3. Study title: An excretion balance and metabolic profiling study after an intravenous dose of asenapine (Org 5222) to male and female NWZ rabbits. (Study No. INT00006333)

Male and female NWZ rabbits received a single intravenous dose of 0.17 mg/kg/1.1 MBq/kg [^{14}C]-asenapine. Administration resulted in extensive metabolism as indicated by the number of peaks observed and the small amount of asenapine in urine and feces (3.5% of the dose). In plasma at least 33 peaks were found in the radioactivity profiles. Unchanged asenapine accounted for 9% of total radioactivity in plasma. In addition, 9 other metabolites (accounting for less than 5% of the total radioactivity) were observed in plasma from both male and female rabbits, including N-desmethylasenapine and the N-oxide of asenapine. Unchanged asenapine accounted for around 3% of dose in urine and feces together. There were no sex differences in metabolite profiles. Several metabolites remained unidentified.

The following study was reviewed by Dr. Lois Freed under the IND 51,641 (please see her review dated June 11, 1997 for further details). The summary is taken directly from Dr. Freed's review:

4. Study title: "Excretion and metabolic profiles after a single intravenous or single oral dose of [^{3}H]-Org 5222 to male and female Beagle dogs (SDGRR No. 3214, Organon, Inc., study dates: 10/91-3/92, non-GLP)

In this study, [^{3}H]-Org 5222 (batch no. H, unlabeled compound) was administered to Beagle dogs (3/sex) at doses of 0.5 mg/kg i.v., and 0.1, 0.6, and 3.6 mg/kg p.o. Each dog received all doses (i.v., p.o.), with 13-14 days wash-out between successive doses. Urine and fecal samples were collected for 4 days (24-hr intervals) after each dose. There was a complicated set of rules governing selection and pooling of urine and fecal samples for metabolic profiling. Suffice it to say that determinations were not based on individual samples of either urine or feces. Metabolites were quantitated and identified using HPLC with radioactivity detection.

The data are summarized in the following Sponsor's Table 1. Following both i.v. and p.o. dosing, the primary route of elimination was via the feces. Total radioactivity recovered ranged from 67-97%. Three metabolites and the parent compound were detected in urine and fecal samples. Metabolites 1 and 2 were unknown. Metabolite 2 appeared to represent more than one compound, and accounted for the majority of sample radioactivity following both i.v. and p.o. dosing. The demethyl derivative was the only identified metabolite in urine or feces. The data were fairly comparable following i.v. and p.o. dosing.

Table 1 Total 96-h excretion of radioactivity with urine and faeces after a single intravenous or single oral administration of [^{3}H]-Org 5222 to male and female Beagle dogs[a].

A. Male dogs

Dose ($mg \cdot kg^{-1}$)	Route	Excretion (% of dosed radioactivity) Urine	Faeces	Total
0,5	Intravenous	8,9 ± 3,8	58,0 ± 7,2	67,0 ± 4,7
0,1	Oral	15,4 ± 6,9	70,1 ± 22,3	85,5 ± 15,6
0,6	Oral	8,3 ± 2,7	63,8 ± 5,6	72,1 ± 3,8
3,6	Oral	9,3 ± 2,8	71,8 ± 6,3	81,1 ± 4,2

B. Female dogs

Dose ($mg \cdot kg^{-1}$)	Route	Excretion (% of dosed radioactivity) Urine	Faeces	Total
0,5	Intravenous	13,7 ± 3,0	66,5 ± 6,6	80,2 ± 3,9
0,1	Oral	15,3 ± 1,2	81,9 ± 3,3	97,1 ± 3,3
0,6	Oral	10,9 ± 2,0	66,9 ± 1,7	77,8 ± 0,2
3,6	Oral	10,3 ± 2,2	69,3 ± 3,4	79,6 ± 4,5

[a] Data are expressed as a percentage of dosed radioactivity and are given as means ± SD, n=3.

2.6.4.6 **Exeretion** (see the Metabolism section above)

2.6.4.7 Pharmacokinetic drug interactions (based on the Sponsor's summary)

1. Study title: <u>The inhibition of the human cytochrome P450 enzymes CYP1A2 and CYP2D6 by Org 5222 (*in vitro*)</u>. (Study No. NL0017588)

The inhibition of human cytochrome P450 enzymes CYP1A2 and CYP2D6 by asenapine was investigated using microsomes derived from insect cells expressing human cytochrome P450 enzymes CYP1A2 and CYP2D6. Formation of fluorescent products (7-ethoxy-3-hydrocoumarin and 3-[2[(N,N-diethyl-N-methylamino)ethyl]-7-hydroxy-4-methylcoumarin) was measured using a fluorometer, in the presence and absence of asenapine and reference inhibitors (furafylline and quinidine, respectively). Asenapine was found to be a competitive inhibitor of CYP1A2 and CYP2D6 with K_i's of 2.06μmol/L and 6.75 nmol/L, respectively.

2. Study title: <u>The inhibition of the human cytochrome P450 enzymes CYP2C19 and CYP3A4 by Org 5222 (*in vitro*).</u> (Study No. NL0013163)

The inhibition of human cytochrome P450 enzymes CYP2C19 and CYP3A4 by asenapine was investigated using microsomes derived from insect cells expressing human cytochrome P450 CYP2C19 and CYP3A4. Formation of products (4-hydroxyme-phenytoin and 6β-hydroxytestosterone, respectively) was measured by HPLC, in the presence and absence of asenapine and reference inhibitors (tranylcypromine and ketoconazole, respectively). Asenapine was found to be an uncompetitive inhibitor of CYP2C19 with a K_i of 25.2 μmol/L and asenapine was found to be a mixed-competitive inhibitor of CYP3A4 with K_{i1} of 91.4 μmol/L and a K_{i2} 125.6 μmol/L.

3. Study title: The assessment of inhibition of the human cytochrome P450 enzyme CYP2D6 with Org 5222 and its metabolites Org 30526 and Org 31437 *in vitro*. (Study No. NL0048836)

The inhibition of human cytochrome P450 enzyme CYP2D6 with asenapine, N-desmethylasenapine (Org 30526), and asenapine N-oxide (Org 31437) *in vitro* was determined using recombinant heterologously expressed human cytochrome P450 2D6 and AMMC (3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin) with quinidine as positive control inhibitor. All three agents tested have inhibitory activity *in vitro* towards CYP2D6 in the nanomolar range. K_i values were 16.0 nmol/L, (competitive), 62.1 nmol/L (non-competitive) and 82.6 nmol/L (competitive), for asenapine, N-desmethylasenapine, and asenapine N-oxide, respectively.

4. Study title: The assessment of inhibition of the human cytochrome P450 enzyme CYP2D6 with Org 10968 and Org 10969 (both enantiomers of asenapine (Org 5222) *in vitro.* (Study No NL0050307)

The inhibition of human cytochrome P450 enzyme CYP2D6 with Org 10698 and Org 10969 (both enantiomers of asenapine) was determined using heterologously expressed human cytochrome P450 2D6 and AMMC (3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin) as substrate. Org 10968 and Org 10969 were found to be competitive inhibitors of CYP2D6 with K_i's of 26.72 nmol/mL and 12.43 nmol/mL, respectively.

5. Study title: The assessment of inhibition of the human cytochrome P450 enzymes with asenapine (Org 5222) and its metabolites Org 30526 and Org 31437 *in vitro*. (Study No NL0050059)

The inhibition of human cytochrome P450 enzymes by asenapine, N-desmethylasenapine (Org 30526), and asenapine N-oxide (Org 31437) *in vitro* was determined using heterologously expressed recombinant human cytochrome P450 enzymes using fluorogenic probes. Asenapine and N-desmethylasenapine demonstrated inhibitory activity towards CYP1A2, CYP2C8, CYP2C9, CYP2C19 and CYP3A4 in the micromolar range. N-Desmethylasenapine also showed inhibitory activity towards CYP2A6 in the micromolar range. The inhibitory activity of asenapine was highest for CYP1A2 (K_i of 1.5 μmol/L) followed by CYP2C19 (K_i of 2 μmol/L) and CYP3A4 (K_i of 33.2 μmol/L). Low inhibitory activity was observed for CYP2C8 and CYP2C9 (K_i of 360 and 105 μmol/L, respectively). Asenapine showed no inhibitory activity towards CYP2A6. The inhibitory activity of Org 30526 was highest for CYP1A2 (K_i of 1.4 μmol/L) followed by CYP2C19 (K_i of 1.8 μmol/L) and CYP3A4 (K_i of 3.5 μmol/L). Low inhibitory activity was observed for CYP2A6 (K_i of 70.3 μmol/L), CYP2C8 (K_i of 80.3 μmol/L) and CYP2C9 (K_i of 172.3 μmo/L). Asenapine N-oxide showed no inhibitory activity towards CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4.

