5.2 Overview of Clinical Development Program

To be filled in post briefing.

5.3 In Vitro Pharmacology

5.3.1 Receptor Binding

pKis and IC_{50}s for various receptors from humans and other species are shown in Table 4, Table 5, and Figure 2 on the following pages.

Asenapine has high receptor affinities for all dopamine, serotonin, alpha-adrenergic, and histamine receptors tested, as well as for norephinephrine and dopamine reuptake transporters based upon typical Cmaxs in the range of 3 - 30 nMol/L (1 - 10 ng/ml) with doses of 5 – 10 mg SL BID, (see Table 53, Table 55 and Figure 51) and typical IC_{50}'s in the range of 0.1 – 4 nMol/L, (see Table 4 and Table 5). Although this estimate does not take into account free concentrations which are around 5% of total it's likely that the receptor binding experiments were conducted in the presence of albumin and so a correction is not needed. In addition, the results of PET studies also suggest that corrections for protein binding are unnecessary, (see Table 155).

In addition to the receptors mentioned, above Figure 2 also shows that asenapine has effects on potential down-stream intracellular mediators.

Unfortunately the sponsor does not indicate whether binding at the various receptors result in antagonism or agonism, and this would be needed to predict potential pharmacologic effects such as cardiac valvulopathy with agonism of $5HT_{2B}$ receptors.

Table 4 Reported pKis for Human Receptor Binding and Transporters

	R&DRR INT00002643					Study 00003223				
Receptor	Asenapine	(-)asenapine	(+)asenapine	N-desmethyl	N-oxide	Org 191634-0 N-sulfated-N-Desmethyl	Org 213772-0 11-OH	Org 214025-0 11-O-sulfate	Org 216761-0 N-Gluc	Org 220473-0 7-OH
5-HT1A	8.60 ± 0.04	8.04 ± 0.03	8.57 ± 0.02	8.21 ± 0.09	5.97 ± 0.01	8.0	8.4	7.5	<5	7.6
5-HT1B	8.40 ± 0.08	8.77 ± 0.11	8.60 ± 0.02	6.70 ± 0.01	7.45 ± 0.02					
5-HT2A	**10.15 ± 0.09**	**10.21 ± 0.08**	**10.40 ± 0.11**	8.62 ± 0.04	8.22 ± 0.14	7.6	10.0	**9.9**	<6	**9.9**
5-HT2B	**9.75 ± 0.03**	**9.42 ± 0.29**	**9.04 ± 0.40**	8.61 ± 0.27	7.42 ± 0.09	8.0	10.0	**9.4**	<6	**9.5**
5-HT2C	**10.46 ± 0.15**	10.00 ± 0.13	10.38 ± 0.28	8.73 ± 0.25	8.22 ± 0.04	7.7	9.9	**9.4**	<6	**9.9**
5-HT5A	**8.84 ± 0.21**									
5-HT6	**9.60 ± 0.04**	9.58 ± 0.11	9.90 ± 0.08	7.86 ± 0.07	7.07 ± 0.02	7.7	**10.0**	**9.7**	<6	**9.1**
5-HT7	**9.94 ± 0.04**	**10.04 ± 0.05**	9.67 ± 0.13	7.98 ± 0.05	7.24 ± 0.08	7.5	9.8	9.6	<6	8.8
D1	**8.85 ± 0.04**	**8.80a**	**8.82 a**	6.92 a	6.69 a					
D2L	**8.90 ± 0.08**	**8.69 ± 0.13**	**8.72 ± 0.14**	7.26 ± 0.04	6.20 ± 0.14					
D2S	**8.84 ± 0.05**	**8.86 ± 0.13**	**8.96 ± 0.16**	7.32 ± 0.09	6.32 ± 0.15	7.0	8.4	7.8	<6	8.4
D3	**9.38 ± 0.06**	**9.37 ± 0.29**	**9.32 ± 0.07**	7.72 ± 0.05	6.69 ± 0.03	7.4	8.4	8.1	<6	**9.1**
D4	**8.95 ± 0.07**	8.98 ± 0.08	8.61 ± 0.07	7.01 ± 0.11	6.35 ± 0.08					
D4.7						6.9	9.0	8.4	<5	8.6
α1A	**8.93 ± 0.04**	**8.84 ± 0.04**	**8.99 ± 0.06**	7.56 ± 0.07	6.50 ± 0.04	7.8	9.0	8.3	<6	8.4
α2A	**8.94 ± 0.05**	**9.07 ± 0.07**	8.62 ± 0.05	7.76 ± 0.02	6.26 ± 0.03	7.1	8.2	7.7	<6	8.2
α2B	**9.49 ± 0.02**	**9.66 ± 0.03**	**9.40 ± 0.11**	8.64 ± 0.10	6.89 ± 0.05					
α2C	**8.91 ± 0.12**	**8.96 ± 0.09**	8.31 ± 0.02	7.43 ± 0.02	6.21 ± 0.05	7.2	8.0	7.8	<6	8.0
H1	**9.00 ± 0.13**	8.48 a	8.92a	7.20 a	6.48 a	7.7	8.9	8.8	<6	**9.9**
H2	8.21 ± 0.10	7.92 a	7.25 a	5.39 a	5.48 a					
M1	5.09 ± 0.03	5.14 ± 0.01	4.99 ± 0.12	5.08 ± 0.04	4.22 ± 0.04					
M2	4.50 ± 0.09	4.41 ± 0.09	4.48 ± 0.08	4.44 ± 0.08	4.19 ± 0.01					
M3	4.67 ± 0.03	4.81 ± 0.06	4.66 ± 0.27	4.59 ± 0.05	4.17 ± 0.01					
M4	5.04 ± 0.10	5.14 ± 0.07	5.21 ± 0.05	5.03 ± 0.08	4.43 ± 0.01					
M5	<5					<5	<5	<5	<5	<5
SERT	<5					<5	<5	<5	<5	<5
NET	<5.5					<5.5	<5	<5	<5	<5
DAT	<5					<5	<5	<5	<5	<5

Table 5 Estimated IC_{50}s (nMol/L) for Human Receptor Binding and Transporters Based on Reported pK_is

	R&DRR INT00002643					Study 00003223				
Receptor	Asenapine	(-)asenapine	(+)asenapine	N-desmethyl	N-oxide	Org 191634-0 N-sulfated- N-Desmethyl	Org 213772-0 11-OH	Org 214025-0 11-O-sulfate	Org 216761-0 N-Gluc	Org 220473-0 7-OH
5-HT1A	2.5	9.1	2.7	6.2	1,071.5	10.0	4.0	31.6		25.1
5-HT1B	4.0	1.7	2.5	199.5	35.5					
5-HT2A	0.1	0.1	0.0	2.4	6.0	25.1	0.10	0.13		0.13
5-HT2B	0.2	0.4	0.9	2.5	38.0	10.0	0.10	0.40		0.32
5-HT2C	0.03	0.1	0.0	1.9	6.0	20.0	0.13	0.40		0.13
5-HT5A	1.4									
5-HT6	0.3	0.3	0.1	13.8	85.1	20.0	0.1	0.2		0.8
5-HT7	0.1	0.1	0.2	10.5	57.5	31.6	0.2	0.3		1.6
D1	1.4									
D2L	1.3	2.0	1.9	55.0	631.0					
D2S	1.4	1.4	1.1	47.9	478.6	100.0	4.0	15.8		4.0
D3	0.4	0.4	0.5	19.1	204.2	39.8	4.0	7.9		0.8
D4	1.1	1.0	2.5	97.7	446.7					
D4.7										
α1A	1.2	1.4	1.0	27.5	316.2	15.8	1.0	5.0		4.0
α2A	1.1	0.9	2.4	17.4	549.5	79.4	6.3	20.0		6.3
α2B	0.3	0.2	0.4	2.3	128.8					
α2C	1.2	1.1	4.9	37.2	616.6	63.1	10.0	15.8		10.0
H1	1.0					20.0	1.3	1.6		0.1
H2	6.17									
M1	8,128	7,244	10,233	8,318	60,256					
M2	31,623	38,905	33,113	36,308	64,565					
M3	21,380	15,488	21,878	25,704	67,608					
M4	9,120	7,244	6,166	9,333	37,154					
M5	2.5	9.1	2.7	6.2	1,071.5	10.0	4.0	31.6		25.1
SERT	4.0	1.7	2.5	199.5	35.5					
NET	0.1	0.1	0.0	2.4	6.0	25.1	0.10	0.13		0.13
DAT	0.2	0.4	0.9	2.5	38.0	10.0	0.10	0.40		0.32

Figure 2 Asenapine Enantiomer Binding to Various Receptors by Species – Report SDGRR 4393

Table 1. Pharmacology of Org 5222 and enantiomers *in vitro*

Test	Receptor	Parameter	Org 5222	Org 10968 (-)	Org 10969 (+)
Serotonin					
5-HT binding (human receptor clone)	5-HT_{1A}	pK_i	7.1		
8-OH-DPAT binding (rat hippocampus)	5-HT_{1A}	pK_i	7.6		
8-OH-DPAT binding (human receptor clone)	5-HT_{1A}	pK_i	8.1	7.7	8.1
GTPγS binding (human receptor clone)	5-HT_{1A}	pIC_{50}	*	7.0	*
		α	0.3	0	0.5
cAMP turnover (human receptor clone)	5-HT_{1A}	pIC_{50}	7.1	7.0	7.2
		α	0.5	0	0.5
5-HT binding (pig striatum)	5-HT_{1D}	pK_i	7.1	7.1	7.3
5-HT release (guinea pig cortex)	5-HT_{1D}	%increase	50% at 10^{-7}mol/L		
Ketanserin binding (rat cortex)	5-HT_{2A}	pK_i	10		
Ketanserin binding (human receptor clone)	5-HT_{2A}	pK_i	10.4	10.3	10.3
PI turnover (human receptor clone)	5-HT_{2A}	pIC_{50}	10.6	10.3	10.4
5-HT binding (pig choroid plexus)	5-HT_{2C}	pK_i	10.1	10.0	10.1
5-HT binding (human receptor clone)	5-HT_{2C}	pK_i	9.1	9.6	9.8
Mesulergine binding (human receptor clone)	5-HT_{2C}	pK_i	10.1	10.0	10.5
PI turnover (human receptor clone)	5-HT_{2C}	pIC_{50}	8.9	8.6	8.5
Dopamine					
Spiperone binding (human receptor clone)	D_{2S}	pK_i	8.8		
Spiperone binding (human receptor clone)	D_{2L}	pK_i	8.8		
Spiperone binding (human receptor clone)	D_3	pK_i	9.1		
Spiperone binding (human receptor clone)	D_4	pK_i	8.9		
Antagonism of quinpirole adenyl cyclase inhibition (human receptor clone) control	D_{2L}	pIC_{50}	6.3		
+Org 5222	D_{2L}	pIC_{50}	4.1		
	agonist/antagonist shift		138		
Acetylcholine					
Oxotremorine M binding (rat cortex)	$M_{1,2}$	pK_i	5.2		
Pirenzepine binding (rat brain)	M_1	pK_i	4.4		
QNB binding (rat brainstem)	$M_{1,2}$	pK_i	6.0		

* Accurate IC_{50} value could not be calculated due to a biphasic effect

Effects of acute and chronic (21 days, b.i.d.) s.c. treatment of rats with Org 5222 on the levels of dopamine (DA) and serotonin (5-HT) and their major metabolites [3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (HVA) and 5-hydroxy indoleacetic acid (5-HIAA)] in the nucleus accumbens with olfactory tubercles and the caudate nucleus.

		DA	DOPAC	HVA	5-HT	5-HIAA
Acute[1]	60 min	NC	increase	increase	NC	NC
	12 h	NC	increase	NC	NC	NC
Chronic[2]	21 days(A)	NC	NC	NC	NC	NC
	21 days(B)	NC	increase	increase	NC	NC

NC = no significant change

1) Single doses were administered 60 min or 12 h before decapitation and the subsequent determination of DA, 5-HT and their major metabolite levels in the brain areas.

2) The last injection was given 12 h (A) or 30 min (B) before decapitation and the subsequent determination of DA, 5-HT and their major metabolite levels in the brain area.

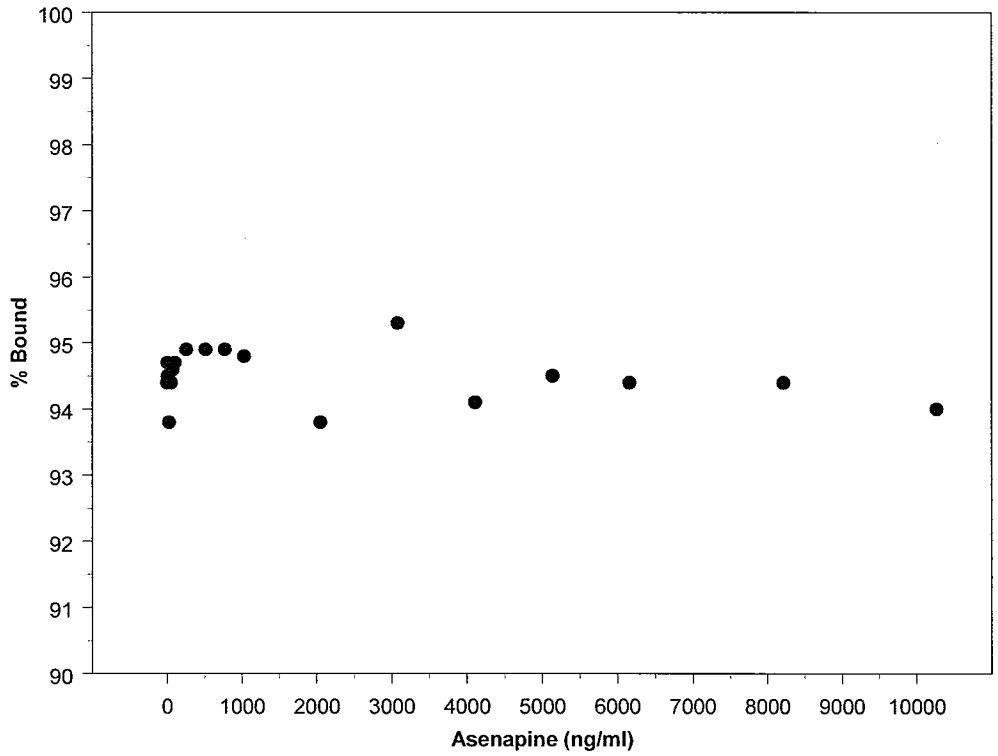
5.3.2 Protein Binding

Asenapine binding to human plasma proteins assessed by equilibrium dialysis was non-saturable over a concentration range of 1.4 to 10,268 ng/ml, with a mean free fraction of 5.5%, (see Table 6 and Figure 3).

