr. K. Mizumori and T. Hara, Inst. of Environ. Tox., Tokyo, Japan

48 virgin, 31 weeks old

48 virgin, 57 weeks old

23 virgin, 83 weeks old

149 virgin, 109 weeks old

F—Data courtesy of Dr. R. Beem, TNO-CIVO Inst. Netherlands

49 virgin, 3 mo old

30 virgin, 12 mo old

77 virgin, 24 mo old

G—Data from Dearberg et al. (1981)

305 virgin

H—Data from Kross et al. (1981)

192 virgin

I—Data courtesy of Dr. S. Jones, Hazleton Labs, America

80 virgin, 58 weeks old

289 virgin, 110 weeks old

J—Data from Mastawa et al. (1983) J Toxicol Sci 8:279-290

88 virgin

K—Data from Murakita et al. (1977a)

49 virgin, up to 36 mo old

Data from Murakita et al.

L—Data from Rubin et al. (1984)

299 virgin/296 animal breeder

M—Data from Mechanick (1973)

1278 rats

N—Data courtesy of Dr. K. Krieg, Hoechst, Germany, maximal rate given

522 virgin

P—Data from Tang and Tang (1981) Gynecol Oncol 12:51-63

20 virgin, 20 mo old

40 virgin, 31 mo old

T—Data from Prejean et al. (1973)

181 virgin

X—Data courtesy of Dr. E. Gillard, Charles River Labs.

11 F344/26 Sprague, 21 days old (virgin)

12 F344/12 Sprague, 80 days old (breeder)

12 F344/30 Sprague, 240 days old (retired breeder)

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European Pathology Services (EPS) Historical Control Tumor Incidence Data

HISTORICAL CONTROL TUMOR INCIDENCE WISTAR RAT

ORGAN: KIDNEYS

ADENOMA (synonyms: tubular adenoma; cortical adenoma)

PROJECT	STUDY TYPE	REPORT PATH.	ANIMALS EXAMINED		ANIMALS WITH TUMORS		INCIDENCE IN %	
			M	F	M	F	M	F
00521	104 WEEK FEEDING	1985 JMA	30	30	-	-	0	0
00521	104 WEEK FEEDING	1985 JMA	30	30	-	-	0	0
00521	104 WEEK FEEDING	1986 RNB	30	30	-	-	0	0
006390	104 WEEK FEEDING	1986 RNB	30	30	-	-	0	0
017820	104 WEEK FEEDING	1986 HW	30	30	-	-	0	0
024300	104 WEEK FEEDING	1986 JMA	99	100	-	-	0	0
027753	104 WEEK I. N.	1989 JMA	52	50	-	-	0	0
027753	104 WEEK FEEDING	1989 VIL	30	30	-	-	0	0
046980	104 WEEK FEEDING	1989 BSC	60	60	-	-	0	0
082252	104 WEEK FEEDING	1990 JMA	60	58	-	-	0	0
041993	112 WEEK FEEDING	1989 GPZ	30	30	-	-	0	0
046912	114 WEEK FEEDING	1989 BSC	30	30	-	-	0	0
008831	116 WEEK FEEDING	1986 PAC	30	30	-	-	0	0
027472	120 WEEK FEEDING	1987 BSC	30	30	1	1	2	2
071212	120 WEEK FEEDING	1988 HJC	100	100	-	-	0	0
041192	120 WEEK FEEDING	1989 HJC	30	30	-	-	0	0
041727	122 WEEK FEEDING	1990 HW	30	30	1	-	2	0
004285	130 WEEK FEEDING	1985 BSC	30	30	1	1	2	2
014387	130 WEEK FEEDING	1986 JAW	30	30	1	-	2	0
014387	130 WEEK FEEDING	1986 JAW	49	49	-	-	0	0
018205	130 WEEK FEEDING	1986 JAW	30	30	-	-	0	0
046923	130 WEEK FEEDING	1990 JMA	30	30	-	-	0	0
071526	130 WEEK FEEDING	1990 BSC	30	30	-	-	0	0
071537	130 WEEK FEEDING	1990 HW	30	30	-	-	0	0
085487	130 WEEK FEEDING	1990 JMA	30	49	-	-	0	0
086672	112 WEEK FEEDING	1990 PAC	30	30	-	-	0	0
086672	112 WEEK FEEDING	1990 PAC	30	30	-	-	0	0

• 2nd control group.

HISTORICAL CONTROL TUMOR INCIDENCE WISTAR RAT

ORGAN: KIDNEYS

CARCINOMA (synonyms: adenocarcinoma; tubular carcinoma; renal carcinoma)