6. Study title: Effect of asenapine on human drug metabolizing enzymes *in vitro.* (Study No DM2005-005222-009)

Asenapine was tested as a potential inhibitor for seven human cytochrome P450 enzymes in pooled human liver microsomes. Cytochrome P450 activities assessed were phenacetin O-deethylase (CYP1A2), bupropion hydroxylase (CYP2B6), amodiaquine N-deethylase (CYP2C8), diclofenac 4'-hydroxylase (CYP2C9), S-mephenytoin 4'-hydroxylase (CYP2C19), dextromethorphan O-demethylase (CYP2D6), and three activities for CYP3A: felodipine dehydrogenase, testosterone 6β-hydroxylase, and midazolam 1'-hydroxylase. Asenapine was tested up to a maximum concentration of 0.030 mM (~8600 ng/mL. The only enzymes appreciably inhibited were CYP2D6 (IC_{50} = 0.044 μM; ~ 13 ng/mL) and CYP1A2 (0.61 μM; ~ 175 ng/mL).

7. Study title: Induction potential of asenapine (Org 5222) on cytochrome P450 1A2 and 3A4 in human hepatocytes. (Study No 764-04914)

Asenapine was tested as a potential inducer of cytochrome P4501 and 3A enzymes in primary human hepatocytes (N=4) in culture for 3 days. Cytochrome P450 activities assessed were phenacetin O-deethylase (CYP1A2) and testosterone 6β-hydroxylase (CYP3A4), and measurements of transcription of CYP1A2 and CYP3A4 genes were also made. Asenapine was tested up to a maximum concentration of 0.030 mM (~8600 ng/mL), and comparisons to positive control inducers (lansoprazole for CYP1A2 and rifampin for CYP3A4) were made. Treatment of hepatocytes with multiple doses of asenapine indicates that there is no significant increase in CYP1A2 and CYP3A4 activities at concentrations relevant to expected therapeutic concentrations. However, there were concentration-dependent increases in mRNA for both CYP1A2 and CYP3A4 observed at asenapine concentrations well in excess of therapeutically relevant values. Because concentrations tested exceed therapeutic concentrations by 10-1000x, it is predicted that asenapine will not cause clinically meaningful induction of CYP3A or 1A2.

8. Study title: An induction of cytochrome P450 with Org 5222 in Wistar rats. (Study No. NL0017615)

Microsomes were isolated from the livers of male and female Wistar rats derived from a 13-week subcutaneous study with asenapine (Org 5222). Daily doses of 2.12, 2.82, and 3.53 mg/kg were used. Microsomal suspensions were characterized for cytochrome P450 activity and total protein. Changes in hepatic cytochrome P450 activity were evaluated by incubations with testosterone, aniline, pentoxyresorufin, ethoxyresorufin and methoxyresorufin. Asenapine was shown to be an inducer of cytochrome P450 enzyme activity. Increased activities were found for CYP1A1, CYP1A2, CYP2B1/CYP2B2 (10-80%) from 2.12 mg/kg onwards in male rats and only in the 3.53 mg/kg group in female rats. Aniline metabolism (CYP2E1 and/or CYP1A2 activity) was increased 20-90% in male and female rats, especially apparent in the 2.82 and 3.53 mg/kg groups. Decreased activities of CYP2C11 (only present in male) and CYP3A1 were found in male rats with increasing dose but not in female rats.

2.6.4.8 Other Pharmacokinetic Studies

There were no other pharmacokinetic studies.

2.6.4.9 Discussion and Conclusions (based on the Sponsor's summary)

The absorption, distribution, metabolism, and excretion (ADME) of asenapine were assessed in several species in order to provide a comparison of the overall metabolism and disposition in animals utilized in safety studies with that in humans. Additionally, several in vitro studies were run in order to determine plasma protein binding, enzymes responsible for metabolism, effects on drug metabolizing enzymes, and to determine the potential for PgP transport. The pharmacokinetics of asenapine in animals and humans are characterized by moderate tissue distribution and high systemic clearance. The pharmacokinetics of asenapine in animals and humans are characterized by moderate tissue distribution and high systemic clearance. There are significant differences in oral bioavailability of asenapine among species. (i.e., between 20-65% in rats, maximally 10% in dogs and <2% in humans). No data on oral bioavailability in rabbits were provided. No pharmacokinetic differences were observed between the enantiomers in dog and man. In mice, rats, and rabbits, higher levels (up to 1.9 fold) of Org 10969, the (+) enantiomer were observed as compared to Org 10968, the (-) enantiomer. Asenapine appears to readily penetrate membranes and is therefore well absorbed from the sublingual route and it can readily cross the blood-brain barrier. High absorption is also consistent with findings in Caco-2 cells in which the apparent permeability (P_{app}) of asenapine through the Caco-2 cell monolayer was found to be 1×10^5 cm/sec on average or greater. Asenapine is not a substrate of the transport protein P-glycoprotein, as assessed through the use of MDCK cells stably transfected with the human MDR-1 gene. It distributes to a variety of tissues and although it has been shown to be eliminated slightly slower from tissues containing melanin, no retention was found, as assessed in rat and dog. Serum/plasma protein binding is high in all species with free fraction values of 0.040, 0.030, 0.031, 0.022, 0.034, and 0.027 in mouse, rat, rabbit, dog, monkey, and human, respectively. In human, both albumin and α_1-acid glycoprotein bind asenapine. Binding to brain tissue is very high, which is consistent with the high brain/plasma ratio observed in rats and monkeys. The main clearance mechanism of asenapine in animals and humans is via metabolism. The metabolism of asenapine is complex, with over 40 metabolites observed in circulation and excreta of laboratory animals and humans. Most metabolites are present as small percentages. Initial asenapine metabolites arise via oxidation, demethylation and direct glucuronidation reactions. Unchanged asenapine accounted for 2–12 % of total drug related material in circulation in mice, rats, rabbits, dogs, and humans. After subcutaneous or intravenous administration to animals, unchanged asenapine was found in the feces accounting for 0.2–12% total drug related material. This might partly be explained by excretion of conjugates via bile and subsequent deconjugation. After sublingual administration to man, unchanged asenapine was found in the feces too. This also might partly be explained by excretion via bile. Most metabolites observed in humans are also observed in laboratory animals. In humans, asenapine N^+-glucuronide appears to be of greater relative abundance than in animals. Of the other circulating metabolites in human, only N-desmethylasenapine and asenapine-11-O-sulfate showed receptor binding, but neither is expected to contribute to the pharmacological activity due to lower affinity, low plasma concentrations and, for the sulfate, the inability to cross the blood brain barrier. The N-oxide metabolite of asenapine was found in abundance in animals but is not important in human. Phase I metabolism of asenapine in vitro yielded N-desmethylasenapine, asenapine N-oxide, 11-hydroxyasenapine, and if supplemented with cofactors and conditions required for activity of uridine diphosphoglucuronyl transferase (UGT), asenapine N^+-glucuronide is observed. For Phase I metabolism, studies suggest that CYP1A2 is the most important of the cytochrome P450 enzymes in the metabolism of asenapine, in addition to contributions from CYP3A4 and CYP2D6. Metabolism occurs via several routes and enzymes with no single clearance mechanism dominating the profile. Asenapine does not inhibit most human cytochrome P450 enzymes, but does inhibit CYP2D6 (IC_{50} = 0.044 µM) and (IC_{50} = 0.61 µM). Asenapine does not cause induction of CYP1A2 or CYP3A activities in cultured human hepatocytes. However, it did exhibit some induction of cytochrome P450 enzyme activity, assessed *ex vivo* in livers from rats treated subcutaneously for 13 weeks with asenapine. In *in vivo* excretion/mass balance studies asenapine drug-related material was observed in both urine and feces, and total recovery values were in excess of 80%. In the mouse (M/F, s.c.), rat (M/F, s.c.), rabbit (M/F, i.v.), dog (M/F, i.v.), and human (M, s.l.), the percentage of total dose recovered in urine was 15/27, 18/18, 67/63, 10/12, and 49%, respectively. The percentage in feces was 65/54, 75/75, 25/29, 82/79, and 39%, of the dose, respectively.