Table 6 Asenapine Plasma Protein Binding over a Concentration Range of 1.4 to 10,268 ng/ml – Study SDGRR 2972

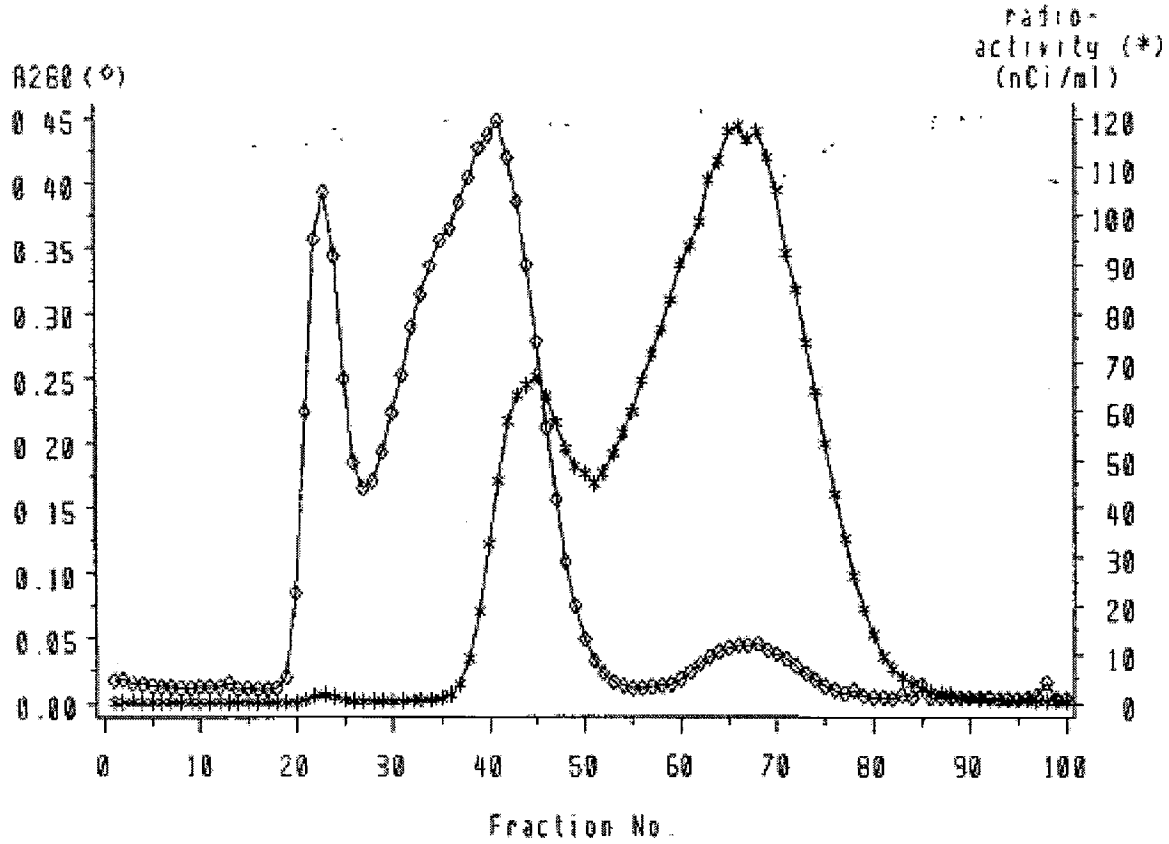
Fraction Bound f_{Bnd} (%)	Fraction Unbound fu (%)
94.5 + 0.4 (0.4) 93.8 – 95.3	5.5 + 0.4 (7.0) 4.7 – 6.2

Figure 3 Asenapine Plasma Protein Binding over a Concentration Range of 1.4 to 10,268 ng/ml – Study SDGRR 2972



The elution profile of radiolabeled [^{3}H]-asenapine from a Sephadex G-200 column indicates that the majority of radioactivity comes off the column as unbound radioactivity whereas a small fraction comes off with a retention time similar to that of low molecular weight proteins. This indicates that asenapine is likely primarily bound to albumin, (see Figure 4).

Figure 4 Elution Profiles of Plasma Proteins (A_{280}) and Radiolabeled [^{3}H]-Asenapine 80 ng/ml from a Sephadex G-200 Column — Study SDGRR 2972



In two other studies, high binding (>95%) to plasma proteins was shown for asenapine, desmethyl-asenapine and asenapine 11-O-sulfate. For asenapine and desmethyl-asenapine total binding was higher in women than in men. However the binding to albumin and to AAG is much lower and these should only account for binding of around 81.5% and 53.2% of asenapine and desmethyl-asenapine respectively. Consequently, a significant fraction of the binding of these species is due to some other unidentified plasma protein, (see Table 7 - Table 9).

Thus it's unclear what changes in plasma proteins might result in changes in free fraction. Since asenapine is a high intrinsic clearance compound, changes in protein binding might result in differences in kinetics.

Table 7 Asenapine and Desmethyl-Asenapine Binding in Human Plasma by Equilibrium Dialysis over 4 hours – Study DM2005-005222-007

Conc. (ng/ml)	Fu (%)			
	Asenapine		Desmethyl-Asenapine	
	Males	Females	Males	Females
1	4.01 ± 2.01	1.66 ± 0.23	3.97 ± 2.30	2.45 ± 0.46
25	2.81 ± 1.03	1.72 ± 0.38	0.872 ± 0.111	1.84 ± 0.48
500	3.10 ± 1.30	2.07 ± 0.55	3.24 ±4.10	1.86 ± 0.90
Average	3.28 ± 1.47	1.81 ± 0.40	2.86 ± 2.89	2.08 ± 0.65

N = 3 - 7

Table 8 Equilibrium Dialysis Plasma Protein Binding of 11-Hydroxy- Asenapine Sulfate (ORG-214025) 200 ng/mL – Study DM2006-005222-015

Species	% Free	% Bound
Human	2.88 ± 0.12 (4.05) 1.75 - 3.03	97.1± 0.12 (0.12) 97.0 - 97.2
Rat	0.98± 0.05	99.0± 0.05
Rabbit	0.23± 0.02	99.8± 0.02

Table 9 Asenapine and Desmethyl-Asenapine Binding to Human Serum Albumin and α1-Acid Glycoprotein by Equilibrium Dialysis over 4 hours – Study DM2005-005222-007

Conc. (ng/ml)	Fu (%)			
	Human Serum Albumin		α1-Acid Glycoprotein	
	Asenapine	Desmethyl-Asenapine	Asenapine	Desmethyl-Asenapine
1	47.1 ± 1.9	38.3	25.4 ± 4.6	45.8 ± 5.1
25	45.8 ± 3.0	36.9 ± 3.1	18.9 ± 3.5	58.0 ± 16.2
500	45.4 ± 1.4	37.2 ± 2.9	25.2 ± 2.1	68.7 ± 14.1
Average	46.1 ± 2.1	37.3 ± 2.5	23.0 ± 4.6	57.5 ± 15.1

Table 10 Reviewer's Estimated Total Asenapine and Desmethyl-Asenapine Binding to Plasma Proteins based on Binding to HSA and AAG in Study DM2005-005222-007

Substrate	Asenapine		Desmethyl - Asenapine	
Protein	HSA	AAG	HSA	AAG
f_{Bnd} (%)	46.1 ± 2.1	37.3 ± 2.5	23.0 ± 4.6	57.5 ± 15.1
Additional % Bound due to AAG	(100 – 46.1) * 37.3 = 20.1%		(100 – 23.0) * 57.5 = 44.1%	
Estimated Total % Bound	46.1 ± 20.1 = 60.2%		23.0 ± 44.1 = 67.1%	
Estimated Total % Free	39.8%		32.9%	

5.3.3 Binding to Red Blood Cells

Asenapine and or a metabolite binds to and sequesters in red blood cells such that the radioactivity measured in RBCs is higher than expected concentration based on passive diffusion alone. Table 11 shows the sponsor's value for the extent of binding, whereas Table 12 shows the reviewer's calculations.

Even though they differ slightly there is probably minimal to any pharmacokinetic significance, although pharmacodynamic significant is unknown.

N.B. These calculations do not account for free concentrations consequently the fraction bound is approximately 20 fold higher.

Table 11 Sponsor's Calculated *In Vitro* Binding of [^{3}H]-Asenapine to male human erythrocytes Study R&DRR NL0029630

[^{3}H]-Org 5222[a] (ng/mL)	Time (min)	Hcrit	Whole blood Radioactivity (Bq/mL)	Plasma Radioactivity (Bq/mL)	R	E
0	60	0.395	3772	5185	0.73	0.18
5		0.395	3855	5280	0.73	0.18
25		0.405	3766	5123	0.73	0.18
200		0.400	3905	5471	0.72	0.17
1000		0.400	3941	5304	0.74	0.19
10000		0.410	3814	5001	0.76	0.22
Mean ± SD (%CV)					73.5 ± 1.4 (1.9%)	18.7 ± 1.75 (9.4%)

[a] Blood samples were spiked with 0, 5, 25, 200, 1000 and 10000 ng/mL unlabeled Org 5222 and 3.66 kBq/mL [^{3}H]-Org 5222 (equivalent to 2.1 ng·mL^{-1})
E = fraction bound to erythrocytes
R = whole blood to plasma radioactivity ratio

Table 12 Reviewer's Calculated *In Vitro* Binding of [^{3}H]-Asenapine to male human erythrocytes Study R&DRR NL0029630

[^{3}H]-Org 5222[a] (ng/mL)	Time (min)	Hcrit	1- Hcrit	Plasma Radioactivity (Bq/mL)	Expected Whole Blood Radioactivity with Passive Diffusion [Plasma Radioactivity/(1-Hcrit)]*Hcrit	Measured Whole blood Radioactivity (Bq/mL)	RBC:Plasma Ratio
0	60	0.395	0.605	5185	3137	3772	1.114
5		0.395	0.605	5280	3194	3855	1.118
25		0.405	0.595	5123	3048	3766	1.080
200		0.400	0.600	5471	3283	3905	1.071
1000		0.400	0.600	5304	3182	3941	1.114
10000		0.410	0.59	5001	2951	3814	1.097
Mean ± SD (%CV)							1.099 ± 0.02 (1.8%)

[a] Blood samples were spiked with 0, 5, 25, 200, 1000 and 10000 ng/mL unlabeled Org 5222 and 3.66 kBq/mL [^{3}H]-Org 5222 (equivalent to 2.1 ng·mL^{-1})
E = fraction bound to erythrocytes
R = RBC to plasma radioactivity ratio

5.3.4 Cell Transport - Pgp

The sponsor reported the following results for cell transport studies with asenapine and N-desmethyl-asenapine:

'Bi-directional transport studies were performed in MDCK and MDR1-MDCK (MDR1) cells to determine the extent of P-glycoprotein (P-gp) mediated transport of [^{3}H]- asenapine and [^{3}H]-N-desmethyl asenapine. The bi-directional transport studies were carried out at 31.6, 100 and 316 nM of asenapine and N-desmethyl asenapine. In addition, [3H]-diazepam (1 µM), [^{3}H]-prazosin (2 µM) and [^{3}H]-quinidine (2 µM) were included as negative, weak positive, and moderate positive P-gp controls, respectively.

The apical to basolateral (A→B) transport of asenapine across the MDCK and MDR1 cell monolayers was characterized by mean effective permeability (Pe, ×106 cm/s) values of 3.12 – 3.51, and 1.90 – 2.43, respectively, over the concentration range studied (31.6 – 316 nM). The corresponding values for N-desmethyl asenapine are 2.24 – 2.94, and 1.82 – 2.25, respectively. The efflux ratios of asenapine in MDCK and MDR1 cells ranged from 0.862 – 1.02, and 0.914 – 1.29, respectively, and the corresponding values for N-desmethyl asenapine were 0.677– 0.836, and 0.596 – 0.720, respectively.

The MDCK normalized efflux ratio of asenapine and N-desmethyl asenapine in MDR1 cells ranged from 1.02 – 1.34 and 0.767 – 1.06, respectively. The corresponding values for P-gp control substrates were 0.903, 0.982, and 2.49 for diazepam, prazosin and quinidine, respectively.' (See Table 13 and Figure 5).

These results suggest that asenapine and N-desmethyl asenapine have low to moderate effective permeability under our experimental conditions and at best are weak substrates of the human P-gp transporter. Thus, it is unlikely P-gp will have a significant impact on the in vivo disposition of asenapine and N-desmethyl asenapine.'

Due to the high binding to the cell membranes effective permeability coefficients, (*Pe*), are reported for asenapine and desmethyl-asenapine, whereas apparent permeability coefficients, (*Papp*), are reported for the control substrates.

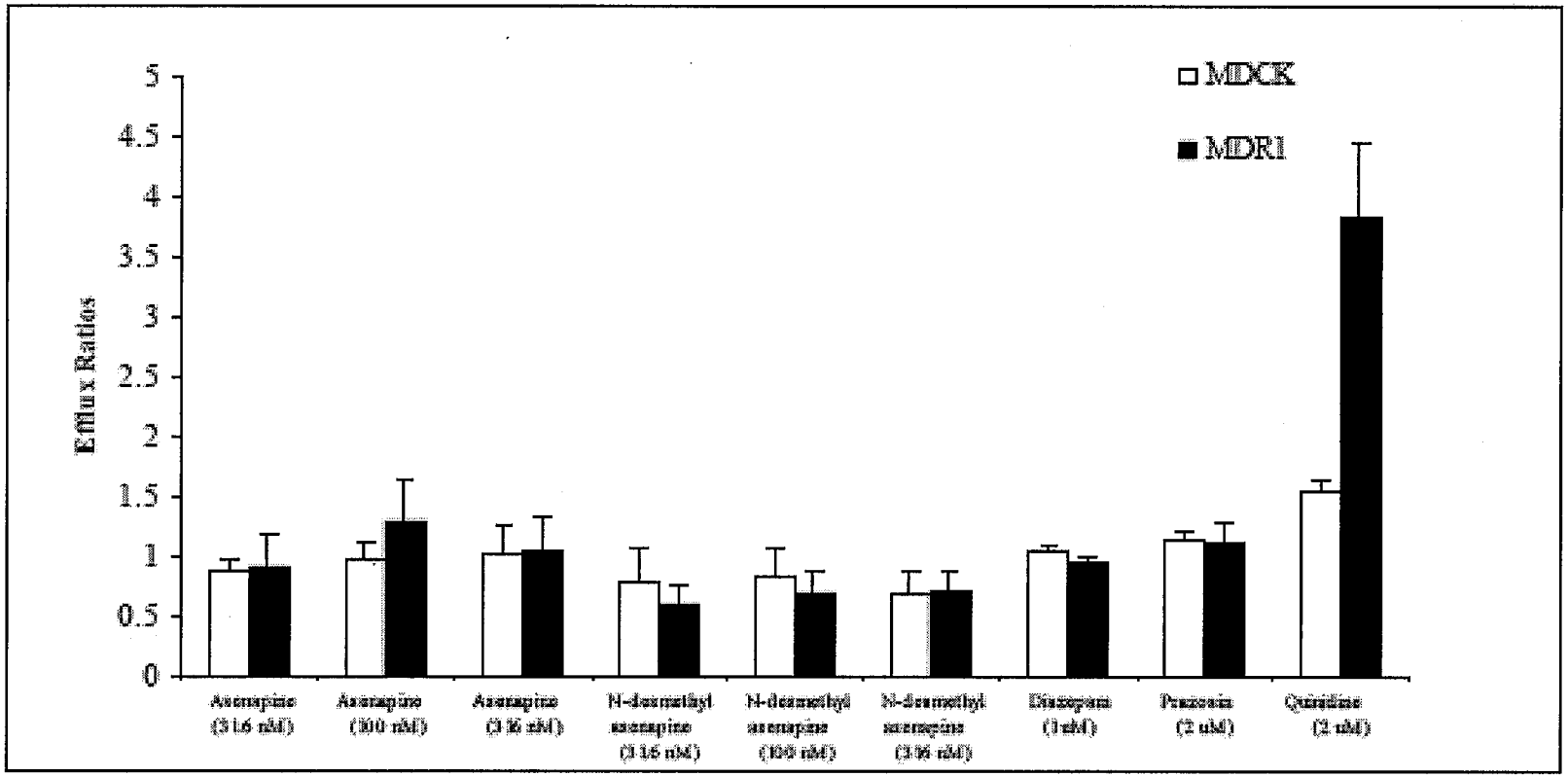
As a highly lipophilic substances these results are expected, however different results might be found for the 7- and 11- Hydroxy metabolites and especially for the sulfate and glucuronide conjugates. Also, other transporters in addition to P-gp may be involved and these other potential substrates and transporters have not been examined.