PROJECT	STUDY TYPE	REPORT PATH.	ANIMALS EXAMINED		ANIMALS WITH TUMORS		INCIDENCE IN %	
			M	F	M	F	M	F
00521	104 WEEK FEEDING	1985 JMA	30	30	-	-	0	0
00521	104 WEEK FEEDING	1985 JMA	30	30	-	-	0	0
00521	104 WEEK FEEDING	1986 RNB	30	30	-	-	0	0
006390	104 WEEK FEEDING	1986 RNB	30	30	-	-	0	0
017820	104 WEEK FEEDING	1986 HW	30	30	-	-	0	0
024300	104 WEEK FEEDING	1986 JMA	99	100	-	-	0	0
027753	104 WEEK I. N.	1989 JMA	52	50	-	-	0	0
027753	104 WEEK FEEDING	1989 VIL	30	30	-	-	0	0
046980	104 WEEK FEEDING	1989 BSC	60	60	-	-	0	0
046980	104 WEEK FEEDING	1989 BSC	60	60	-	-	0	0
082252	104 WEEK FEEDING	1990 JMA	60	58	-	-	0	0
041993	112 WEEK FEEDING	1989 GPZ	30	30	-	-	0	0
046912	114 WEEK FEEDING	1989 BSC	30	30	-	-	0	0
008831	116 WEEK FEEDING	1986 PAC	30	30	-	-	0	0
027472	120 WEEK FEEDING	1987 BSC	30	30	-	-	0	0
071212	120 WEEK FEEDING	1988 HJC	100	100	-	-	0	0
041192	120 WEEK FEEDING	1989 HJC	30	30	-	-	0	0
041727	122 WEEK FEEDING	1990 HW	30	30	-	-	0	0
004285	130 WEEK FEEDING	1985 BSC	30	30	-	-	0	0
014387	130 WEEK FEEDING	1986 JAW	30	30	-	-	0	0
014387	130 WEEK FEEDING	1986 JAW	49	49	-	-	0	0
018205	130 WEEK FEEDING	1986 JAW	30	30	-	-	0	0
046923	130 WEEK FEEDING	1990 JMA	30	30	-	-	0	0
071526	130 WEEK FEEDING	1990 BSC	30	30	-	-	0	0
071537	130 WEEK FEEDING	1990 HW	30	30	-	-	0	0
085487	130 WEEK FEEDING	1990 JMA	30	49	-	-	0	0
086672	112 WEEK FEEDING	1990 PAC	30	30	-	-	0	0
086672	112 WEEK FEEDING	1990 PAC	30	30	-	-	0	0

• 2nd control group.

HISTORICAL CONTROL TUMOR INCIDENCE WISTAR RAT

ORGAN: UTERUS

ADENOCARCINOMA

PROJECT	STUDY TYPE	REPORT PATH.	ANIMALS EXAMINED		ANIMALS WITH TUMORS		INCIDENCE IN %	
			M	F	M	F	M	F
00521	104 WEEK FEEDING	1985 JMA	49	4	-	-	0	0
00521	104 WEEK FEEDING	1985 JMA	30	1	-	-	0	2
00521	104 WEEK FEEDING	1986 RNB	49	1	-	-	0	2
006390	104 WEEK FEEDING	1986 RNB	49	-	-	-	0	0
017820	104 WEEK FEEDING	1986 HW	30	4	-	-	0	0
024300	104 WEEK FEEDING	1986 JMA	100	1	-	-	0	1
027753	104 WEEK I. N.	1989 JMA	31	-	-	-	0	0
046980	104 WEEK FEEDING	1989 VIL	30	2	-	-	0	4
046980	104 WEEK FEEDING	1989 BSC	60	3	-	-	0	5
082252	104 WEEK FEEDING	1990 JMA	38	2	-	-	0	4
041993	112 WEEK FEEDING	1989 GPZ	30	-	-	-	0	0
046912	114 WEEK FEEDING	1989 BSC	30	1	-	-	0	2
008831	116 WEEK FEEDING	1986 PAC	30	-	-	-	0	0
027472	120 WEEK FEEDING	1987 BSC	30	4	-	-	0	0
071212	120 WEEK FEEDING	1988 HJC	100	1	-	-	0	1
041192	120 WEEK FEEDING	1989 HJC	49	-	-	-	0	0
041727	122 WEEK FEEDING	1990 HW	30	1	-	-	2	2
004285	130 WEEK FEEDING	1985 BSC	30	3	-	-	0	6
014387	130 WEEK FEEDING	1986 JAW	30	-	-	-	0	0
014387	130 WEEK FEEDING	1986 JAW	47	1	-	-	0	2
018205	130 WEEK FEEDING	1986 JAW	30	3	-	-	0	6
046923	130 WEEK FEEDING	1990 JMA	30	1	-	-	0	2
071526	130 WEEK FEEDING	1990 BSC	49	-	-	-	0	0
071537	130 WEEK FEEDING	1990 HW	30	1	-	-	0	2
085487	130 WEEK FEEDING	1990 JMA	47	2	-	-	0	4
086672	112 WEEK FEEDING	1990 PAC	30	2	-	-	0	4
086672	112 WEEK FEEDING	1990 PAC	30	1	-	-	0	2

• 2nd control group.

2 pages

PURGED

(DRAFT LABELING)

RECOMMENDATIONS

1. The NDA is approvable.
2. Labeling recommendations are in the preceding section.
3. Additional genotoxicity and metabolism studies of tolcapone are warranted to more adequately assess the human risk of tolcapone-induced renal toxicity and tumorigenicity. Appropriately designed genotoxicity studies using either kidney cells and/or kidney metabolizing fractions should determine if a kidney-specific metabolite is genotoxic. The use of kidney metabolizing fractions from rat, human, dog and mouse would clarify the species susceptibility profile for the renal toxicity of tolcapone. The speculated intermediate metabolite, the 4'-alcohol, should be included in these studies. Additional comparative metabolism studies (human, rat, dog, mouse) focusing on the urinary formation of sulfate conjugates of tolcapone are also recommended. These studies should be conclusive with respect to metabolite identification and quantitation.



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Pharmacologist/Toxicologist

Original NDA 20697

cc.: /Division File, HFD-120
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