2.6.4.9 Tables and figures to include comparative TK summary

Multiple dose AUC and C_{max} values determined in various species and compared with human exposure are shown in the following Sponsor's table:

Study/species/dosing route (multiple dose)	Dose (AE mg·kg^{-1})	AUC (ng·h·mL^{-1})		C_{max} (ng·mL^{-1})	
		Male	Female	Male	Female
13 week mouse SC[a,21]	2.12	302	259	236	223
	2.82	359	308	273	246
	5.29	647	623	528	639
104 week mouse SC[b,22]	0.35	-	-	31.2	28.2
	1.06	-	-	88.4	80.4
	3.55	-	-	201	166
52 week rat oral[c,18,23]	0.21	38.3	-	15	-
	1.27	93.3	-	26	-
	7.61	355	-	110	-
2 week rat iv[d,24]	0.3	-	92.3	-	86
	0.9	-	226	-	147
	1.5	-	413	-	314
13 week rat SC[e,25]	2.12	279	183	162	115
	2.82	335	256	165	95
	3.53	458	362	209	166
104 week rat SC[f,26]	0.2	-	-	34	25.2
	0.9	-	-	132	103
	3.5	-	-	317	354
12 days rabbit iv[g,27]	0.018	-	4.9	-	8
	0.088	-	41.5	-	49
	0.44	-	179.0	-	232
13 week dog iv[h,28]	0.07	23	18	14	15
	0.35	94	94	65	64
	1.76	575	537	385	410
39 week dog iv[i,29]	0.07	37	33	18	33
	0.78	121	123	58	78
	1.13	594	759	272	351
Man[j]	0.08	28.6		4.23	
	0.15	43.4		6.58	

[a] $AUC_{(0-2.5h)}$, [b] Highest concentration of three measured time point after 27 weeks

[c] $AUC_{(0-7h)}$; [d] $AUC_{(0-t)}$; [e] $AUC_{(0-2.5h)}$; [f]: highest concentration of three measured time point after 98 weeks; [g]: $AUC_{(0-1h)}$ for 0.018 mg·kg^{-1} and $AUC_{(0-2h)}$ for 0.088 and 0.44 mg·kg^{-1}, C_{max} is average plasma concentration and first sampling time point (5 min)[h]: $AUC_{(0-30h)}$, C_{max} is average plasma concentration and first sampling time point (5 min); [i]: $AUC_{(0-t)}$ - not determined; [j]: Data from the thorough QTc study (see Clinical Trial Report on A7501001, Table 48, $AUC_{(0-12h)}$).

The summary of representative pharmacokinetic and excretion properties of [^{14}C]-asenapine drug-related material in animals as compared to humans is shown in the following Sponsor's table:

Species:	Mouse[a]	Rat[a]	Rabbit[a]	Dog[a]	Human[b]
Dose ($mg \cdot kg^{-1}$) (Route)	0.74 (sc)	3.0 (sc)	0.17 (iv)	0.1 (iv)	10.3 mg (sl)**
Pharmacokinetic Parameters					
C_{max} ($ngeq \cdot mL^{-1}$)	nd	235	104/122	nd	78***
t_{max} (h)	nd	4.0			4.0***
$T_{1/2}$ (h)	nd	9.7	9.5/11.9	nd	39***
Excretion parameters					
Dose in Urine (%)*	15/27	18/18	67/63	10/12	49
Dose in Feces (%)*	65/54	75/75	25/29	82/79	39
Total Recovery (%)*	80/82	93/93	93/93	93/91	88

ND: not determined; NR: not reported; [a]male/female; [b]healthy male subjects SD.
* 0-168h excretion;
** a dosing schedule was used from 0.3 mg to 10 mg in 5 days and then 5 days of 10 mg asenapine per day.
*** based on total radioactivity

The summary of metabolites observed in animal studies and in humans is shown in the following Sponsor's table:

Table 2 Summary of Representative Metabolites of Asenapine in Preclinical Species and Human[a] (2.6.4.5)

	% of Dose in Excreta[b]					% of Total Radioactivity in Circulation[b,c]				
Metabolite/Identity	Mouse	Rat	Rabbit	Dog	Human[e]	Mouse	Rat	Rabbit	Dog	Human[e]
Asenapine	3.0/0.6	10/6.2	3.4/3.3	1.0/1.5	4.5-10	6/10	9.0/15	10/10	10/10	2
N-Desmethylasenapine	10/8.7	20/30	2.0/1.5	11/12	0-1.7	+[b]	ND/6.0	4/4	10/10	+
Asenapine N-Oxide	2.0/2.2	1.0/1.5	0.4/0.5	0.1/1	-	14/6	ND/15	ND/1	7/8	
Asenapine N[+]-Glucuronide	+	+[f]	4.7/5.8	+	10.3-21.3			+[b]		13-18
N-Desmethylasenapine N-Carbamoyl Glucuronide	+	ND/1.8		+	2-3		7.5/8.2			5
Asenapine 11-O-Sulfate	+	+	+/+	+	+	+[b]				+/-
Asenapine-O-glucuronide	+	+	+/+	+	+					
N-desmethylasenapine-O-glucuronide	+			+	+					
OH-asenapine-O-glucuronide			+/+							
conjugated-dihydroxylated-asenapine			+/+							
dihydroxylated asenapine	+	+		+	+					
conjugated-dihydroxylated-desmethylasenapine	+	+		+	+					
dihydroxylated desmethylasenapine	+	+		+	+					
[illegible]-Hydroxy-N-formyl-N-desmethylasenapine	+	+		+	+					
Hydroxylated and conjugated asenapine N-oxide					+					

[illegible]
[b]Doses and routes studied were: mouse: 0.74 mg/kg[-1], sc; rat: 3.0 mg/kg[-1] sc; rabbit: 0.17 mg/kg[-1], iv; dog: 0.1 mg/kg[-1], iv; human: 10 mg SL.
[c]ND denotes that the metabolite was not detected by radiometric HPLC; [d]: rough estimates in most cases; [e] in bile; [f]: only males; +: present, not quantifiable; +/-: present in some individuals.
Based on at least retention time comparison the major human metabolites were covered in pre-clinical species. Only some metabolites from the rabbit urine and rat could be isolated and partly identified. For all other samples the amount of metabolites was too low and/or the amount of impurities was too high to perform identification.

The proposed metabolic pathways in all species examined are shown in the Sponsor's figure below:

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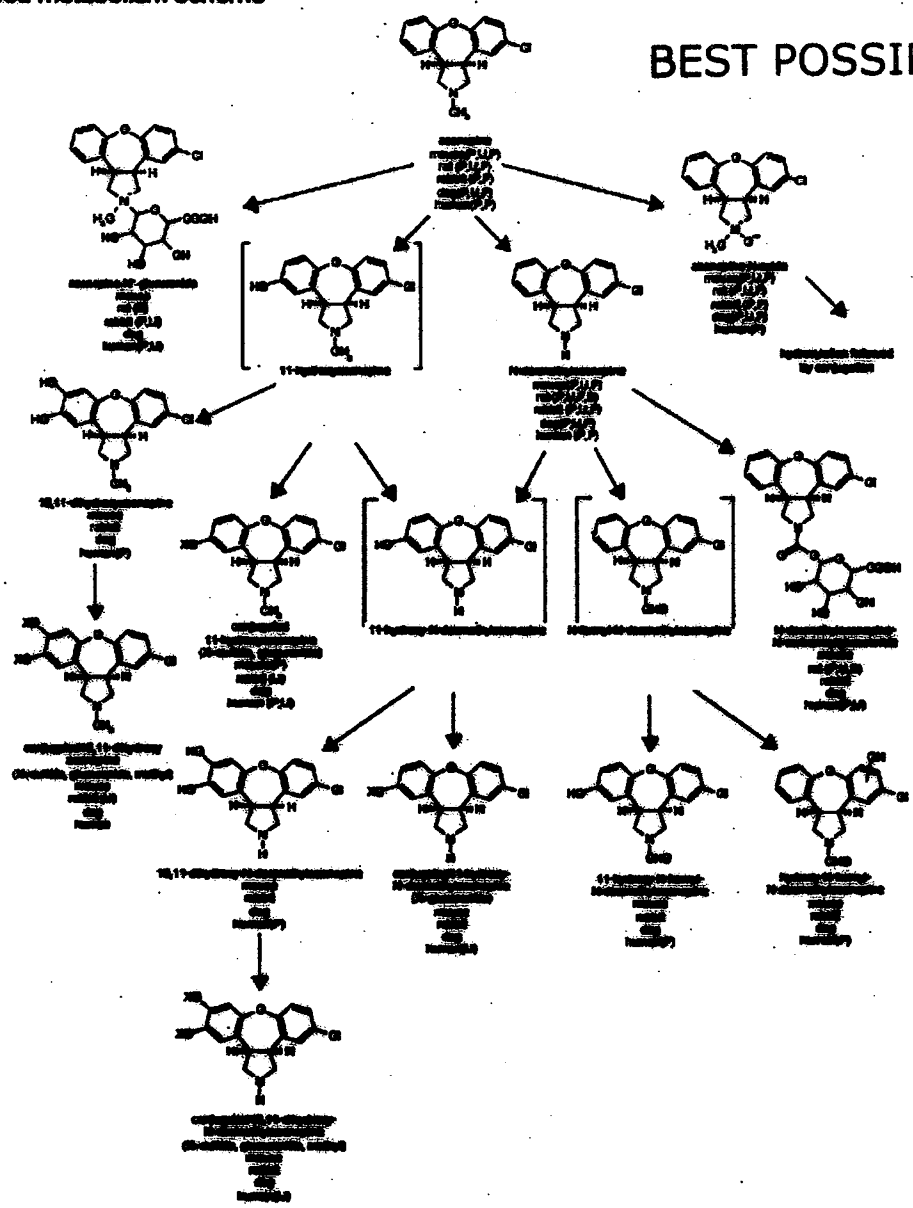
Proposed metabolism scheme