Table 13 P-gp Cell Transport of [^{3}H] Asenapine and [^{3}H] N-Desmethyl-Asenapine – Study DM2005-005222-008

	Substrate	Type of Control	Concentration	A → B Pe or Papp (cm/sec x 10^6 cells)	B → A Pe or Papp (cm/sec x 10^6 cells)	P-gp Efflux Ratios $\frac{B \rightarrow A\ P_e}{A \rightarrow B\ P_e}$
				MDCK	MDR1	MDR1/MDCK
Controls	Diazepam	Negative	1.0 µM	1.04 ± 0.04	0.939 ± 0.047	0.903
	Prazosin	Weak positive	2.0 µM	1.14 ± 0.08	1.12 ± 0.18	0.982
	Quinidine	Strong positive	2.0 µM	1.54 ± 0.11	3.83 ± 0.63	2.49
Test Substrates	Asenapine		31.6 nM	0.862 ± 0.101	0.914 ± 0.256	1.06
			100.0 nM	0.960 ± 0.152	1.29 ± 0.35	1.34
			316.0 nM	1.02 ± 0.23	1.04 ± 0.29	1.02
	N-Desmethyl asenapine		31.6 nM	0.777 ± 0.291	0.596 ± 0.160	0.767
			100.0 nM	0.836 ± 0.226	0.698 ± 0.185	0.835
			316.0 nM	0.677 ± 0.186	0.720 ± 0.158	1.06

a Mean ± SD, n = 4

Figure 5 Efflux Ratios of [^{3}H]-Asenapine, [^{3}H]-N-desmethyl asenapine, and [^{3}H]-P-gp Control Substrates for MDCK and MDR1 Cells – Study DM2005-005222-008



*: Efflux ratios for asenapine and N-desmethyl asenapine were calculated using Pe due to extensive membrane retention, while Papp was used for calculating efflux ratios of diazepam, prazosin, and quinidine.

5.4 Drug Metabolism

5.4.1 Overview of Human Drug Metabolism

Come back to post briefing.

5.4.2 *In Vivo* Drug Metabolism

5.4.2.1 Location of Information

In vivo drug metabolism and mass balance was formally examined at steady-state in study 25532. Results were reported in the clinical trial report for study 25532, (including sub-reports) as well as the reports INT00008145 (AKA 040105) and INT00003211 (AKA 40218). The additional reports were not always cross referenced appropriately and were found by accident. The manner of reporting the information from the mass balance study was confusing as it required extensive cross checking of documents that were labeled with different report codes on the page headers. For future reference these are included in Table 14.

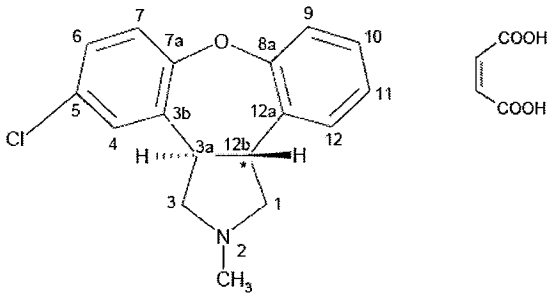
Table 14 Cross References of Reports of Differing Aspects of Mass Balance Study 25532

Study Report Code	Additional Coding inside Main Report	Report Title
25532		Open, non-randomized, single center trial to determine the excretion balance, metabolic profile and pharmacokinetics of asenapine after a sub-lingual dose of [14C]-labeled asenapine.
	NL0057152	Bioanalysis of Asenapine, Org 30526 and Org 31437 in human plasma samples from Clinical Trial 25532
	PBR-041201	The Determination of [14C]-Asenapine in Human Plasma, Urine and Faeces Samples Originating From a Human ADME Study With Liquid Scintillation Counting
INT00003211	040218	Profiling of a Metabolism Study with [14C]-Labeled Asenapine in Healthy Volunteers (Additional to Clinical Trial Protocol 25532)
INT00008145	040105	Isolation and Identification of Metabolites of Asenapine (ORG 5222) in Various Types of Samples

5.4.2.2 Study Design

Study 25532 utilized a single 0.3 mg dose of ^{14}C-Asenapine [56 µCi] administered on day 10 of asenapine administration by placing an ethanolic solution containing the radioactive dose on a 10 mg tablet of unlabeled asenapine and administering it sublingually. This resulted in a total dose of 10.3 mg in six healthy male volunteers that included three smokers and three nonsmokers. Figure 6 shows the numbering of asenapine and location of the ^{14}C label.

Figure 6 Asenapine Numbering and Location of ^{14}C Label



* is the place of the [^{14}C]- label in [^{14}C]-asenapine maleate.

Plasma was sampled through 72 hours post dose. In addition feces and urine were to be collected until >90% of the total radioactivity was recovered; although this was not done, possibly due to partial loss of the collected sample, a technical issue, or inaccurate dosing.

Subjects were also phenotyped for CYP1A2, 2C19, 2D6, and 3A4 using the cocktail shown in Table 15.

Table 15 Phenotyping Cocktail – Study 25532

CYP P450	Substrate	Dose (mg)	Measurement	Matrix
1A2	Caffeine	100	Paraxanthine / caffeine ratio at 6 h	Plasma
2C19	Mephenytoin	100	4-OH S-Mephenytoin / S-Mephenytoin excreted over 8 h	Urine
2D6	Dextromethorphan	30	Dextrorphan / dextromethorphan at 4 h	Plasma
3A4	Cortisol	Endogenous	6 β-OH Cortisol / Cortisol excreted over 8 h	Urine

Results for phenotyping are stated as being reported in report INT00003211, however this reviewer was unable to find any data on phenotype.

Subject Demographics

Table 16 shows the demographics of the enrolled subjects, Subjects 5 and 6 were withdrawn from the study due to opisthotonus on day 5. It's noteworthy that these two subjects had the lower weights and thus possibly higher concentrations. Although there were supposed to be 3 smokers and 3 non-smokers, the smoker who dropped out had nicotine metabolite exposures that were inconsistent with smoking.

Table 16 Demographics of Subjects in Mass Balance Study – Study 25532

Subject	Age	Gender	Race	Height (cm)	Weight (kg)	BMI (kg/m^2)	Smoker (Yes/No)	Serum Nicotine Metabolites (ng/mL)
1	40	Male	White	177	87.6	28.0	Yes	724.0
2	23	Male	White	179	90.1	28.1	No	<10.0
3	54	Male	White	176	82.1	26.5	No	<10.0
4	33	Male	White	180	80.1	24.7	Yes	424.0
5[a]	23	Male	White	184	69.5	20.5	No	<10.0
6[a]	21	Male	Asian	167	62.3	22.3	Yes	<10.0
N = 4[a]	**38 ± 13**			**178 ± 1.8**	**85.0 ± 4.7**	**26.8 ± 1.6**		

a Dropped out due to severe opisthotonus on day 5

b Mean ± SD of study completers

5.4.2.3 Analytic Methodology

Initially a µ-Bondapak phenyl column was used (HPLC system 1) however the metabolite profile was not reproducible on a replacement column. Consequently, a new HPLC system was developed on a µ-Bondapak C18 column (HPLC system 2). This necessitated a change in the mobile phase gradient. The two HPLC systems used are shown in Table 17.

Table 17 Comparison of HPLC Systems Used for Metabolic Profiling of Mass Balance Study 25532

System	*HPLC System 1*	*HPLC System 2*
Guard-column	µ-Bondapak phenyl	µ-Bondapak C18
Column	µ-Bondapak phenyl (internal length: 300 mm; internal diameter: 7.8 mm)	µ-Bondapak C18 (internal length: 300 mm; internal diameter: 7.8 mm; particle size: 10 µm)
Solvents	A. 0.1 mol·L^{-1} Ammonium acetate buffer, adjusted to pH=4.2 with acetic acid	A. 0.1 mol·L^{-1} Ammonium acetate buffer, adjusted to pH=4.2 with acetic acid
	B. Methanol/ Acetonitrile (1/3 v/v %)	B. Methanol/ Acetonitrile (1/3 v/v %)
Gradient	5% B isocratic during 5 minutes	10% B isocratic during 3 minutes
	5 to 35% B in 30 minutes (linear)	10 to 40% B in 17 minutes (linear)
	35 to 90% B in 20 minutes (linear)	40 to 90% B in 30 minutes (linear)
	90 to 100% B in 1 minutes (linear)	90 to 95% B in 1 minute (linear)
	100% B isocratic during 9 minutes	95% B isocratic during 8 minutes
	100% to 5% B in 5 minutes (linear)	95 to 10% B in 1 minute (linear)
Flow	2.0 mL/min	2.0 mL/min
Temperature	50°C	50°C
LS-Flow	3.5 mL/min	3.5 mL/min
LS Cell-volume	0.5 mL	0.5 mL

System 1 and system 2 employed different HPLC numbering systems.

Metabolite numbering, except for human metabolites, was generally performed based on the retention time for each separate matrix. For human metabolites numbering for HPLC system 2 for all matrices were based on the retention times for peaks from chromatograms from all matrices. Thus the retention time of metabolite U10 is comparable with the retention time of metabolite F10. In contrast HPLC System 1 utilized a different peak numbering system.

Multiple metabolites have been identified and associated with a single or even two overlapping peaks from HPLC system 2. The identification of multiple metabolites associated with these peaks was based on further characterizations of the pooled urine and feces peak components. To do this the sponsor subjected the effluent of the initial radiochromatography (HPLC system 2) to a second HPLC-UV chromatographic process (HPLC system 3) and collected fractions of the effluent of this second chromatographic process. Fractions of effluent representing separate peaks were recombined and the metabolite(s) contained in them were identified. Although it might be possible to further identify amounts associated with these individual fractions, time constraints prevent this. See Figure 7 for an example of the HPLC fraction chromatogram of urine peak 35 (aka PC2) showing two subpeaks, U35A and U35B.

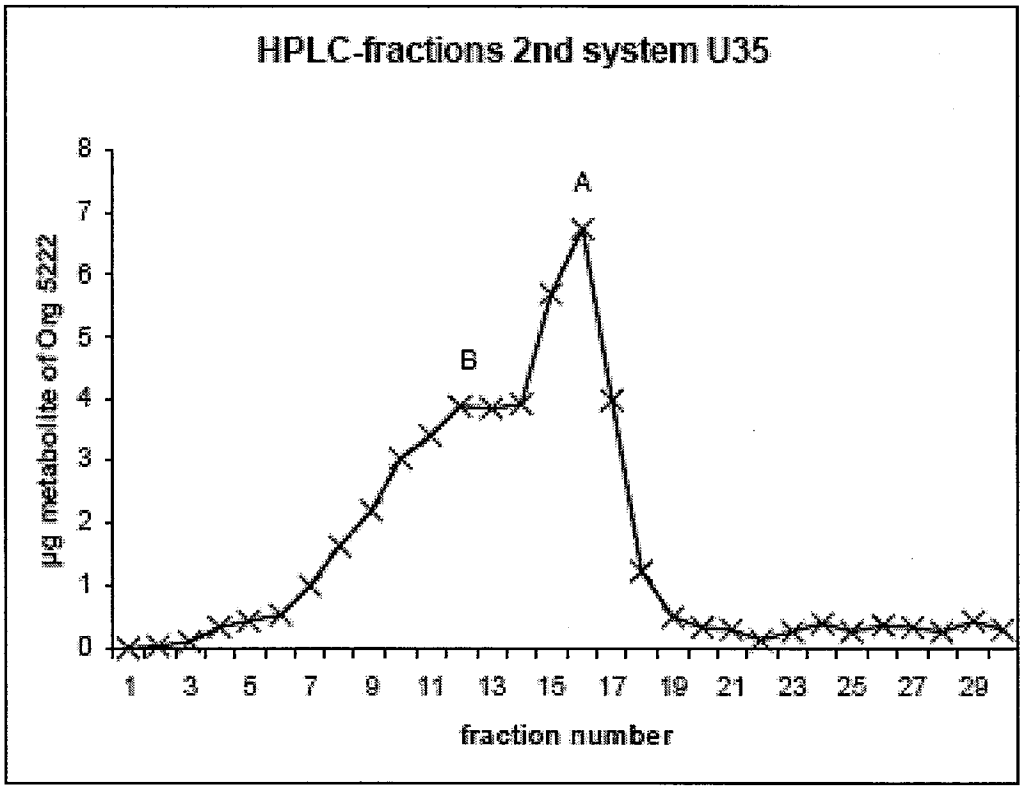
In addition, a fourth HPLC system was also utilized for radio-chromatograms, that included purification and identification of the subpeaks of HPLC system 2 but the description is confusing and will not be discussed further.

Retention times, numbering and identified metabolites associated with various peaks and HPLC systems are shown in Table 18.

Table 18 Peak Numbering Found in HPLC Chromatograms from Plasma, Urine and Feces Samples Collected after Sublingual Administration of [^{14}C]-Asenapine – Study 25532

Nominal Description	HPLC System 2										HPLC System 1	
	Combined Peak #	Urine			Feces			Secondary Isolation (HPLC Systems 3 and 4)			Peak no.	Mean RT (minutes)
		Peak no.	Mean RT (minutes)	Relative RT	Peak no.	Mean RT (minutes)	Relative RT	Combined Peak #	Isolation codes	Mean RT (minutes)		
	PC1	U1	15.2	0.45								
N(2)-des-methyl asenapine 10-Methoxy 11-O-Glucuronide & N(2)-des-methyl asenapine 10-O-Glucuronide 11-Methoxy	PC2	**U2**	**16.6**	**0.49**				PC2	U35	16.6	1	24.5
	PC3	U3	17.6	0.52								
	PC4	U4	18.5	0.55								
	PC5	U5	19.3	0.57								
N(2)-des-methyl asenapine 10-methoxy 11-O-Sulfate & N(2)-des-methyl asenapine 10-O-Sulfate 11-Methoxy N-des-methyl asenapine 11-O glucuronide Asenapine-11-O-glucuronide Plus some other sulphates and glucuronides	PC6	**U6**	**22.0**	**0.65**				PC6	U80	22.0	4	32.1
	PC7	U7	22.7	0.68								
U8/9 contained some conjugated metabolites (sulphates and glucuronides)	PC8	U8	23.3	0.69								
	PC9	**U9**	**23.6**	**0.70**				PC8/9	U87	23.3/23.6	6/7	35.1/36.2
U10/11 Asenapine 11-O-Sulfate N-oxide asenapine sulphates and glucuronides F10/11 is identified as the 10, 11-dihydroxy-des-methyl asenapine and 10, 11-dihydroxy-asenapine.	PC10	**U10**	**25.1**	**0.74**	**F10**	**25.1**	**0.75**	PC10/11	U108	25.2/25.5	10	38.9
	PC11	**U11**	**25.6**	**0.76**	**F11**	**25.6**	**0.76**		P72	22.4		
U12/13 asenapine glucuronide	PC12	**U12**	**26.8**	**0.80**				PC12/13	F71b	24.9/25.6		
	PC13	**U13**	**27.2**	**0.81**	F13	27.2	0.81		U117	26.8/27.3	11	40.8
									P84 and P88	24.6/25.1/25.3		
	PC14				**F14**	**28.4**	**0.85**					
	PC15	U15	29.0	0.86	**F15**	**29.0**	**0.86**					
U16- N(2)-des-methyl asenapine glucuronide	PC16	**U16**	**30.7**	**0.91**	F16	30.7	0.91	PC16	U151	30.7	13	44.6
									P107 & P110	28.4/28.9		
	PC17				F17	31.4	0.93					
	PC18				F18	32.1	0.96					
F19 co-elutes with the N(2)-des-methyl of asenapine	PC19				F19	32.6	0.97	PC19	P116	29.7		
asenapine	PC20				**F20**	**33.6**	**1.00**	PC20	F127	33.6	15	47.5
									P115 & P120	29.7/30.6		
	PC21				F21	34.6	1.03					
11-hydroxy N-formyl asenapine	PC22				**F22**	**35.1**	**1.04**	PC22	F151c	35.1		
X-hydroxy N-formyl asenapine (the position of the hydroxyl group could not be assigned)	PC23				**F23**	**36.2**	**1.08**	PC23	F159c	36.2		

Figure 7 HPLC Fractions from HPLC System 3 associated with Urine Peak 35 (AKA PC2) from HPLC System 2 – Report 040218



5.4.2.4 *Extent of Recovery of Radioactivity*

Cumulative radioactivity recovery was >85% in 3 of the 4 subjects with approximately 40% of the dose recovered in feces and 50 – 60% of the dose recovered in urine. The low recovery of radioactivity in subject 3 was attributed by the sponsor as likely due to inadvertent loss of part of the urine sample. This is a reasonable possibility. Total individual and mean recoveries by route are show in Table 19, Figure 8, and Figure 9.

Table 19 Cumulative Radioactivity Recovery in Urine and Feces after Sublingual Administration of Asenapine 10 mg plus [^{14}C]-Asenapine 0.3 mg – Study 25532

	Excreted Radioactivity (% of the Radioactive Dose)					
	Subject 1	Subject 2	Subject 3	Subject 4	Mean ± SD	Mean ± SD (excluding subject 3)
Urine	50.7	58.8	37.0	49.0	48.9 ± 9.0 37 - 59	52.8 ± 5.3
Feces	36.2	37.1	34.8	47.0	38.8 ± 5.6 35 - 47	40.1 ± 6.0
Total	86.9	95.9	71.8	96.0	87.7 ± 11.4 (72 – 96)	93.0 ± 5.2

Figure 8 Cumulative Radioactive Excretion Profile by Subject after Sublingual Administration of Asenapine 10 mg plus [^{14}C]-Asenapine 0.3 mg – Study 25532

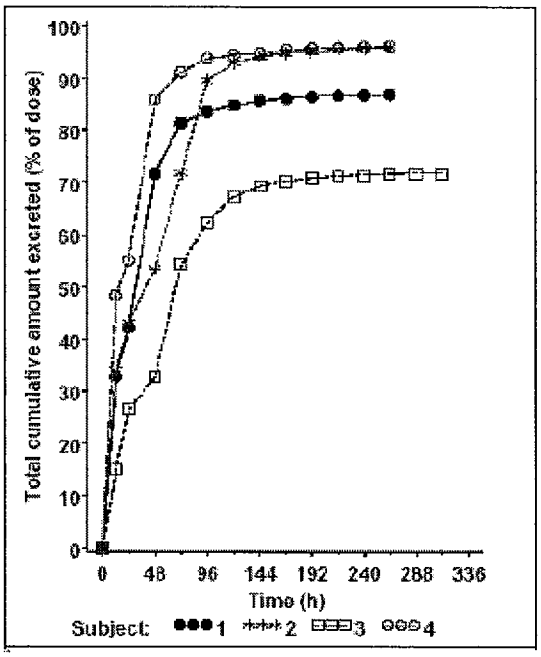
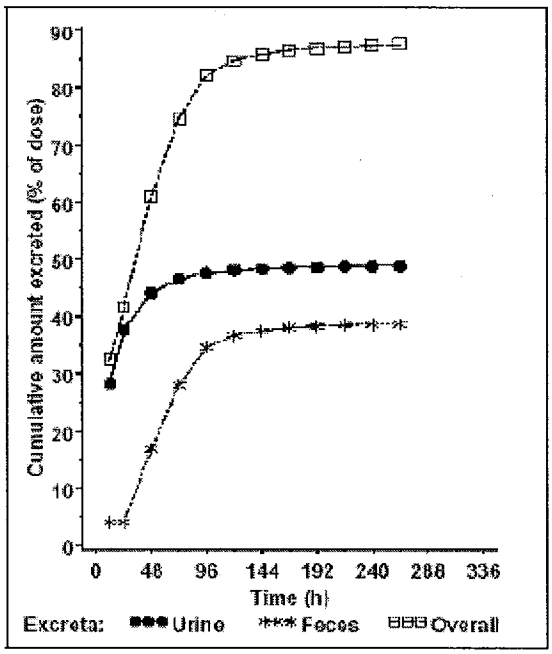


Figure 9 Cumulative Radioactive Excretion in Urine and Feces after Sublingual Administration of Asenapine 10 mg plus [^{14}C]-Asenapine 0.3 mg – Study 25532



5.4.2.5 *Plasma Metabolic Profiles*

5.4.2.5.1 HPLC System 1

At first plasma samples at selected time points between 1.5 – 12 hours were analyzed per subject on HPLC system 1. These data were used to give quantitative data. Representative radiochromatograms and quantitative data are shown in Figure 10 and Table 20 respectively.

Figure 10 Radio-Chromatograms at 1.5 and 4 hours from Subjects 1 & 4 – Study 25532 / Report 040218

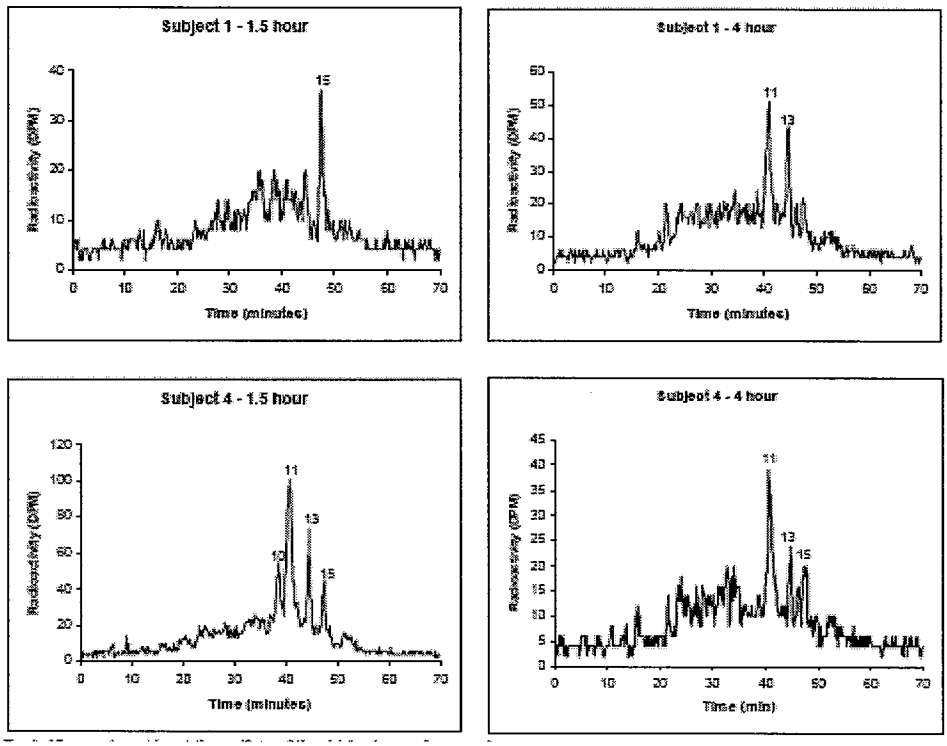


Table 20 Individual Plasma Concentrations by Time Point Detected by HPLC System 1 after Sublingual Administration of ^{14}C-Asenapine – Study 25532 / Report 040218

Subject	Peak	Analyte Identity	RT	1.5 h	2.0 h	4.0 h	8.0 h	12.0 h
1	10	11-OH-Asenapine	38.7	—	—	—	—	—
	11	Asenapine-Glucuronide	40.8	—	—	6.3	3.5	—
	13	N-Desmethyl-Glucuronide	44.6	—	—	2.6	2.9	—
	15	Asenapine	47.5	2.2	2.2	—	—	—
2	10	11-OH-Asenapine	38.7	2.0	—	—	—	—
	11	Asenapine-Glucuronide	40.8	13.2	12.5	10.1	—	—
	13	N-Desmethyl-Glucuronide	44.6	1.9	—	—	—	—
	15	Asenapine	47.5	2.8	3.1	—	—	—
3	10	11-OH-Asenapine	38.7	—	—	—	—	—
	11	Asenapine-Glucuronide	40.8	7.6	10.7	15.9	10.5	3.4
	13	N-Desmethyl-Glucuronide	44.6	—	—	—	—	—
	15	Asenapine	47.5	3.8	—	—	—	—
4	10	11-OH-Asenapine	38.7	4.5	2.3	—	—	—
	11	Asenapine-Glucuronide	40.8	12.0	11.8	9.3	6.7	—
	13	N-Desmethyl-Glucuronide	44.6	3.8	3.0	3.3	—	—
	15	Asenapine	47.5	2.1	1.4	3.0	—	—

Figure 10 and Table 20 only show the three metabolites that the sponsor also measured by standard analytic methodologies, yet in the study reports the sponsor states that at least 9 different peaks could be identified using system 1. The sponsor then further explains that the resolution of the obtained metabolite signals of urine and feces samples obtained on HPLC system 1 was sub-optimal (see Figure 10), and the integration of the metabolite profiles was inconclusive (see Table 20).

5.4.2.5.2 LSC and Bioanalysis of Selected Metabolites

In addition to comparing radioactivity via HPLC system 1, the sponsor also compared the plasma concentrations of selected species determined by standard bioanalytic methods, (i.e. asenapine, desmethyl-asenapine, and asenapine N-oxide) to total plasma radioactivity as determined by scintillation counting.

Figure 11 shows the mean plasma concentration vs. time profile for asenapine, desmethyl-asenapine, asenapine-N-oxide, and total ^{14}C in asenapine, ng-eq/mL. Since this study was conducted at steady-state the total radioactivity reflects the radioactivity for a single dose, whereas the concentrations of asenapine and the two metabolites can readily be seen to be superimposed on concentrations from prior dosing. Thus even though the relative exposures to asenapine and the metabolites are at best only a few % of the exposure to all species just based on the relative concentrations, if corrected for superpositioning the relative exposures would be even lower and the amount of unidentified species would account for nearly all of the circulating radioactivity. In addition, asenapine was administered at a dose of 10 mg, whereas the radioactive dose was less than 0.3 mg, yet the peak asenapine concentration is around 10 ng/mL, which is what we expect from a 10 mg dose. Thus it appears that the relative exposures for asenapine the metabolites and the radioactivity were not corrected for the disparate doses.

Figure 11 Mean Plasma concentration-versus-time curves – Study 25532

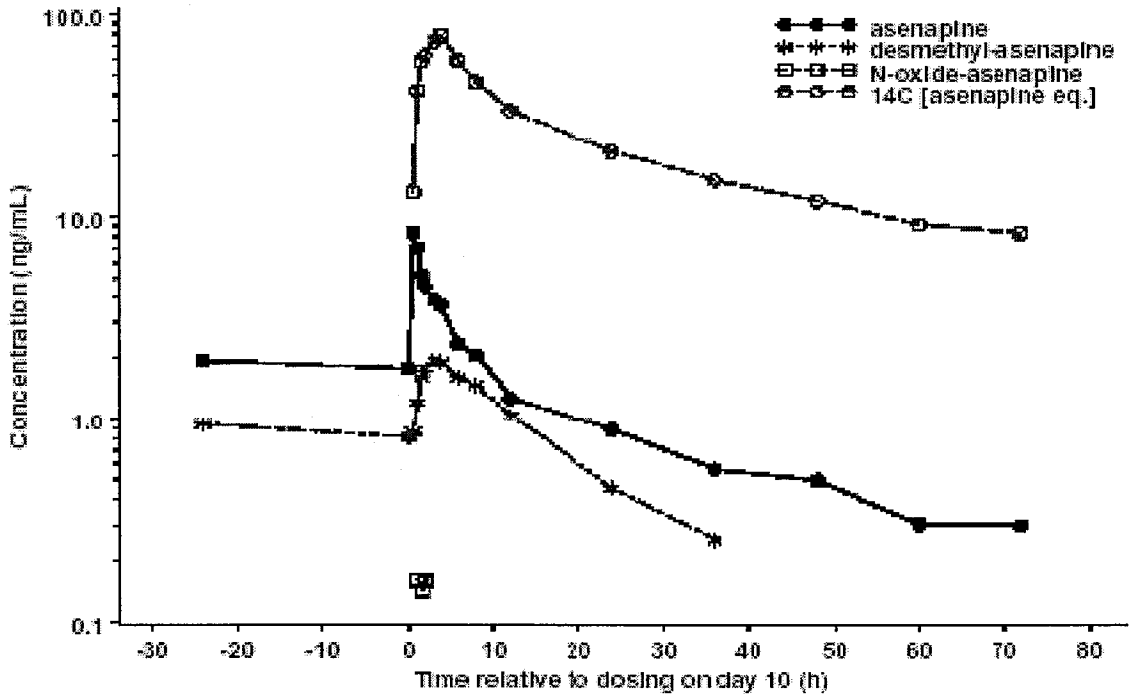


Table 21 shows the concentration vs. time data for total radioactivity both in terms of raw data and dose normalized, and the raw data for asenapine, desmethyl-asenapine, and asenapine N-oxide. When dose normalized radioactive Cmax is compared to the Cmax of asenapine the total radioactivity is 223 – 552 fold higher, (i.e. 3145/14.1 and 3008/5.44).

In addition when appropriate dose normalized AUCs are compared the unidentified radioactivity is clearly even larger with 99.9% of the circulating radioactivity unidentified. The pharm/tox reviewer was advised of this a few days after the midcycle meeting held at the end of Janurary 2008.

Table 21 Plasma Concentration vs. Time Data for Total Radioactivity, Asenapine, Desmethyl-Asenapine, and Asenapine N-Oxide – Study 25532

Day	Normalized Dose	Subject	10										11		12		13
Time (h)			0	0.5	1	1.5	2	3	4	6	8	12	24	36	48	60	72
^{14}C [asenapine equivalents] (ng/mL)*	(0.3 mg)	1	0	6.67	13.7	23.6	39.8	65.7	69.7	60.7	46.9	36.1	22.9	16.2	10.9	9.01	7.02
		2	0	19.6	53.8	64.7	64.2	61.7	64.7	43.7	31	27	16.2	13	10.2	7.27	8.36
		3	0	15.1	45.2	75.2	77.7	84.6	91.6	77.2	62.2	40.9	24.6	18.1	16.2	13.5	11.3
		4	0	11.7	55.7	69.2	68.7	77.2	87.6	55.3	44.9	30.5	20	13.3	10.7	7.02	6.67
Dose Normalized ^{14}C [asenapine equivalents] (ng/mL)* (DN to 10.3 mg)		1	0	229	470	810	1366	2256	2393	2084	1610	1239	786	556	374	309	241
		2	0	673	1847	2221	2204	2118	2221	1500	1064	927	556	446	350	250	287
		3	0	518	1552	2582	2668	2905	3145	2651	2136	1404	845	621	556	464	388
		4	0	402	1912	2376	2359	2651	3008	1899	1542	1047	687	457	367	241	229
Asenapine (ng/mL) 10.3 mg		1	1.29	7.16	7.24	4.49	4.35	3.48	3.37	2.14	1.77	1.09	0.629	0.398	0.365	0.173	0.246
		2	1.24	6.82	6.07	4.5	3.55	2.99	2.67	1.61	1.39	0.881	0.657	0.374	0.348	0.234	0.187
		3	2.55	14.1	9.5	6.92	5.39	4.77	4.49	3.15	3.01	1.9	1.33	1.06	0.853	0.617	0.524
		4	2.06	5.23	5.44	4.71	4.54	4.3	4	2.48	2.14	1.21	0.922	0.425	0.424	0.197	0.246
Desmethyl-asenapine (ng/mL)		1	0.435	0.459	0.567	0.639	0.859	1.37	1.58	1.24	1.14	0.787	0.304	0.144	0	0	0
		2	0.363	0.412	1.01	1.43	1.51	1.34	1.28	1.39	1.08	0.811	0.305	0.207	0	0	0
		3	1.66	1.75	1.97	2.65	3.05	3.15	2.75	2.31	2.26	1.58	0.784	0.398	0.287	0.204	0.122
		4	0.782	0.842	1.3	1.71	1.72	1.95	2.05	1.49	1.42	1.01	0.434	0.27	0	0	0
Asenapine N-oxide (ng/mL)		1	0	0	0.137	0	0.135	0	0	0	0	0	0	0	0	0	0
		2	0	0.247	0.217	0.12	0.171	0.133	0	0	0.117	0	0	0	0	0	0
		3	0	0.256	0.233	0.189	0.191	0	0.174	0	0	0	0	0	0	0	0
		4	0	0	0	0.205	0.129	0.175	0.174	0	0	0	0	0	0	0	0

Table 22 Plasma Exposures to Asenapine and Selected Metabolites Relative to Total ^{14}C Radioactivity after Asenapine 10 mg and 0.3 mg 14-C-Asenapine at Steady-State - Study 25532

Dose		10.3 mg			0.3 mg	10.3 mg	
Metric	Subject	Asenapine	Desmethyl – Asenapine	Asenapine N-oxide	^{14}C [asenapine equivalents]	Dose Normalized ^{14}C [asenapine equivalents]	% extrap
AUCτ [a] (ng/mL x hr^{-1})	1	33.3	12.8	0.2	1523.2	52297	11.5
	2	27.8	13.6	0.9	1282.6	44036	25.6
	3	50.6	27.7	0.7	1952.8	67046	16.5
	4	35.7	17.7	0.6	1470.0	50470	8.5
Fraction of ^{14}C (%)	1	2.2	0.8	0.01	–	–	–
	2	2.2	1.1	0.07	–	–	–
	3	2.6	1.4	0.04	–	–	–
	4	2.4	1.2	0.04	–	–	–
	Mean[b]	2.3	1.1	0.04	–	–	–
Fraction of Dose Normalized ^{14}C (%)	1	0.06	0.02	0.000	–	–	–
	2	0.06	0.03	0.002	–	–	–
	3	0.08	0.04	0.001	–	–	–
	4	0.07	0.04	0.001	–	–	–
	Mean[c]	0.067	0.032	0.001	–	–	–

a AUC∞ for ^{14}C. N.B. AUC∞ used because it's a single dose.
b Mean = 3.44 (i.e. minimum without dose normalization 96.6% unidentified)
c Mean = 0.102 (i.e. 99.9% unidentified)

Pharmacokinetic metrics as reported by the sponsor are shown in Table 23. Total ^{14}C is elimination rate limited however what's most interesting is that the elimination of desmethyl-asenapine appears to be more rapid than asenapine which should not be. The reason for this is unclear.