P= plasma, U = urine, F = feces, B = bile

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pivotal studies relevant to the primary pharmacodynamic effect are shown in the following Sponsor's table:

Type of study	Test system	Method of administration	Testing Facility	Report number	Location in CTD
Absorption	mouse	sc	Organon, Schaijk, NL	[illegible]	4.2.2.2
Absorption	rat	iv	Organon, Schaijk, NL	[illegible]	4.2.2.2
Absorption	rat	iv	Pfizer, Groton, USA	[illegible]	4.2.2.2
Absorption	rat	sc	Organon, Schaijk, NL	[illegible]	4.2.2.2
Absorption	[illegible]	iv	Organon, Schaijk, NL	[illegible]	4.2.2.2
Absorption	dog	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.2
Absorption	dog	iv	Organon, Schaijk, NL	[illegible]	4.2.2.2
Absorption	monkey	sc	Pfizer, Groton, USA	[illegible]	4.2.2.2
Absorption	monkey	sc	Pfizer, Groton, USA	[illegible]	4.2.2.2
Distribution	rat	oral	Organon, Oss, NL	[illegible]	4.2.2.3
Distribution	rat	oral	Organon, Oss, NL	[illegible]	4.2.2.3
Distribution	rat	sc	Pfizer, Groton, USA	[illegible]	4.2.2.3
Distribution	dog	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.3
Distribution	monkey	sc	Pfizer, Groton, USA	[illegible]	4.2.2.3
Distribution	[illegible]	Plasma in vitro	Organon, Oss, NL	[illegible]	4.2.2.3
Distribution	[illegible]	Plasma in vitro	Organon, Schaijk, NL	[illegible]	4.2.2.3
Distribution	mouse, rat, rabbit, dog, human	Plasma/protein in vitro	Pfizer, Groton, USA	[illegible]	4.2.2.3
Distribution	rat, rabbit, human	Plasma in vitro	Pfizer, Groton, USA	[illegible]	4.2.2.3
Distribution	mouse, rat, rabbit, dog, human	Blood (erythrocytes) in vitro	Organon, Schaijk, NL	[illegible]	4.2.2.3

Type of study	Test system	Method of administration	Testing Facility	Report number	Location in CTD
Distribution	rat	iv	HLS, UK	[illegible]	4.2.2.3
Distribution	rabbit	iv	HLS, UK	[illegible]	4.2.2.3
Distribution	[illegible] cells	in vitro	Pfizer, Groton, USA	[illegible]	4.2.2.3
Metabolism (interspecies comparison)	mouse	sc	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	rat	[illegible]	Organon, Oss, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	rat	sc	Pfizer, Groton, USA	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	rabbit	iv	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	dog	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	dog	[illegible]	Organon, Oss, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	dog	iv	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	human	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	[illegible]	Liver [illegible]	Organon, Oss, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	mouse, rat, rabbit, dog, human	Liver microsomes	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	rat, human	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	mouse, rat, rabbit, dog, human	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	human	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	human	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	human	[illegible]	Pfizer, Groton, USA	[illegible]	4.2.2.4
Metabolism (interspecies comparison)	[illegible]	[illegible]	Organon, Schaijk, NL	[illegible]	4.2.2.4
Excretion	mouse	sc	Organon, Schaijk, NL	[illegible]	4.2.2.5
Excretion	rat	[illegible]	Organon, Oss, NL	[illegible]	4.2.2.5
Excretion	rat	sc	Pfizer, Groton, USA	[illegible]	4.2.2.5

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Type of study	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

[illegible]

[illegible]

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Asenapine maleate (Org 5222) was tested in the genetic toxicity, reproductive and developmental toxicity, general toxicity, carcinogenicity, phototoxicity, antigenic potential and local irritation studies conducted *in vitro* or in CD-1 mice, Wistar and Sprague-Dawley rats, Beagle dogs or Dutch and New Zealand White rabbits. The majority of the general and reproduction and developmental toxicity studies, as well as the rat micronucleus assay were conducted using the oral route of administration. Asenapine is being developed for sublingual administration in humans. It is not feasible to use the sublingual route in rodents. Therefore, the Sponsor conducted bridging studies in laboratory animals by subcutaneous or intravenous administration to support the intended route in humans. From toxicokinetic point of view, the intravenous route is a route that can be compared best with the sublingual route. Therefore, the intravenous route was used in many pivotal toxicology studies. All pivotal studies have been conducted according to the principles of Good Laboratory Practice (GLP). In addition, toxicity studies were conducted to investigate the toxicity of impurities in the asenapine drug substance. Moreover, the *in vitro* and *in vivo* genotoxicity studies were conducted for two structurally related degradants in drug product present in the commercial batches at levels . Since the overall metabolic profile in humans and laboratory animal species is similar, individual metabolites or enantiomers have not been tested toxicologically. b(4)

Note: Asenapine dosages refer to the maleate salt (approximately 71% active entity) unless otherwise indicated.

General toxicology: Effects of asenapine were assessed in single and repeated dose general toxicology studies. Asenapine was tested in the following single dose toxicity studies in rats and dogs:

Species	Dosage (mg/kg/day)	Route/duration of observation	Number of animals/dose	Reference (study #)
rat	75 to 625	p.o./14 days	6 males, 6 females	SDG RR 2723

rat	50, 100, 200	p.o./14 days	6 males, 6 females	SDG RR 3005
rat	9, 15, 21	i.v./14 days	6 males, 6 females	SDG RR 3006
dog	50, 100, 200	p.o./14 days	2 males	SDG RR 3002

Asenapine was administered orally to rats and dogs in the repeated dose general toxicity studies for up to 52 weeks. Twice daily administration was applied in some studies to attenuate C_{max} related clinical signs. In the pivotal 52-week study in dog, the MTD has not been reached. Therefore, a 39-week study has been performed in dogs by the intravenous administration. Asenapine was tested in the following repeat dose toxicity studies in rats, mice, and dogs:

Species	Dosage (mg/kg/day)	Route/duration of observation	Number of animals/dose	Reference (study #)
Repeat-dose toxicity: non pivotal studies				
mouse	25, 75	p.o./4 weeks	6 males, 6 females	SDG RR 2940
mouse	0.12, 0.6, 3.0	s.c../2 weeks	18 males, 18 females	SDG RR 7013
mouse	0.5, 1.0, 2.0	s.c./13 weeks	12 males, 12 females	SDG RR 7026
rat	18.5, 37.5, 150	p.o./1 or 2 weeks	6 males, 6 females	SDG RR 2724
rat	25, 50, 75	p.o./4 weeks	8 males, 8 females	SDG RR 2664
rat	0.12, 0.6, 3.0	s.c./2 weeks	6 males, 6 females	SDG RR 7014
dog	40, 80, 160	p.o./1-2 weeks	1 male, 1 female	SDG RR 2502
dog	20, 40, 80	p.o./4 weeks	3 male, 3 female	SDG RR 2663
Repeat-dose toxicity: pivotal studies				
mouse	3, 4, 5/7.5	s.c./13 weeks	12 males, 12 females	NL0017870
rat	6.25, 22.5, 75	p.o./ 13 weeks	8 males, 8 females	SDG RR 2320
rat	0.3, 1.8, 10.8 b.i.d.	p.o./52 weeks	20-30 males, 20-30 females	SDG RR 3210 SDG RR 2979
rat	0.5, 1, 2	s.c./13 weeks	12 males, 12 females	SDG RR 7025
rat	3, 4, 5	s.c./13 weeks	10 males, 10 females	NL0017877
rat	0.1, 0.5, 3	i.v./2 weeks	8 males, 8 females	SDG RR 2798
dog	escalating dose part 3.6, 5.4, 7.2, 10.8 b.i.d. fixed dose part 10.8 b.i.d.	p.o. escalating dose 3-5 days fixed dose part 4 weeks	escalating dose 3 males, 3 females fixed dose 3 males, 3 females	NL0001154
dog	1.25, 7.5, 20	p.o./13 weeks	3-5 males, 3-5 females	SDG RR 2285
dog	0.1, 0.6, 3.6 b.i.d.	p.o./52 weeks	4-6 males, 4-6 females	SDG RR 3209
dog	0.1, 0.5, 3.0	i.v./2 weeks	3 males, 3 females	SDG RR 2799
dog	0.1, 0.5, 2.5	i.v./13 weeks	3 males, 3 females	SDG RR 4417
dog	0.1, 0.4, 1.6	i.v./39 weeks	4 males, 4 females	NL00012511

All listed above single dose studies and the following subchronic and chronic toxicity studies in rats and dogs have been submitted to the IND 51,641 and reviewed by Dr. Lois Freed: SDG RR No. 2940, 2724, 2664, 2502, 2663, 2320, 3210, 2798, 2285, 3209, 2799 and 4417 (Dr. Freed's review dated June 11, 1997). The reports of studies NL0017870, SDG RR 7025, and NL0017877 have been submitted later to the IND 51,641 and reviewed also by Dr. Lois Freed (Dr. Freed's review dated April 20, 2000). These reviews are available in the Division's file. Study NL0012511 has been submitted to the IND 70,329 and reviewed by Dr. Sonia Tabacova. Dr. Tabacova's review dated July 20, 2005 is available in the DARRTS. The summaries of these studies taken directly from Drs. Freed's and Tabacova's reviews can be found on page 50 of this review. Other toxicity studies were non-pivotal and will not be discussed further. In addition, Dr. Tabacova reviewed the summary of plasma exposure data from several toxicity studies (rodent and nonrodent; all routes, including oral) for parent compound and major circulating metabolites with respect to a switch in development from oral to sublingual administration of asenapine in humans and concluded that the pivotal 13-week subcutaneous rat study, the 52-oral rat study, and the 39-week dog i.v. study are adequate for safety evaluation of asenapine toxicity upon sublingual administration in humans (Dr. Tabacova's review of June 21, 2007 is available in the DARRTS). The summary taken directly from Dr. Tabacova's review can be found on page 78 of this review.

Genetic toxicology: Asenapine was tested for genotoxic potential in a battery of *in vitro* and *in vivo* tests. Asenapine did not induce mutations in the bacterial reverse mutation tests (Ames tests) conducted with *Salmonella typhimurium* and *Escherichia coli* strains, mutations at the thymidine kinase locus in the mouse lymphoma assay, and non-mutational DNA changes in the sister chromatid exchange test in rabbit lymphocytes *in vitro*. However, in the chromosomal aberration assay in cultured human lymphocytes *in vitro*, asenapine minimally increased the incidence of structural chromosomal aberrations in the presence of metabolic activation and numerical aberrations in the absence and presence of metabolic activation. Therefore, the results of this study are considered equivocal. In the *in vivo* micronucleus test conducted in rats, asenapine did not induce chromosomal damage in the bone marrow cells. The weight of evidence suggests that asenapine lacks genotoxic potential.

Carcinogenicity: In the rat carcinogenicity study, the MTD (maximum tolerated dose) was clearly exceeded in males at all dose levels and in females at the high dose based on significant and dose-dependent decreases in body weight gain and body weight. The incidence of preneoplastic changes and tumors (total number of tumors and tumor-bearing animals) was decreased at the high dose when compared to the vehicle controls. The low dose and medium dose groups were not routinely examined. Since it is known that a significant decrease in body weight can lead to a decrease in tumor development, it is presumed that the sensitivity of this study to detect any asenapine-induced tumors was reduced, and thus this study is considered to be inadequate until a full histopathologic examination of the low and mid dose males and females is completed. (Note that these additional examinations will not completely address the problem in male rats, where the MTD was exceeded at all doses. However, it is concluded that this study does not have to be repeated assuming adequate data are obtained from female rats as well as from both male and female mice).

In the mouse carcinogenicity study, the incidence of pleomorphic malignant lymphomas and all combined lymphomas in the hemolymphoreticular system was statistically significantly increased in the female mice at the high dose compared to the vehicle control (7/57 and 20/60 in the vehicle control and high dose group, respectively). However, the incidence of these tumors in the female mice at the high dose was similar to that in the untreated controls (22/57). The reason for this large difference between the vehicle and untreated controls is not known. The vehicle did not appear to cause a general decrease in other tumor types. Therefore, the large difference in the incidence of lymphomas between vehicle and untreated female controls should be explained. Furthermore, full histopathology examination of the low dose and medium dose female groups should be performed. The final evaluation of the lymphomas will be made after the additional data are received from the Sponsor.

Reproductive toxicology: Effects of asenapine were assessed in a definitive fertility and early embryonic development p.o. study in rats, two definitive (p.o. and i.v.) embryofetal development studies in rats, two definitive (p.o. and i.v.) embryofetal development studies in rabbits, and two i.v. prenatal and postnatal development studies in rats.

In the oral study in Wistar rats designed for the assessment of fertility and reproductive performance asenapine was administered at 0.5, 2.5 and 15.0 mg/kg b.i.d. to males and females before and during mating and gestation to termination on day 21 of gestation or day 21 after birth. There were no statistically significant effects on fertility index, conception rate or gestation index in dosed females (although decreased mating and pregnancy ratios were seen in the pilot study). Dose-related effects included clinical signs of piloerection, sedation, and abnormal posture, effects on food consumption (increase before mating, decrease during gestation and lactation), and lower body weight in males and females. There were also effects on reproductive performance including increased pre-coital time at all dose levels. Embryotoxic effects included increased pre-implantation loss at all dose levels and increased post-implantation loss at the MD and HD, slightly increased neonatal mortality at all dose levels, and slight delays in the development of the surviving offspring (decreased body weight gain of the F1 pups associated with a delayed skeletal development of the fetuses most likely related to the reduced body weights) at the HD. No skeletal teratogenic effects were observed. The evaluation of external and visceral teratogenic effects in this study is considered inadequate.

Oral administration of asenapine to pregnant Wistar rats in a definitive embryotoxicity and teratogenicity study at 0.5, 2.5 and 15.0 mg/kg b.i.d. from day 7 through day 17 of pregnancy resulted in dose-related clinical signs of ruffled fur, somnolence, hunched posture and ventral recumbency in all dose groups. Reduction in food consumption was observed at the MD and HD. Minimal to marked dose-dependent reduction in body weight between days 7 and 8 of pregnancy and body weight gain during the treatment period was noted in all dose groups. At the HD, there was an increase in number of females with total resorptions, increase in post-implantation loss, and slight reduction in fetal body weight. There were no treatment related toxicologically significant changes in the sex ratio of the fetuses and their external, visceral and skeletal abnormalities. Therefore, asenapine was not teratogenic in this study. The evaluation of external and visceral teratogenic effects in this study is considered inadequate. The Sponsor did not address these concerns directly. However, an intravenous embryofetal developmental study in rats, designed to characterize teratogenic effects of asenapine, was submitted under this NDA.

The intravenous embryofetal developmental study in Sprague-Dawley rats was designed to examine the effects of asenapine on pregnant females as well as embryonic and fetal development when administered intravenously from implantation to closure of the hard palate (days 6-17 of pregnancy) at 0.3, 0.9, and 1.5 mg/kg/day. All dams treated with asenapine showed marked reduction in motor activity and increased muscle tone during the course of treatment days starting immediately after administration and decreasing during the course of the respective day. Body weight gains were minimally lower after initiation of the treatment (days 6-12) when compared with that of the control group. However, there was no clear dose-dependence for this effect. Moreover, body weight gain was also lower at the HD on days 0-6 of pregnancy (before the treatment initiation). The reviewer concurs with the Sponsor's conclusion that the MTD for maternal toxicity was not achieved in this study. However, evidence of pharmacological activity was clearly demonstrated at all dose levels. Skeletal examinations demonstrated minimally increased incidence of a variety of abnormal findings in 5 HD litters. However, the majority of the findings occurred in one individual litter and, therefore, is not considered drug-related. There were no test article-related external or visceral findings in fetuses at any dose level.