Table 23 Reported Pharmacokinetic Metrics of Selected Species - Study 25532

Metric (unit)	^{14}C [asenapine equivalents]	Asenapine	Desmethyl-asenapine	Asenapine N-Oxide
Tmax (h)	4.00 (1.50-4.00)	0.75 (0.50-1.00)	3.50 (2.00-4.00)	0.75 (0.50-1.50)
Cmax (ng/mL)	78.4 (13.2)	8.40 (3.88)	2.07 (0.757)	0.211 (0.0543)
AUC_{0-12} (ng /mL x hr^{-1})	n.a.	36.9 (9.72)	17.9 (6.87)	n.c.
AUCtlast (ng /mL x hr^{-1})	1557 (284)	n.a.	n.a.	n.a.
AUC_{∞} (ng /mL x hr^{-1})	2020 (467)	n.a.	n.a.	n.a.
Clapp (L/h)	n.a.	293 (68.9)	n.a.	n.a.
Vz,app (L)	n.a.	11371 (2096)	n.a.	n.a.
$t_{1/2}$ (h)	39.3 (7.55)	27.5 (4.97)	12.9 (4.46)	n.c.

Presented are median (minimum-maximum) for T_{max}; arithmetic mean (SD) for other PK parameters.
#: n=4; n.a..: Not applicable; n.c.: Not calculated.
Source Appendix BI, Table 5-3. Study Report 25532

5.4.2.5.3 HPLC System 2

Later the plasma from the 1 hour sample and from the remaining plasma from all of the plasma samples from 1.5 – 12 hours from all four subjects was pooled. Both pooled plasma samples were analyzed on HPLC system 2. The pooling of these samples was not performed quantitatively (i.e. the sponsor does not report the volumes) and therefore these chromatograms were only evaluated by the sponsor in a qualitative way.

In spite of this it should still be possible to infer approximate exposures to various metabolites since the samples are pooled over time.

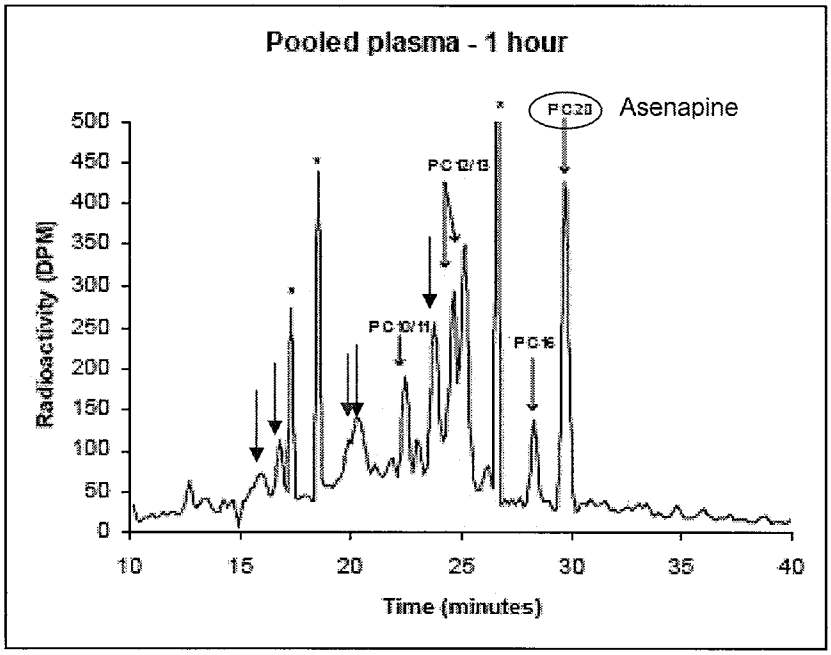
Figure 12 shows the pooled plasma chromatograms from the 1 hour sample and the combined 1.5 – 12 hour samples. The sponsor only labels asenapine and 4 metabolites as being of interest, (i.e. peaks labeled PC#), it's clear that the areas under the peaks identified by this reviewer with red arrows are nearly as great the peak area for asenapine in the pooled 1.5 to 12 hour sample.

Examination of the scale used for the peak heights used for the two different chromatograms reveal that the area under the smaller peaks in the 1.5 to 12 hour sample may be as great as the areas under peaks that appear visually taller in the 1 hour sample. In addition, since asenapine is declining yet radioactivity in plasma continues past 72 hours post dose the relative exposures to these metabolites may be even higher yet.

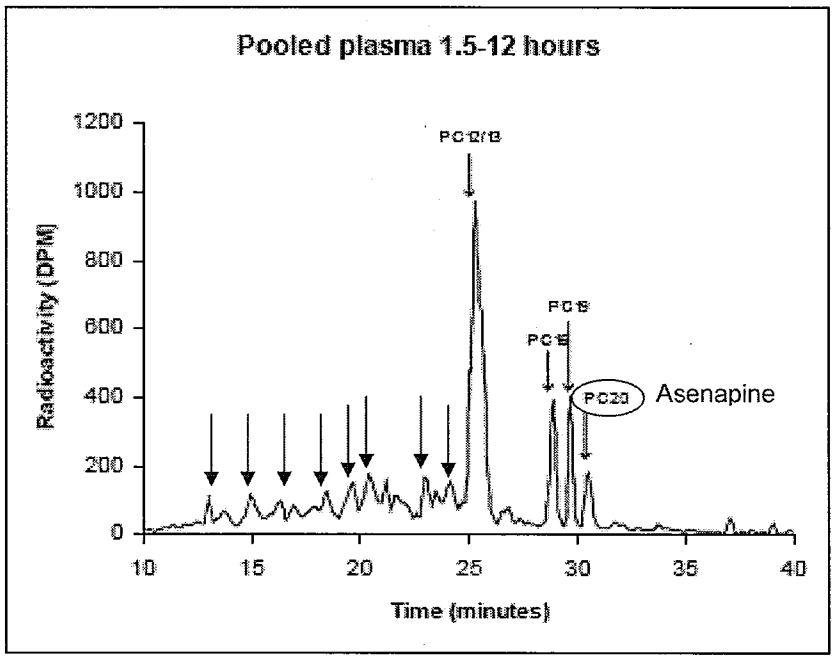
Although the sponsor claims that peaks identified in the 1 hour plasma sample with asterixes are not related to asenapine this seems suspect as they are so tall and the mode of detection is radioactivity. Consequently they should not only be due to the ^{14}C that was incorporated into asenapine. It's possible that their lack of detection in later samples may be secondary to their being formed by CYP2D6, which appears to be mechanistically inactivated by N-desmethyl-asenapine, and their subsequent rapid elimination.

In conclusion it appears that there may be a dozen or more unidentified metabolites circulating in plasma for which the plasma exposure is greater than 10% of the exposure to asenapine. Consequently, a large number of unidentified metabolites may still need to be qualified. The pharm/tox reviewer was also advised of this a few days after the midcycle meeting held at the end of Janurary 2008.

Figure 12 Representative HPLC Metabolite Profiles (HPLC system 2) of Pooled Plasma Samples of Male Human Volunteers after Sublingual Asenapine plus [^{14}C]-Asenapine – Study Report 40218



* These spikes are based on LC-MS analysis not related to asenapine.



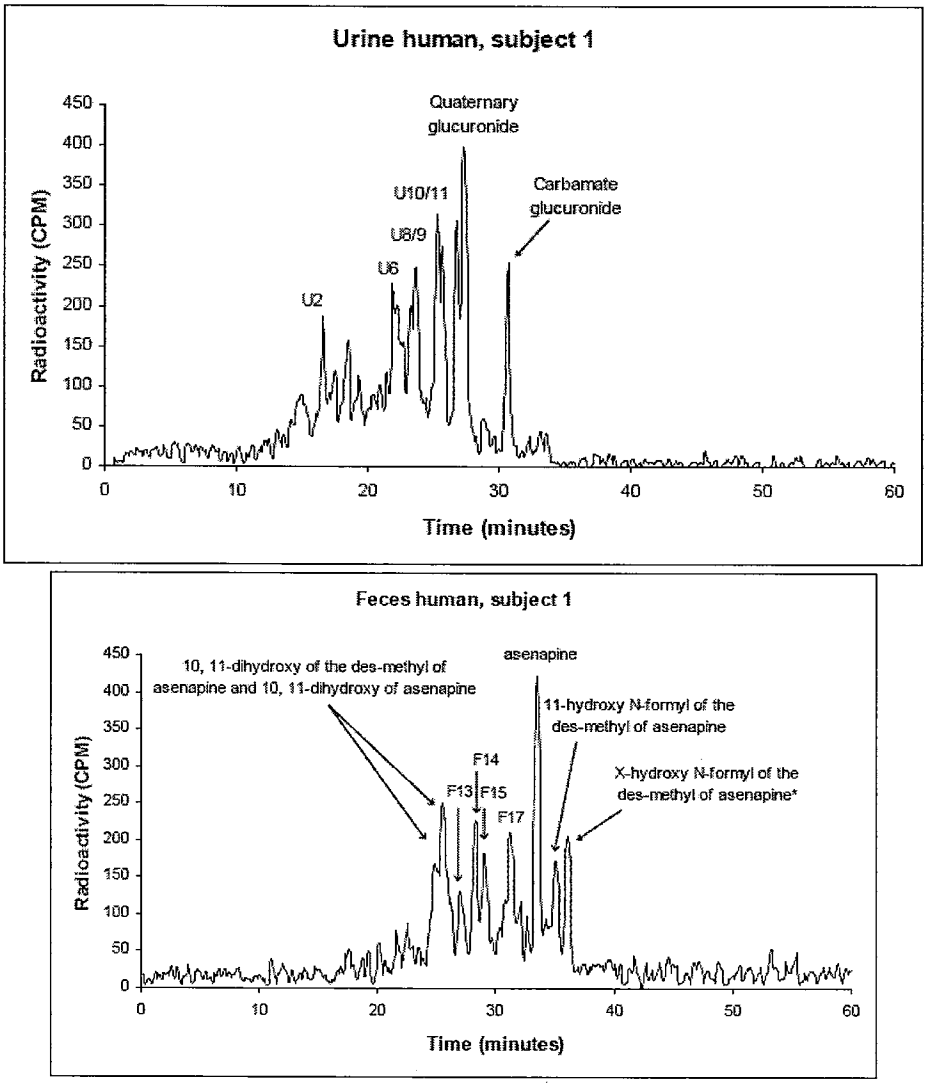
Peak PC10/11 contains at least the sulfate of the 11-hydroxy of asenapine
Peak PC12/13 are identified as the quaternary glucuronide of asenapine
Peak PC16 is identified as the carbamate glucuronide of the N(2)-des-methyl of asenapine
Peak PC19 is identified as the N(2)-des-methyl of asenapine
Peak PC20 is identified as asenapine

5.4.2.6 *Recovery in Urine and Feces, Metabolic Scheme, & Mass Balance*

5.4.2.6.1 Metabolites Identified in Urine and Feces

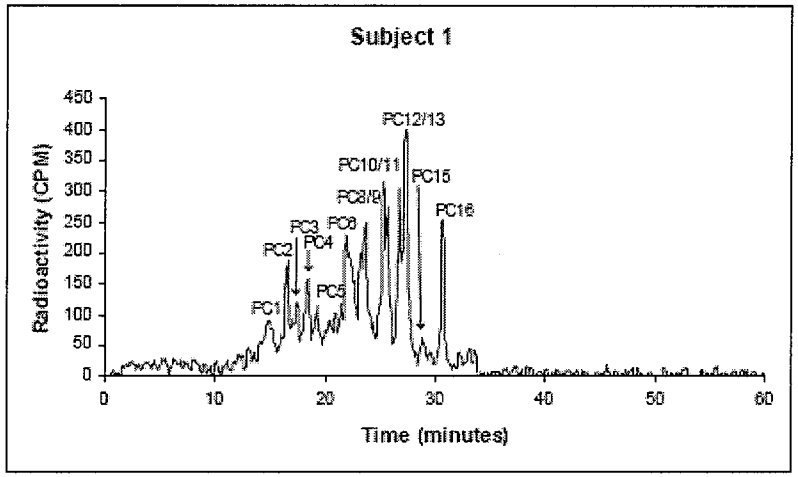
Figure 13 shows 'representative' HPLC system 2 metabolite profiles with separate urine and feces numbering of pooled urine and feces samples collected after sublingual administration of [^{14}C]-Asenapine to a healthy male volunteer in study 25532. Figure 14 on the following page shows 'representative' chromatograms of urine metabolites with HPLC system 2 for all subjects. The collection interval for these urine samples were not described, thus the sponsor's description as 'representative'. Yet it's clear that more than 20 potential peaks are visible yet the peak for asenapine (PC20) is not identifiable.

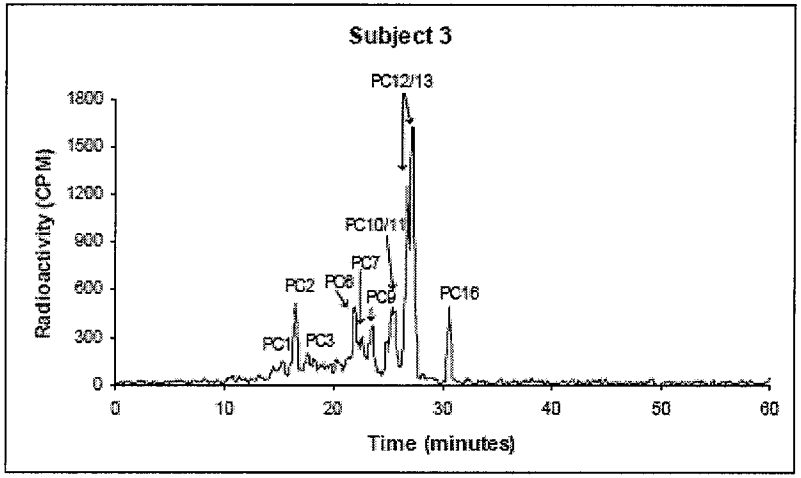
Figure 13 Representative HPLC Metabolite Profiles (HPLC system 2) of Urine and Feces Samples Collected after Sublingual Administration of [^{14}C]-Asenapine to a Healthy Male Volunteer

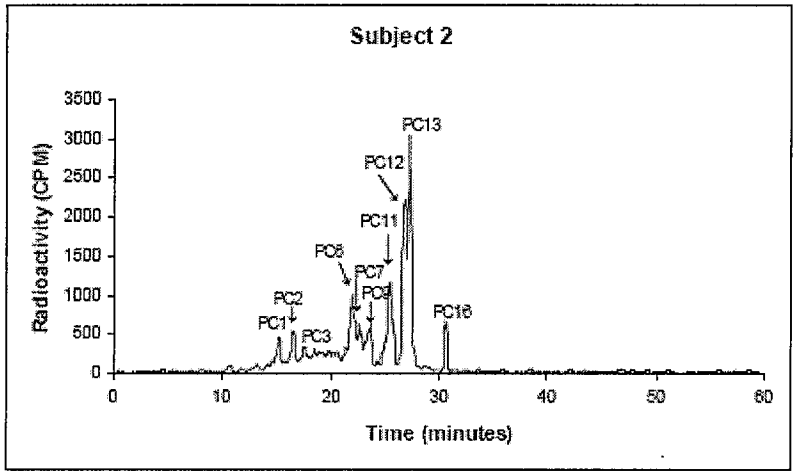


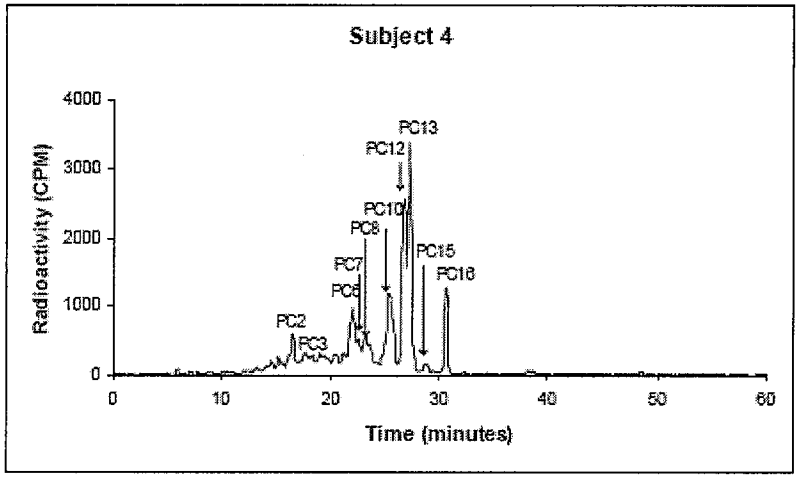
U2 is identified as the methoxy and glucuronide of the 10, 11-dihydroxy of the N-des-methyl of asenapine in which the position of the methoxy and glucuronide is 10, 11 and the reverse.
U6 is identified as the methoxy and sulphate of the 10, 11-dihydroxy of the N-des-methyl of asenapine in which the position of the methoxy and sulphate is 10, 11 or the reverse, the glucuronide of the 11-hydroxy of N-des-methyl of asenapine, the glucuronide of the 11-hydroxy of asenapine plus some other conjugated metabolites (sulphates and glucuronides).
U8/9 contained some conjugated metabolites (sulphates and glucuronides)
U10/11 is identified as the sulphate of the 11-hydoxy of asenapine plus some other conjugated metabolites (sulphates and glucuronides) of most probably the N-oxide of asenapine.
U12/13 is identified as the quaternary glucuronide of asenapine.
U16 is identified as the carbamate glucuronide of the N(2)-des-methyl of asenapine.
* the position of the hydroxyl group could not be assigned but it might be the 6-hydroxy

Figure 14 Representative HPLC Metabolite Profiles (HPLC System 2) of Pooled Urine Samples after Sublingual Administration of Radiolabeled and Unlabeled Asenapine – Study 25532







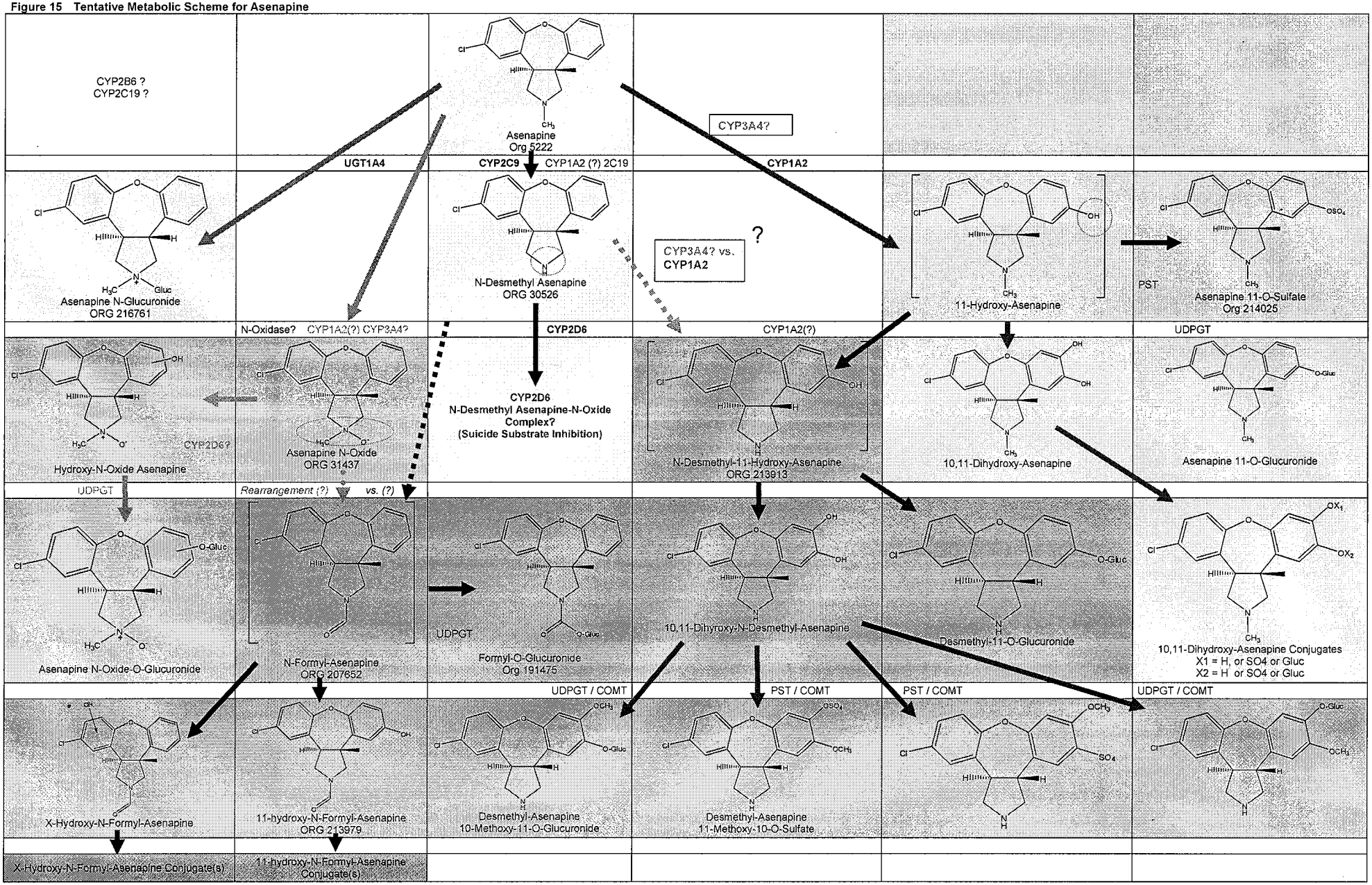


PC2 is identified as the methoxy and glucuronide of the 10, 11-dihydroxy of the N(2)-des-methyl of asenapine in which the position of the methoxy and glucuronide is 10, 11 and the reverse.
PC6 is identified as the methoxy and sulfate of the 10, 11-dihydroxy of the N(2)-des-methyl of asenapine in which the position of the methoxy and sulfate is 10, 11 or the reverse, the glucuronide of the 11-hydroxy of N(2)-des-methyl of asenapine, the glucuronide of the 11-hydroxy of asenapine plus some other conjugated metabolites (sulfates and glucuronides).
PC8/9 contained some conjugated metabolites (sulfates and glucuronides)
PC10/11 is identified as the sulfate of the 11-hydoxy of asenapine plus some other conjugated metabolites (sulfates and glucuronides) of most probably the N(2)-oxide of asenapine.
PC12/13 is identified as the quaternary glucuronide of asenapine.
PC16 is identified as the carbamate glucuronide of the N(2)-des-methyl of asenapine.

5.4.2.6.2 Metabolic Scheme (Tentative)

Figure 15 on the next page shows a tentative metabolic scheme. This scheme is based on the sponsor's more limited proposed scheme with the addition of metabolites only identified nominally by the sponsor, and with the addition of metabolites that can be inferred based on the available data. The scheme is only tentative as the data provided by the sponsor on certain secondary, tertiary and even lower level pathways cannot be identified with certainty. More importantly the enzymes or specific isozymes involved frequently cannot be identified. Consequently, pathways for which the enzymes or isozymes are relatively certain have been identified with bolded text.

Figure 15 Tentative Metabolic Scheme for Asenapine



5.4.2.6.3 Mass Balance

Table 25 on the following page shows the recovery of the radioactive dose by identified peak and by patient in both urine and feces, i.e. the reported mass balance.

Table 24 below summarizes the recovery of the radioactive dose reported in Table 25 and compares it to that reported by the sponsor. At least part of the discrepancy may be due to the radioactive peaks shown in Figure 12 that the sponsor claims was not associated with asenapine or any metabolites.

Table 24 % of Radioactive Dose Recovered in Urine and Feces as Determined from Individual Peaks and as Reported by the Sponsor

Reference	Description	Urine	Feces	Urine and Feces
Table 25	**Tally from Mass Balance Data provided by Sponsor**	43% 33% - 52%	32% 30 – 40%	75% 59% - 83%
Table 19	**Recovery of Radioactivity as Reported by Sponsor**	48.9% 37% - 59%	38.8% 35% – 47%	87.7% (72% – 96%)

Table 26 attempts to figure out relative contributions to the elimination of asenapine from each of the four primary metabolic pathways and shows only one possibility.

When the fraction of the dose that was not recovered, not accounted for, or not identified is totaled the fate of 37% - 56% (average 45%) of the dose is unknown.

Since multiple metabolites were identified for each peak, (see Table 25), and since the metabolic scheme is uncertain, (see Figure 15), except for direct glucuronidation by UGT1A4 which accounts for 12% - 21% of the dose and elimination of unchanged asenapine which accounts for 5% - 16% of the dose, the relative contribution of the 3 primary oxidative pathways cannot be definitively assigned. Thus the primary elimination pathways and enzymes have not been identified for 64.5% – 82.8% of the dose.

Table 25 Mass Balance Recovery of the Radioactive Dose by Identified Peak (HPLC System 2) for each Subject in both Urine and Feces – Study 25532

Peak No.	RT (min)	% of Radioactive Dose Recovered in Urine				% of Radioactive Dose Recovered in Feces				Nominal Description	% of Radioactive Dose Recovered in Urine & Feces			
		Subj 1	Subj 2	Subj 3	Subj 4	Subj 1	Subj 2	Subj 3	Subj 4		Subj 1	Subj 2	Subj 3	Subj 4
PC1	15.2	2.71	2.29	1.19							2.71	2.29	1.19	0
PC2	16.6	2.51	2.7	2.8	2.61					N(2)-des-methyl asenapine 10-Methoxy 11-O-Glucuronide & N(2)-des-methyl asenapine 10-O-Glucuronide 11-Methoxy	2.51	2.7	2.8	2.61
PC3	17.6	1.53	1.93	1.14	1.67						1.53	1.93	1.14	1.67
PC4	18.5	2.85									2.85	0	0	0
PC5	19.3	2.35									2.35	0	0	0
PC6/7	22	6.17	6.01	2.76	3.95					N(2)-des-methyl asenapine 10-methoxy 11-O-Sulfate & N(2)-des-methyl asenapine 10-O-Sulfate 11-Methoxy N-des-methyl asenapine 11-O glucuronide Asenapine-11-O-glucuronide Plus some other sulfates and glucuronides	6.17	6.01	2.76	3.95
PC7	22.7		2.35	1.56	1.86						0	2.35	1.56	1.86
PC8	23.3	2			3.1					U8/9 contained some conjugated metabolites (sulphates and glucuronides)	2	0	0	3.1
PC9	23.6	3.92	4.34	2.83							3.92	4.34	2.83	0
PC10	25.1	4.53		1.27	6.9	2.83	3.33	2.35	2.95	U10/11 Asenapine 11-O-Sulfate N-oxide asenapine sulphates and glucuronides	7.36	3.33	3.62	9.85
PC11	25.6	3.24	8.44	3.62		4.5	6.77	4.46	2.8	F10/11 10, 11-dihydroxy-des-methyl asenapine and 10, 11-dihydroxy-asenapine.	7.74	15.21	8.08	2.8
PC12	26.8	3.88	9.36	5.99	7.43					asenapine glucuronide	3.88	9.36	5.99	7.43
PC13	27.2	6.34	11.97	7.51	9.79	2.17			2.12	asenapine glucuronide	8.51	11.97	7.51	11.91
PC14	28.4					2.87	1.42	2.32	2.5		2.87	1.42	2.32	2.5
PC15	29	1.12			0.73	2.43	3.12	1.85	4.29		3.55	3.12	1.85	5.02
PC16	30.7	3.13	2.42	2.02	3.16			0.77		U16- N(2)-des-methyl asenapine glucuronide	3.13	2.42	2.79	3.16
PC17						3.79	4.05	2.6	2.81		3.79	4.05	2.6	2.81
PC18						1.13					1.13	0	0	0
PC19						0.92	1.65			N-desmethyl-asenapine	0.92	1.65	0	0
PC20						4.79	5.97	7.62	16.2	Asenapine	4.79	5.97	7.62	16.2
PC21							1.17	1.1	1.59		0	1.17	1.1	1.59
PC22						1.97	1.44	1.51	2.31	11-hydroxy N formyl N-desmethyl	1.97	1.44	1.51	2.31
PC23						2.7	1.88	1.71	2.82	X-hydroxy N-formyl of N-desmethyl	2.7	1.88	1.71	2.82
Cumulative Recovery (% of ^{14}C Dose)		46.3	51.8	32.7	41.2	30.1	30.8	26.3	40.4		**76.4**	**82.6**	**59.0**	**81.6**

Table 26 One Possibility for Relative Contributions by Primary Pathways to Mass Balance