The oral embryotoxicity and teratogenicity study in Chinchilla rabbits was designed to examine the effects of asenapine on the pregnant female rabbit as well as embryonic and fetal development when administered daily from day 6 to day 18 of gestation at 0.5, 2.5, and 15 mg/kg b.i.d. Two HD females died about 5 minutes after the second daily administration. Body weight loss of pregnant females from day 19 to day 21 of pregnancy (after treatment period) correlated with reduced food consumption. There were no effects on body weight during pregnancy. No adverse effects of treatment with asenapine on the pregnancy parameters were observed. There were no test article-related visceral or skeletal malformations in the fetuses. There was no clear pattern in changes in ossification. Mean fetal body weight was reduced at the HD. In conclusion, asenapine was not teratogenic under conditions of this study. However, it is unclear whether the external and visceral malformations were properly examined. "Runt" was the only abnormal finding reported upon external or visceral examinations. It appears extremely unlikely that no spontaneous external or visceral findings were detected in any fetus. Therefore, this study is considered inadequate for evaluation of external and visceral teratogenic effects. The Sponsor did not address these concerns directly. However, an intravenous embryofetal developmental study in the rabbits, designed to characterize teratogenic effects of asenapine, was submitted under this NDA.

In the intravenous embryofetal development study, New Zealand White rabbits were administered asenapine intravenously at 0, 0.018, 0.089, and 0.444 mg/kg/day from day 6 to day 18 of gestation. There were two drug-related unscheduled deaths. Clinical signs of polypnea (all animals), occasional motor incoordination, occasional ptosis (all animals), and occasional hyperactivity were observed at HD. There were no adverse effects on maternal body weight or fetal body weight, food consumption, pregnancy performance or gross pathology. There were no skeletal or visceral variants that were clearly attributable to drug. Visceral malformations (major defects) were observed in 1/177, 2/111, 1/97, and 4/164 control, LD, MD, and HD females, respectively. In the HD group, 1 fetus had 2 major defects; the other fetuses had each one malformation. The abnormal litter ratio was 0.5%, 1.4%, 1.0%, and 3.9% in the control, LD, MD, and HD females, respectively. Skeletal malformation (flexure of the forelimb) was observed only in one fetus in the MD group. As the major malformations had a low incidence in drug-treated groups, they were considered incidental in origin and, therefore, not drug related. The exposure achieved at the HD in this study (AUC_{0-24}: 179.02 ng·h/mL) was 2-fold higher than that achieved at steady state following sublingual administration of asenapine at the MRHD of 10 mg b.i.d. (AUC_{0-24}: 86.8 ng·h/mL).

In the intravenous prenatal and postnatal development study in Sprague-Dawley rats administration of asenapine at 0.3, 0.9, and 1.5 mg/kg/day to female rats from implantation (day 6 of gestation) through weaning (day 20 post partum) did not result in any findings indicative of a treatment related effect during gestation except marked reduction of motor activity and increased muscle tone in all treatment groups and slight (up to 15%), but not dose-related, decreases in body weight gain from day 6 to 21 of gestation. Body weight gain of F0 dams was also slightly decreased during lactation. Increased post-implantation loss (2.1, 9.9, 15.5, and 10.9% in the control, LD, MD, and HD groups, respectively) and postnatal loss (3.8, 4.2, 9.2, and 25.2% in the control, LD, MD, and HD groups, respectively, in days 1-4) as well as signs of cannibalizations, were noted at all dose levels at the first litter check. According to the additional analysis conducted by the Sponsor, post implantation loss likely reflected undetected loss of pups during or after parturition i.e. before the first check could have been performed. Although the mean pup weights were initially similar for all groups, body weight gain was minimally to slightly decreased during lactation period in dosed animals compared to controls. There were no other signs which were indicative of embryo- and fetotoxicity.

In a modified prenatal and postnatal developmental study with asenapine in Sprague-Dawley rats at 1.5 mg/kg/day, cross-fostering was included to determine whether the neonatal mortality observed in the first prenatal and postnatal study was a result of the effects on the mother or a toxicological effect on offspring. The results indicate that peri- and postnatal pup loses after pre- and postnatal treatment of the dams with asenapine are due to effects on pups (pre-impairment of the pups) rather than to the changed nursing behavior of the dams. Neurobehavioral functional development of the offspring was not impaired in this study.

Administration of asenapine to juvenile Sprague-Dawley rats resulted in reduction of body weight and increased activity at all dose levels. The NOAEL for the juvenile toxicity was not determined in this study.

<u>Local tolerance</u>: Local tolerance of asenapine sublingual tablets up to 15 mg b.i.d. was tested in Beagle dogs. Administration for 7 days did not induce any histopathological changes at the site of treatment.

<u>Special toxicology</u>: The following special toxicology studies were conducted with asenapine: (1) antigenicity study in guinea pigs, (2) phototoxicity 3T3 neutral red uptake assay, and (3) prolactin release in male rats using risperidone as comparator. Asenapine

did not cause any sign of antigenicity or phototoxicity. The increases in prolactin release following s.c. administration to rats were similar to those after oral treatment with risperidone.

Qualification of impurities in drug substance:

b(4)

Impurity [redacted]. Drug substance impurity [redacted] has been present in the drug substance commercial size clinical/stability batches [redacted]. However, the Sponsor proposed to set a specification limit for this impurity in asenapine drug substance at [redacted], thus above the ICH Q3A(R) qualification limit [redacted]. The content of [redacted] in relevant asenapine batches used in the preclinical program was below the limit of detection. A non-GLP pilot segment II study in rabbits was performed with this impurity; however, this study is considered inadequate for several reasons, including the following: (1) only a single dose [redacted] was employed which did not result in any maternal toxicity; (2) the number of animals per group was less than the recommended 16 per group, with only 34 fetuses examined in the [redacted] group; (3) relatively high post-implantation loss was observed in the control group; (4) no information on drug analysis was provided; (5) no toxicokinetic data were obtained; (6) [redacted] was administered orally, although asenapine is being administered by the sublingual route; and (7) unclear terminology was used to describe fetal findings. Moreover, a 9-fold increase in the incidence of malformations and signs of embryotoxicity demonstrated as a 2-fold increase in post-implantation loss, were observed in fetuses of female rabbits dosed with [redacted] at 80 mg/kg/day during the period of organogenesis in this non-GLP pilot study. The NOAEL has not been identified for these effects. Therefore, the Sponsor should perform an embryofetal development study with [redacted] in the rabbit to qualify this impurity during phase IV or reduce the specification of [redacted] to the ICH Q3A(R) qualification limit of [redacted]

b(4)

Other studies conducted to qualify [redacted] included direct testing in genotoxicity studies (Ames tests, mutations at HGPRT locus in CHO cells, mouse lymphoma assay, and *in vivo* rat micronucleus assay) and 4-week oral toxicity studies in rats and dogs. Based on these studies [redacted] is considered non-genotoxic and qualified for general toxicity.

Impurity [redacted] Studies conducted to qualify the impurity [redacted] directly included genotoxicity studies (Ames tests and chromosomal aberrations study in cultured human lymphocytes) and an intravenous 2-week toxicity study with [redacted] in Wistar rats. Reproductive toxicology study (embryofetal toxicity study in rats) recommended by the Agency for qualification of impurities was not conducted with [redacted]. However, this impurity is considered to be qualified based on reproductive toxicology study conducted with asenapine.

b(4)

Qualification of degradants in drug product:

The following degradation products have been identified in asenapine tablets:

[redacted]

[redacted], the N-oxide of asenapine, is a metabolite found in abundance in animals but not in humans. According to the reviewing chemist Dr. Chhagan Tele, [redacted] is an aliphatic N-oxide (usually aromatic N-oxides are known for structural alerts for mutagenicity). [redacted] was present in mice (14% and 8% of total circulating radioactivity in males and females, respectively), rats (not detected and 15% of total circulating radioactivity in males and females, respectively), rabbits (not detected and 1% of total circulating radioactivity in males and females, respectively) and dogs (7% and 6% of total circulating radioactivity in males and females, respectively). The exposure to [redacted] achieved in rats and dogs following long term treatment (oral and intravenous administration) is similar to or exceeds that to the asenapine. In human plasma, C_{max} is ~0.2 ng/mL compared to 3.6 ng/mL for asenapine at 5 mg b.i.d. This degradation product is much less pharmacologically active compared to asenapine. As indicated by the reviewing chemist Dr. Tele, stability tests of 5 mg and 10 mg asenapine tablets indicate that the level of [redacted] will significantly increase with time up to [redacted] respectively. It is concluded that [redacted] is qualified with respect to its general toxicity, reproductive toxicity and genotoxicity based on its presence as the metabolite in laboratory animals. *b(4)* b(4)

[redacted]: As indicated by the reviewing chemist Dr. Tele, [redacted] is present at levels less than [redacted] in 5 mg and 10 mg asenapine tablets. Therefore, this degradation product does not exceed a toxicology-based specification limit (ICH Q3B (R) *b(4)*

[redacted]: According to the Sponsor, the chemical structures of both degradants do not carry structural alerts for genotoxicity. A battery of *in vitro* and *in vivo* genotoxicity studies have been conducted for each of those two structurally related degradants in drug product because early stability data indicated that these degradants could exceed the qualification limit [redacted]. However, according to the reviewing chemist Dr. Tele, stability data up to 12 month on registration batches now indicate that it is unlikely that these degradants would reach levels higher than [redacted] at the end of shelf life.

Both degradants were negative in bacterial reverse mutation test, *in vivo* rat bone marrow micronucleus test, and *in vivo* comet assay. However, both degradants induced structural chromosome aberrations predominantly chromatid type (deletions and some exchanges) in cultured human peripheral blood lymphocytes in both the absence and presence of metabolic activation. In addition, significant increase in the frequency of cells with numerical aberrations (polyploidy cells) was also observed in cultures treated with [redacted] in the absence of metabolic activation. Based on the weight of evidence approach, the Division concluded previously that the degradants have been qualified for genotoxicity b(4)

2.6.6.2 Single-dose toxicity

The following summary and evaluation of the single dose toxicity studies reviewed by Dr. Lois Freed is taken directly from her review of the IND 51,641 dated June 11, 1997 (please see Dr. Freed's full review for additional details):

"Acute: the acute toxicity of Org 5222 was tested in rats (Wistar, Sprague-Dawley) and Beagle dog.

In Wistar rats (6/sex/grp), Org 5222 was administered at doses of 0, 75, 115, 160, 200 (males only), 225, 315, 440, and 625 mg/kg p.o. In Sprague-Dawley rats (6/sex/grp), Org 5222 was administered orally at doses of 0, 50, 100, and 200 mg/kg, and by i.v. bolus at doses of 0, 9, 15, and 21 mg/kg. With p.o. dosing (both studies), unscheduled deaths occurred at doses ≥75 mg/kg in females and ≥160 mg/kg in males; all animals (with one exception) died at doses ≥200 mg/kg p.o. [The highest doses not associated with death were 50 and 100 mg/kg in males and females, respectively.] The LD_{50} was determined to be ~176 and 139 mg/kg in male and female Wistar rats, respectively, and 150 and 110 mg/kg in male and female Sprague-Dawley rats, respectively. Drug-related clinical signs were evident at all doses, and consisted of labored respiration, decreased reaction to stimuli, ptosis, sedation, and clonic convulsions. Body tremors were reported at all doses in Wistar rats. Convulsions were observed in all animals that died prematurely. In Sprague-Dawley rats, microscopic findings in animals dying prematurely consisted of superficial necrosis of the glandular epithelium of the stomach, lymphocytolysis in spleen, thymus, and lymph nodes, and granulocytic cell aggregation in the uterus.

Acute i.v. toxicity was tested in Sprague-Dawley rats (6/sex/grp) at doses of 0, 9, 15, and 21 mg/kg. There were no unscheduled deaths during this study. Drug-related clinical signs, evident at all doses, included sedation and ataxia. Convulsions were noted at 15 and 21 mg/kg. The Sponsor indicated that there were no drug-related microscopic findings; however, microscopic examination was limited to those tissues containing gross abnormalities (i.e., uterus and lung from 1 MDF).

In Beagle dog (n = 2 M/grp), Org 5222 was administered at doses of 50, 100, and 200 mg/kg p.o. There were no unscheduled deaths during the study. Drug-related clinical signs, consisting of sedation and miosis, were evident at all doses. At 100 and 200 mg/kg, ataxia, trembling, and compulsive behavior were also observed. At 200 mg/kg, compulsive behavior resulted in facial abrasions and bleeding. A number of findings were noted on hematology, clinical chemistry, and urinalysis parameters; however, the small number of animals precludes a definitive assessment of drug-related effects".

2.6.6.3 Repeat-dose toxicity

The following summary and evaluation of the repeat dose toxicity studies reviewed by Dr. Lois Freed is taken directly from her review of the IND 51,641 dated June 11, 1997 (please see Dr. Freed's full review for additional details):

"Subchronic: subchronic toxicity was tested in NMRI mice (2-wk, 4-wk pilot studies), Wistar rats (2-wk, 4-wk, 13-wk p.o., 2-wk i.v. bolus), and Beagle dogs (4-wk, 13-wk p.o., 2-wk, 13-wk i.v. bolus). [Both subchronic studies in mice were preliminary and will not be discussed further.]

Since the intended clinical route is sublingual, the preclinical i.v. toxicity studies may be more relevant than the oral, depending on how much of the drug is absorbed through the oral cavity and how much is swallowed. The only comparable studies are the 2-wk i.v. bolus and p.o. studies.

Rat

Comparison of 2-wk p.o. and i.v. studies: Org 5222 was administered orally at doses of 0, 18.75, 37.5, 75, and 150 mg/kg and by i.v. bolus at doses of 0, 0.1, 0.5, and 3 mg/kg. There were 6/sex/grp in the p.o. study and 8/sex/grp in the i.v. study. Unfortunately, toxicokinetic data were not collected in either study; therefore, plasma exposure between the two routes cannot be compared. In the p.o, study, HD survivors were sacrificed at the end of Wk 1 of dosing.

In the oral study, there were deaths at 150 mg/kg in 2/6 HDM and 6/6 HDF. These deaths were attributed to drug-induced respiratory failure by the Sponsor. No premature deaths occurred in the i.v. study. Drug-related clinical signs were markedly more severe in the oral study. In that study, reduced activity, hunched posture, ventral recumbency, reduced reactivity to stimuli, decreased respiratory frequency, miosis, and aggressiveness (in females were noted at all doses. In addition, hemorrhagic drainage around the mouth and nostrils were observed at doses ≥37.5 mg/kg, body tremors were observed at doses of 75 and 150 mg/kg, and hypothermia and weakness were noted at 150 mg/kg. Similar findings were observed in animals that died. In contrast, only miosis was noted at all doses in the i.v. bolus study. Additional signs consisted of reduced activity and lacrimation (at 0.5 and 3 mg/kg), and vocalization at handling (3 mg/kg). Body weight was reduced (compared to C animals) at all doses in a dose-related manner (6-14%) in the oral study. In the i.v. study, body weight was reduced in males (3-11%, compared to CM), but not necessarily in a dose-related manner; body weight was not affected in females. Changes in food consumption were fairly consistent with those noted in body weight. There were no hematology findings related to drug in the oral study. In the i.v. study, slight, but dose-related, increases were noted in rbc parameters in males and females (all doses affected); in males, dose-related decreases in PDW, MPV, and PLC, and a slight decrease in thrombocytes at the HD were also noted. On clinical chemistry parameters, the primary findings in the oral study were (1) increases in SGOT in males and females at all doses (HD could not be evaluated because of deaths) and (2) decreases in glucose at 37.5 and 75 mg/kg. In the i.v. study, only slight effects on glucose (increase, not dose-related, in females) and serum lipids (decrease at the HD), and a dose-related increase in plasma hgb in females were noted. On urinalysis parameters, reducing substances were detected at 75 mg/kg p.o.; no findings were noted in the i.v. study. There were no clear drug-related gross pathology findings in either the p.o. or i.v. study. On organ/tissue wt, the following findings were noted in the p.o. study (in survivors): (1) a decrease in thymus wt at doses ≥37.5 mg/kg in males and females, (2) an increase in adrenal wt at all doses examined in males and females, (3) decrease in prostate wt at 75 mg/kg, (4) increases in liver and kidney wt at 75 mg/kg, and (5) decreases in ovary and uterus wt at all doses. In HD animals sacrificed at Wk 1, there was a decrease in thymus wt, and increases in liver, kidney, thyroid, adrenal, and pituitary wts. In the i.v. study, an increase in adrenal wt (3 mg/kg), and decreases in thymus (0.5 and 3 mg/kg) and lung (all doses) wt were noted in males, and increases in heart (0.5 and 3 mg/kg) and adrenal (1 F at 3 mg/kg) wt, and a decrease in ovary wt (all doses) were noted in females. Summary histopathology tables were not provided for the oral study. Summary tables were provided for the i.v. study, however, it should be confirmed that these tables contained all histopathology findings and not only findings considered drug-related by the Sponsor (or pathologist). Microscopic findings reported in the i.v. study consisted of the following: (1) eosinophilic infiltration of lung at 3 mg/kg in females, (2) an increase in mild hemosiderin deposition in spleen in females at 3 mg/kg, (3) an increase in adipose tissue and mast cells in bone marrow in males and females at 3 mg/kg, and (4) an increase in mast cells in lymph nodes in females at 3 mg/kg.