Peak No.	Description	Subj 1	Subj 2	Subj 4	Subj 4
11 Hydroxylaton (CYP1A2)					
PC2	N(2)-des-methyl asenapine 10-Methoxy 11-O-Glucuronide & N(2)-des-methyl asenapine 10-O-Glucuronide 11-Methoxy	2.51	2.7	2.8	2.61
PC6	N(2)-des-methyl asenapine 10-methoxy 11-O-Sulfate & N(2)-des-methyl asenapine 10-O-Sulfate 11-Methoxy N-des-methyl asenapine 11-O glucuronide Asenapine-11-O-glucuronide Plus some other sulphates and glucuronides	6.17	6.01	2.76	3.95
PC10	Asenapine 11-O-Sulfate	7.36	3.33	3.62	9.85
PC11	N-oxide asenapine sulfates and glucuronides 10, 11-dihydroxy-des-methyl asenapine and 10, 11-dihydroxy-asenapine.	7.74	15.21	8.08	2.8
Subtotal		**23.78**	**27.25**	**17.26**	**19.21**
N-Demethylation ?					
PC22	11-hydroxy N formyl N-desmethyl asenapine	1.97	1.44	1.51	2.31
PC23	X-hydroxy N-formyl of N-desmethyl asenapine	2.7	1.88	1.71	2.82
PC19	N-desmethyl-asenapine	0.92	1.65	0	0
PC16	N(2)-des-methyl asenapine glucuronide	3.13	2.42	2.79	3.16
Subtotal	**N.B. it's uncertain if formyl metabolites should be included under N-Demethylation or not. Or alternatively under N-oxidation or even another pathway.**	**8.72**	**7.39**	**6.01**	**8.29**
quaternary glucuronide of asenapine UGT1A4					
PC12	asenapine. glucuronide	3.88	9.36	5.99	7.43
PC13	asenapine. glucuronide	8.51	11.97	7.51	11.91
Subtotal		**12.39**	**21.33**	**13.5**	**19.34**
Unidentified					
PC1		2.71	2.29	1.19	0
PC3		1.53	1.93	1.14	1.67
PC4		2.85	0	0	0
PC5		2.35	0	0	0
PC7		0	2.35	1.56	1.86
PC14		2.87	1.42	2.32	2.5
PC15		3.55	3.12	1.85	5.02
PC17		3.79	4.05	2.6	2.81
PC18		1.13	0	0	0
PC21		0	1.17	1.1	1.59
Subtotal		**20.78**	**16.33**	**11.76**	**15.45**
Unidentified Sulfate and Glucuronide Conjugates					
PC8	some conjugated metabolites (sulfates and glucuronides)	2	0	0	3.1
PC9	some conjugated metabolites (sulfates and glucuronides)	3.92	4.34	2.83	0
Subtotal		**5.92**	**4.34**	**2.83**	**3.1**
PC20	**Asenapine**	**4.79**	**5.97**	**7.62**	**16.2**
Total Recovery from Individual Peaks		**76.38**	**82.61**	**58.98**	**81.59**
	Identified	***49.68***	***61.94***	***44.39***	***63.04***
	Unidentified	26.7	20.67	14.59	18.55
	Not Accounted For in report of Urine and Feces Recovery	23.62	17.39	41.02	18.41
Total Recovery per Sponsor		**86.9**	**95.9**	**71.8**	**96.0**
	Not Recovered	13.1	4.1	28.2	4.0
Difference between amount reported as not recovered by sponsor and amount not accountable for in report of urine and feces recovery		10.52	13.29	12.82	14.41
Unidentified, Unaccounted, and Not Recovered		***50.32***	***38.06***	***55.61***	***36.96***
(Average)		***(45.2%)***			

5.4.3 *In Vitro* Drug Metabolism Studies

5.4.3.1 Hepatocytes

Metabolism in isolated human hepatocytes will be discussed first as the intact cells provides the best information on the overall metabolic profile as they include cytosolic enzymes in addition to microsomal enzymes. However it should be remembered that a hepatocyte system lacks the anatomical structure found *in vivo* and thus may not be accurate in terms of relative abundance of each metabolite or the importance of various metabolic pathways.

5.4.3.1.1 Study 5067 (1997) - AKA NCL Study

Study 5067 conducted in 1997 incubated [^{3}H]-Asenapine labeled at the 11 position, (see Figure 16), at a concentration of 149 ng/mL (521.4 nMol/L) for 3 hours with isolated human hepatocytes from a 41 yo female.

Results are shown in Table 27. Recoveries were reported for both the cell medium as well as the cell extract, unfortunately the relative amounts in the cell extract compared with the cell medium were not reported so only tentative conclusions may be drawn. The following tentative conclusions are made based upon the relative retention times:

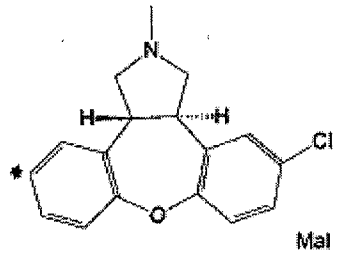
Peach Table Cells – greater amount found in cell medium and also eluting earlier, thereby indicating greater hydrophylicity and possible active secretion.
Yellow Table Cells - approximately equal amounts found in cell medium and cell extract possibly indicating passive diffusion.
Light Blue Table Cells - greater proportion found in cell extract indicating possible binding to cellular components or greater lipophilicity.

Table 27 Percent Radioactive Recovery by Peak after Asenapine Incubation with Human Hepatocytes at 521 nMol – Study 5067 (1997)

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13 & H14	H15	Asenapine	H16
	Unknown												N-Oxides	Desmethyl Asenapine		Unknown
Cell Medium	37.7	9.3	3.2	12.5			2.8	6.9	11.9	1.1			9.1	4.0	1.6	
Cell Extract	3.1					2.1	3.9	8.2	12.0			3.9	10.4	46.7	9.6	

Metabolite H1 is highly polar and accounts for the majority of the recovery in the cell media. In addition 80% of the radioactivity in the cell media was volatile suggesting that much of the radioactivity was tritiated water. Taken together these facts suggest that the majority of asenapine's metabolism in this system is via is 11-oxidation and that H1 is likely the 11-O-sulfate.

Figure 16 Position of Asenapine ^{3}H Radiolabel - Study 5067



5.4.3.1.2 Study NL0060905 (2006)

Study NL0060905 conducted in 2006 incubated [^{14}C]-Asenapine at concentrations of 4.7 nMol/mL (μM) and 19.5 nMol/mL (μM) in a final ethanol concentration of 2% (v/v) performed in duplicate with isolated human male hepatocytes.

Results are shown in Table 28. Unfortunately only total recoveries were reported even though the sponsor stated that recoveries were determined in both the cell medium as well as the cell extract. Where feasible, recoveries that can be attributed to a single primary pathway are combined to show the relative importance of that primary pathway. This reveals that over 50% of asenapine's metabolism in this system proceeds via N-desmethylation.

Table 28 Fractional Recovery after Asenapine Incubation with Human Hepatocytes - Study NL0060905 (2006)

Peak			Total recovery	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17
m/z								384.1		464.2	290.1	450.2		274.1	288.1	318.1	304.1		302.1	
Code #														30526	5222		34137			
Nominal Structure								O-SO4		N-Gluc	OH-N-Des	N-Des-Gluc[c]		N-Des	As	N-formyl OH	N-oxide		N-formyl	
B	4.7 nMol/mL (μM)	Total	88.5	—[a]	—	5.17	3.76[b]	27.19	4.03	2.51	6.15	19.27	2.29	21.29	4.89	5.35	—	—	—	—
		Media	[redacted]																	
		extract																		
	Combined							31.2		2.5				52.0	4.9	5.35				
A	19.5 nMol/mL (μM)	Total	83.5	—	—	1.81	1.31	13.07	1.85	2.47	2.55	19.2	1.53	13.63	31.1	2.4	1.32	1.88	4.81	1.11
		Media	[redacted]																	
		extract																		
	Combined							15.0		2.5				42.7	31.1	7.2	3.1			

a – not detected
b - observed in only 1 duplicate
c –structure not identified by sponsor

It should be noted that the sponsor did not identify the structure of metabolite H9, however from the molecular weight it is readily apparent that it is the glucuronide conjugate of N-desmethyl-asenapine. By not identifying this structure, if this reviewer had not realized that the N-formyl metabolites proceed via N-desmethylation the relative contribution of N-desmethylation would have been capped at half of what the results truly show. Consequently the clinical importance of inhibition of this pathway would not have been as apparent.

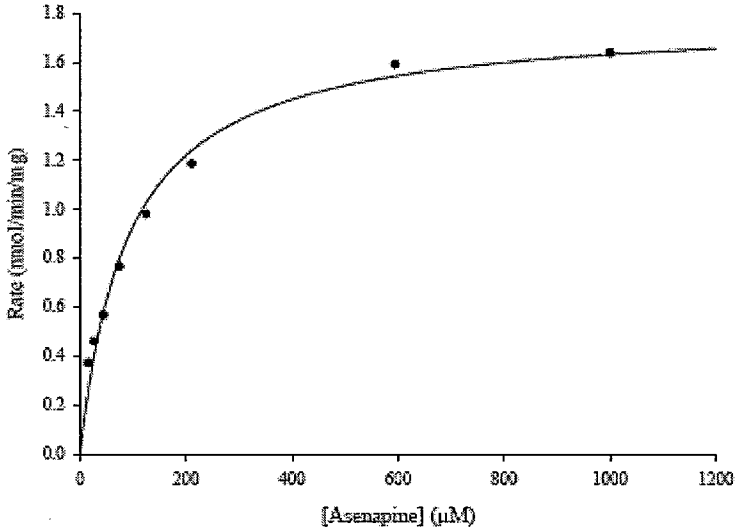
5.4.3.2 N-Glucuronication

The Uridine Glucuronosyl Transferase isozymes (UGT) involved in the N-glucuronidation of asenapine were identified in study DM2006-005222-013. Glucuronidation of metabolites was not examined.

Incubations were first conducted with pooled human liver microsomes (HLM-13) to determine apparent instrinsic enzyme kinetic parameters, followed by incubations with the recombinant UGT enzymes (UGT1A1, 1A3, 1A4, 1A6, 1A8, 1A9, 1A10, 2B4, 2B7, and 2B15). Incubation times were 1 hour, and the duration of incubation and the protein concentration used were established in preliminary experiments that assessed the linearity of the relationship with the reaction velocity. The formation of asenapine N-glucuronide was determined by mass spectrometry.

Data from incubations with pooled human hepatic microsomes were fit to a Michaelis-Menten model resulting in a Km of 92.6 μM and Vmax of 1.8 nMol / min / mg, (see Figure 17). Since asenapine's *in vivo* concentrations peak between 10 – 70 nMol/L glucuronidation should be a linear *in vivo*,

Figure 17 Mean Asenapine N-Glucuronide Formation Rate vs. Concentration in Pooled Human Hepatic Microsomes – Study DM2006-005222-013



After the apparent instrinsic kinetic parameters in pooled microsomes were determined, various recombinant UGT isozymes were incubated under nonlinear conditions with asenapine at a concentration equal to the apparent instrinsic Km, (i.e. 92 μM).

Based on these experiments UGT1A4 was identified as the isozyme with the greatest intrinsic affinity to glucuronidate asenapine, (see Table 29).

Table 29 Formation Rate of Asenapine N-Glucuronide by Recombinant UGT Isozymes at the Apparent Km (92 μM) – Study DM2006-005222-013

UGT Isozyme	1A1	1A3	1A4	1A6	1A8	1A9	1A10	2B4	2B7	2B15
Formation Rate[a] (nmol / min / mg)	<LLOQ	<LLOQ	**0.49**	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ

a LLOQ = 0.03 nmol/min/mg

UGT1A1 glucuronidates bilirubin and is also known as bilirubin-UGT-1 (BUGT1).

Despite high sequence identity, UGT1A3 and UGT1A4 differ in terms of substrate selectivity. UGT1A3 glucuronidates planar phenols such as 1-naphthol (1-NP) and 4-methylumbelliferone (4-MU). Whereas UGT1A4 converts the tertiary amines, such as lamotrigine (LTG) and trifluoperazine (TFP), to a quaternary ammonium glucuronide. Thus the finding that UGT1A4 glucuronidates asenapine is not surprising.

5.4.3.3 Microsomal Oxidative Metabolism

In vitro studies were conducted examining the microsomal oxidative metabolism of asenapine. Studies utilized the following test systems:

a) Human Liver Microsomes
b) Supersomes (i.e. microsomes from P450 isozyme specific cDNAh expressed in intact insect cells)

There were typically at least two study reports for each test system, an initial study conducted by Organon during their initial development, and a later study conducted by Pfizer within a few years of submission.

Unfortunately almost all of the studies were conducted at asenapine concentrations ~ 1000 fold higher than *in vivo* concentrations (2 – 28 nMol). Therefore results are somewhat suspect.

5.4.3.3.1 Human Liver Microsomes

The following three studies were conducted with human liver microsomes:

1) Study 2874 (1991)
2) NL0060848 (2005)
3) INT00003054 (2006)

5.4.3.3.1.1 Human Liver Microsomes – Study 2874 (1991)

Study 2874 examined the fractional recovery of radioactivity after incubation of 25 µM of ^{3}H-Asenapine in human liver microsomes from two Dutch males. Table 30 shows that recovery as metabolites is primarily as the N-Desmethyl. Three other metabolites including the diasteromeric N-oxide and 2 unidentified metabolites are recovered at lower fractions.

Table 30 Fractional Recoveries of Extracted Radioactivity after Incubation of ^{3}H-Asenapine 25 µM with Human Liver Microsomes for 30 Minutes – Study 2874

% of Extracted Radioactivity				
N-Oxide (Diastereomeric)	M2	M3	Desmethyl-Asenapine	Asenapine
5.8	8.3	4.5	12.7	68.9

5.4.3.3.1.2 Human Liver Microsomes – Study HLM NL0060848 (2005)

5.4.3.3.1.2.1 NADPH Dependence

In study NL0060848 (2005) male human liver microsomes (microsomal protein concentration: 500 µg/mL) were incubated for 15 minutes at 37°C with [^{14}C]-asenapine at 2 and 20 µmol/L in the presence and the absence of NADPH.

Table 31 shows that at lower asenapine concentrations of 2 µM biotransformation is NADPH dependent, indicating that only P450 is involved in oxidation of asenapine at clinical concentrations which are much lower. At higher concentrations of 20 µM asenapine turnover is not entirely NADPH dependent, likely indicating the involvement of FMO, in addition the turnover is lower than at 2 µM, indicating the possibility

of a mechanism based inhibitor. Based on the structure of asenapine and the likely involvement of FMO there is a good likelihood that this is an N-oxide metabolite.

Table 31 % Biotransformation of Asenapine in Human Liver Microsomes 500 mcg/ml – Study NL0060848 (2005)

	2 µmol/L [^{14}C]-asenapine			20 µmol/L [^{14}C]-asenapine		
	Sample-1	Sample-2	Mean	Sample-1	Sample-2	Mean
Control[a]	0.00	--	--	5.06	--	--
(-) NADPH	0.00	0.00	0.00	5.48	6.12	5.80
(+) NADPH	17.50	24.96	21.23	9.65	11.31	10.48

5.4.3.3.1.2.2 Inhibition by Isozyme Specific Inhibitors

Table 32 shows the degree of inhibition of asenapine biotransformation by CYP450 isozyme specific inhibitors in human liver microsomes. As expected the degree of inhibition is less at the higher asenapine concentrations as the I/Ki : C/Km ratio is smaller at the higher asenapine concentration. The results show that 3A4, 1A2, and likely 2D6 are involved in the metabolism of asenapine and 2D6 might cause autoinhibition at higher concentrations, (also possibly 2C19 but this is less certain). Plus inhibition of 3A4, 1A2, and 2D6 might occur *in vivo* but the importance of these can only be determined from *in vivo* data since the specific metabolites and relative importance of the pathways need to be considered.

Table 32 % Inhibition of Asenapine Biotransformation by CYP450 Isozyme Specific Inhibitors in Human Liver Microsomes - NL0060848 (2005)

Asenapine (µmol/L)	1.8	20.5	2.4	24.3	2.4	24.3	2.4	24.3	2.4	24.3	0.2	2	1.8	22.7
Isozyme	1A2		2B6		2B6		2C19		2C19		2D6		3A4	
Inhibitor (µM)	Furafylline		MPEP		Orphenadrine		Benzylnirvanol		Tranylcypromine		Quinidine		Ketoconazole	
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1											1.84	2.66	55.76	3.23
0.2			10.04	3.23										
0.5							-9.17	1.32			17.62	2.91	59.53	5.08
1	11.51	2.39	15.16	-0.97			11.09	-4.71	11.29	12.66	19.93	0.15	77.37	13.26
2			10.34	7.30										
2.5					1.87	4.55								
5	45.83	10.90					6.98	-8.45	2.70	16.24			77.23	25.20
10	15.69	12.70	14.22	4.26			0.12	-10.02	4.60	17.03			86.14	36.08
11											24.32	2.96		
12.5					6.94	4.73								
20			14.08	4.44										
50	40.63	25.68			6.54	5.14	-12.92	-18.78	11.18	21.40				
53											31.71	7.72		
100	100.0	30.29												
125					11.96	6.69								
250									27.27	24.39				
500					7.29	6.28								

5.4.3.3.1.3 Human Liver Microsomes – Study INT00003054 (2006)

Study INT00003054 examined the fractional HPLC peak recoveries of radioactivity after Incubation of ^{14}C-Asenapine at ~ 5 and ~20 nMol/L with human liver microsomes at a protein concentration of 500 mcg/mL for 30 minutes.

Two sets of experiments were performed each using a different batch of microsomes. An initial set where the final concentration of ethanol used to dilute asenapine was 5% and a second set at a lower final ethanol concentration of 1%. The second set of experiments were conducted as ethanol interfered with the metabolism of asenapine and resulted in no turnover at the higher asenapine concentration.

The mechanism for alcohol's inhibition could be either nonspecific or specific inhibition of 2E1, 3A4, or alcohol and aldehyde dehydrogenase.

Similar to study 2874 (1971) the metabolites recovered included the N-desmethyl, the N-oxide and two other unidentified metabolites, (see Table 33).

Table 33 Fractional Recoveries of Radioactivity after Incubation of ^{14}C-Asenapine at ~ 5 and ~20 nMol/L with Human Liver Microsomes - Study INT00003054 (2006)

		EtOH	kBq	nMol/L	M1	M2	M3	M4	M5	M6	M7 DesMe	M8 Asenapine	M9	M10 N-Ox	M11	M12	M13	M14
A	1	5%	10.5	5.5				2.5			1.5	94.5		1.5				
	2	1%	10.1	5.3			2.3	5.0			5.2	81.7		5.9				
B	1	5%	42.4	22.3								100						
	2	1%	35.8	18.8				10.5			10.9	63.8	6.0	8.8				

5.4.3.3.2 Supersomes

5.4.3.3.2.1 Supersome Study NL0010293 (1998)

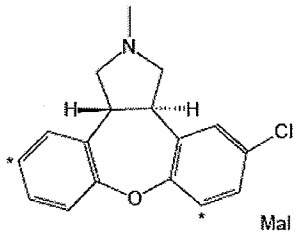
5.4.3.3.2.1.1 Initial Formation Rates

The initial formation rates of asenapine N-oxide and desmethyl-asenapine from [^{3}H]-Asenapine by cDNAh P450 isozymes expressed in insect cells (i.e. Supersomes) was examined in study NL0010293.

[^{3}H]-Asenapine labeled in two positions as shown in Figure 18 was incubated with CYP1A2, CYP2A6, CYP2D6, CYP2E1, CYP2C9, CYP2C19 and CYP3A4 supersomes at a microsomal protein concentration of 250 µg/mL for 15 min at 37 °C at concentrations of 2 and 20 µM.

Results are shown in Table 34. From this data it appears that CYP1A2 is involved in the formation of the reactive N-Oxide as well as is the primary isozyme responsible for formation of the N-Desmethyl metabolite, although CYP2C19, which is polymorphic, and CYP3A4 may be involved. It should be noted that the actual importance of these isozymes will also depend on their relative abundance *in vivo*. Consequently, CYP3A4 may be more important than CYP2C19.

Figure 18 ^{3}H Radiolabeling of Asenapine Used in Study NL0010293 (1998)



* position of label

Table 34 Initial Formation Rates of Asenapine N-Oxide and Desmethyl-Asenapine by Supersomes - Study NL0010293 (1998)

Supersomes	Formation Rates[a] (pMol-/ nMol P450 x min^{-1})			
	Asenapine - N(2)-Oxide		N(2)-Desmethyl Asenapine	
[^{3}H]-Asenapine Conc.	2 µM	20 µM	2 µM	20 µM
CYP1A2	*	*	376.91	1277.17
CYP2A6	–	–	–	–
CYP2C9	–	–	–	123.73
CYP2C19	–	–	85.77	725.44
CYP2D6	–	–	–	181.30
CYP2E1	–	–	–	–
CYP3A4	–	*	10.52	155.82

a Data are presented as mean values of duplicate incubations
– Below limit of detection
* Showed activity.

According to the sponsor, *'It was not possible to quantify the formation of the N(2)-oxide metabolite of Org 5222, because in the HPLC metabolite profiles of the higher substrate concentration an impurity was present at a detectable level, which eluted at the retention time of the N(2)-oxide metabolite of Org 5222. However the activity of CYP1A2 towards the N(2)-oxidation was higher as compared with CYP3A4 activity.'*

5.4.3.3.2.1.2 Enzyme Kinetic Parameters

Enzyme kinetic parameters for the formation of the N-oxide and the N-desmethyl metabolites were also determined for each of these isozymes, and the results are shown in Table 35. This data tends to confirm the previous conclusions.

Table 35 Enzyme Kinetic Parameters for the Formation of Asenapine N(2)-oxide and N(2)-Desmethyl Asenapine by CYP1A2, CYP2C19 and CYP3A4 Supersomes - Study NL0010293 (1998)[a]

Supersome Isozyme	Enzyme Kinetic Parameter	N(2)-oxide	N(2)-Desmethyl Asenapine
CYP1A2	Vmax (pMol / min / nMol P450)	942 ± 47	1556 ± 251
	Km (nMol/mL) (μM)	0.7 ± 0.2	16.6 ± 8.4
	Clint (L/hr x μMol^{-1})	83.3	5.62
CYP2C19	Vmax	—[b]	6052 ± 6 42
	Km	—	99.1 ± 14.7
	Clint (L/hr x μMol^{-1})	—	3.66
CYP3A4	Vmax	572 ± 67	5735 ± 1156
	Km	77.0 ± 27.9	453.5 ± 166.0
	Clint (L/hr x μMol^{-1})	0.44	0.78

a Values are presented as mean ± standard error (SE) of the fit.
b not determined

5.4.3.3.2.1.3 Correlation of Asenapine Metabolite Formation with Isozyme Activity

Spearman Rank correlations between the formation of the asenapine metabolites N-oxide asenapine and N-desmethyl asenapine and the metabolism of cytochrome P450 enzyme selective substrates also tend to confirm the rank order of activity of these isozymes toward the formation of the N-oxide and N-desmethyl metabolite, (see Table 36).

Table 36 Spearman Rank Correlations between the Formation of Asenapine N-oxide asenapine and N-Desmethyl asenapine and the Metabolism of Cytochrome P450 Isozyme Selective Substrates - Study NL0010293 (1998)

Cytochrome P450	Substrate	Reaction	Asenapine N(2)-Oxide		N(2)-Desmethyl Asenapine	
			2 µM	20 µM	2 µM	20 µM
CYP1A2	Phenacetin	O-DeEthyl	0.78**	0.71*	0.92***	0.79**
CYP2A6	Coumarin	7-OH	-0.23	0.07	-0.37	0.09
CYP2C	S-mephenytoin	4-OH	0.28	0.59	0.45	0.56
CYP2D	Dextromethorphan	O-DeMethyl	-0.32	-0.07	-0.22	0.12
CYP2E	Chlorzoxazone	6-OH	-0.23	-0.20	-0.23	-0.33
CYP3A	Testosterone	6β-OH	0.41	0.45	0.48	0.53

Statistical significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

5.4.3.3.2.1.4 Effect of Isozyme Selective Inhibitors on Metabolite Formation

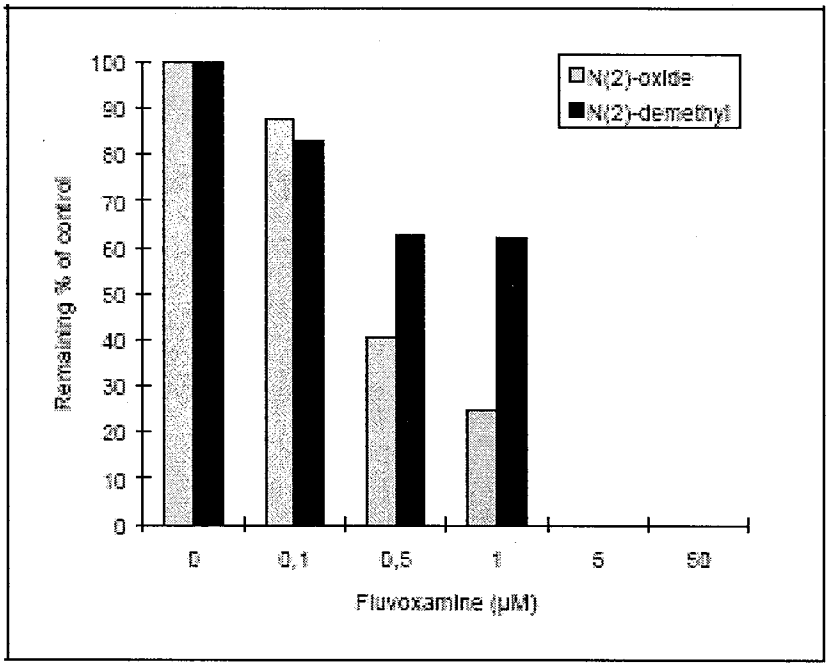
Microsomal incubations with 2 µM and 20 µM of [^{3}H]-asenapine were performed with five different inhibitor concentrations, (0.1, 0.5, 1, 5, 50 µM), of fluvoxamine and ketoconazole, selective inhibitors for CYP1A2 and CYP3A, respectively.

The sponsor's results are shown in Figure 19 and Figure 20. The asenapine concentrations shown in the figures appear to be transposed, as there is less inhibition at lower asenapine concentrations. If we assume that the concentrations are transposed, then the results would be consistent with the other experiments in supersomes which would not be surprising and all the results using the same experimental system should be consistent.

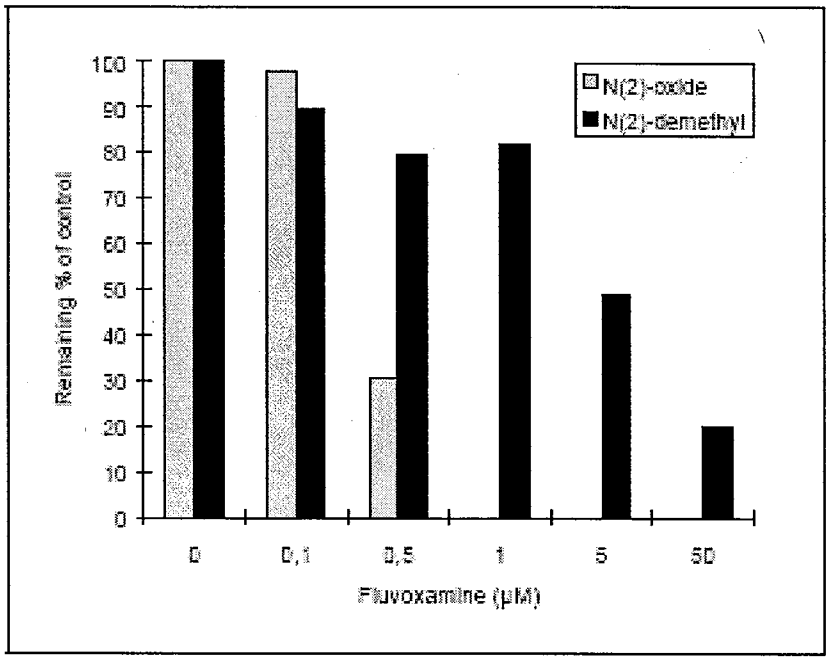
This should be remembered in assessing the weight of evidence for *in vitro* data showing the specific isozymes involved.

Figure 19 Inhibition of N(2)-Oxide and N(2)-Desmethyl Asenapine Formation by the CYP1A2 Selective Inhibitor Fluvoxamine – Study NL0010293 (1998)[a]

- 2 nmol·mL^{-1} Org 5222



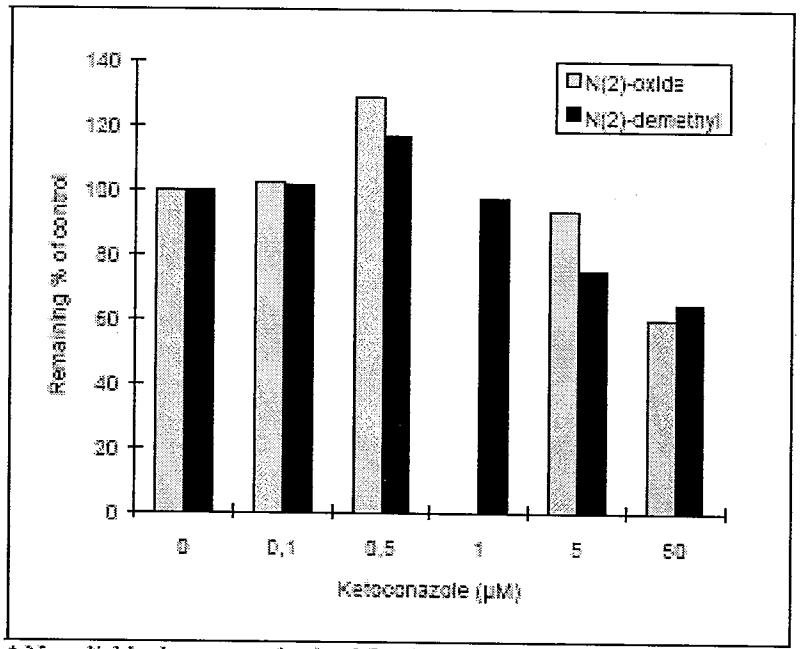
- 20 nmol·mL^{-1} Org 5222



a Asenapine concentrations 2 and 20 µM (nMol/mL))

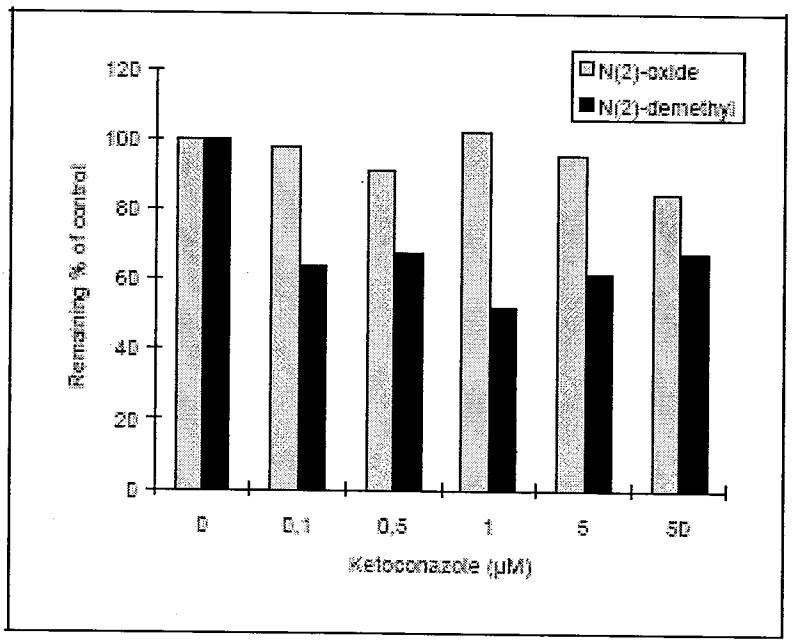
Figure 20 Inhibition of N(2)-Oxide and N(2)-Desmethyl Asenapine Formation by the CYP3A Selective Inhibitor Ketoconazole – Study NL0010293 (1998)

- 2 nmol·mL^{-1} Org 5222



* No reliable data were obtained for the N(2)-oxidation at 1 µM ketoconazole.

- 20 nmol·mL^{-1} Org 5222



a Asenapine concentrations 2 and 20 µM (nMol/mL)