Clearly, the oral route was associated with notably more severe signs of toxicity than the i.v. route. [No dose-limiting toxicity was observed in the i.v. study.] Also, there were different toxicities depending upon the route. Hematology findings (increased rbc parameters) were noted only with i.v. dosing, as were increases in lung (male) and heart (female) wt. In additional, microscopic findings differed between the two routes. Oral administration was associated primarily with liver changes, whereas i.v. dosing was associated with lung, spleen, bone marrow, and lymph node findings. It is unclear how much differences in plasma exposure (for parent compound and metabolites) between the two routes account for the differences noted. PK (AUC) data provided for single i.v. and p.o. doses of 0.3 mg/kg indicated that, in rats, the parent compound was 56-140% (M-F) higher following i.v. than p.o. dosing; levels of the N(2)-demethyl metabolite was ~50% lower following i.v. dosing in males, but similar to that following p.o. dosing in females. Levels of the N(2)-oxides were similar following i.v. and p.o. dosing in males, but 50% higher after i.v. dosing in females. If one assumes linearity of AUC, plasma exposure to parent compound and metabolites would probably have been markedly higher in the p.o. than in the i.v. study.

Oral toxicity of Org 5222: the oral toxicity of Org 5222 was tested in 2-wk, 4-wk, and 13-wk studies in Wistar rats. Org 5222 was administered by gavage at doses of 0, 18.75, 37.5, 75, and 150 mg/kg in the 2-wk study, at doses of 0, 25, 50, and 75 mg/kg in the 4-wk study, and at doses of 0, 6.5, 22.5, and 75 mg/kg in the 13-wk study.

Drug-related deaths occurred primarily in the 2-wk and the 13-wk studies. In the 2-wk study, spontaneous deaths occurred at 150 mg/kg in males (2/6) and females (all); all HD survivors were sacrificed at the end of Wk 1 of dosing. In the 4-wk study, the only potentially drug-related death was observed in 1 HDM (75 mg/kg; Day 15). In the 13-wk study, there were a number of drug-related deaths at 22.5 and 75 mg/kg (the MD and HD). At the MD, 4/6 males and 4/6 females died (1 MDM was sacrificed moribund on Day 77); spontaneous deaths occurred on Days 5-88 of dosing). At the HD, 7/16 males and 4/16 females died (1 HDM was sacrificed moribund on Day 79); spontaneous deaths occurred on Days 1-91. Of the spontaneous deaths in the 13-wk study, only 4 occurred within 5 days of the initial dose, i.e., the majority occurred after 21 wk of dosing. Deaths in the 2-wk study were attributed to respiratory failure. Deaths in the 13-wk study were considered either to be due to a primary respiratory failure or secondary to convulsive episodes, however, cause of death was not established.

Drug-related clinical signs were evident at all doses in all three studies. In the 2-wk study, these consisted of reduced activity, hunched posture, ventral recumbency, reduced reactivity to stimuli, decreased respiratory frequency, miosis and aggressiveness at all doses. At the higher dose, body tremors and hemorrhagic drainage from month and nose were noted, and at the highest dose, hypothermia and weakness were evident. In the 4- and 13-wk studies, similar signs were noted, except that in the 13-wk study, convulsions were observed in 2 HDM and 1 HDF (on Days 10-79, 0.5-4 hr postdosing).

Body weight, hematology, clinical chemistry, organ wt, and histopathology data are summarized in the attached summary table. It should be noted that in the original IND submission, the Sponsor did not provide summary histopathology tables for most of the toxicity studies. At the request of the Division, the Sponsor provided histopathology summary tables for a number of the toxicity studies; however, none was provided for the 2-wk oral study in rats. In addition, of the ones provided, none clearly included all microscopic findings; (at least some of) the summary tables were, instead, based on summary reports prepared by the pathologist. Therefore, the Sponsor should, once again, be asked to provide summary tables for all definitive toxicity studies which are to include all observations, whether they are considered to be drug-related or not. Until these tables are provided, the review of these studies cannot be completed.

Clearly, sufficiently high doses were used in all three oral toxicity studies, based on death at the HD in all three studies, >10% decreases in body weight (as compared to C) in both the 4- and 13-wk studies, and convulsions at the HD in the 13-wk study. No drug-related hematology findings were noted in the 2- and 4-wk studies. In the 13-wk study, a decrease was noted in wbc at all doses (fairly dose-related), with an increase in the % segmented neutrophils and a decrease in the % lymphocytes in MDM and HDM. There was also an increase in the ESR in HDM and HDF. Clinical chemistry findings consisted of the following: (1)increases in AST in all studies at all doses in both males and females; however, all increases in means were <100% of C means, (2) decreases in glucose in all studies, all doses in males and females; all decreases (based on means) were <35% of C means, (3) increases in urea nitrogen was noted in the 4- and 13-wk studies, at all doses in both males and females; all increases were <50% of C means, (4) decreases in TG were noted in the 4- and 13-wk studies at all doses, but only in males, and (5) additional findings noted only in the 13-wk study were increases in creatinine in males (at all doses) and decreases in cholesterol in females (all doses). Urinalysis findings consisted primarily of increases in urinary volume at 50 and 75 mg/kg in the wk study, and at 22.5 and 75 mg/kg in females only in the 13-wk study. At doses of 75 and 150 mg/kg (4-wk, 13-wk), there were detectable reducing substances and organisms, and increases in total protein in urine. The only consistent findings on organ wts were decreases in thymic wt (all studies, all doses, male and female, except for the LD in the 2-wk study) and increases in adrenal wt (all studies, all doses, male and female). An increase in liver wt was noted at doses ≥22.5 mg/kg. As noted above, the sponsor's summary histopathology tables were incomplete. From the summary data provided, the main microscopic findings involved liver and thymus. Thymus findings consisted of sclerotic atrophy, and were noted only in the 13-wk study (MDM, HDM, HDF), although reduced thymus wt was noted almost all doses examined. Liver findings were inconsistent among studies. In the 2-wk study, swollen and vacuolated hepatocytes were detected at doses of 75 and 150 mg/kg. In the 4-wk study, liver changes were noted only in 2 F at 75 mg/kg (necrosis and/or proliferated bile ducts); these were not associated with marked changes in AST, ALT, or alkaline phosphatase. In the 13-wk study, hypertrophy, reduced basophilia and "eccentrically situated nuclei" were noted in males and females at 75 mg/kg. Adrenal hypertrophy and lipid depletion in the zona fasciculata were noted in males and females at 75 mg/kg only in the 13-wk study.

In none of the oral toxicity studies was a true NOEL established based on a number of findings at the LD in each study. The highest non-lethal doses were 75, 50, and 6.5 mg/kg in the 2-, 4-, and 13-wk studies, respectively. Unfortunately, TK data were not available for these studies.

Dog

The subchronic toxicity of Org 5222 was studied in 4- and 13-wk oral studies and in 2- and 13-wk i.v. studies in Beagle dog. For the proposed clinical indication, the i.v. dose is probably the most relevant.

Given as an i.v. bolus, Org 5222 was administered to 3/sex/grp at doses of 0, 0.1, 0.5, and 3.0 mg/kg in the 2-wk study and at doses of 0, 0.1, 0.5, and 2.5 mg/kg in the 13-wk study.

There were no unscheduled deaths in either study. Drug-related clinical signs were evident at all doses and were primarily related to the CNS, e.g., reduced motor activity, behavioral changes ("pushing syndrome", yelping, repetitive head movements). At doses >0.01 mg/kg, motor incoordination, vomiting, trembling, and miosis were also noted. At 2.5-3.0 mg/kg, there was some evidence of "preconvulsive" behavior in 1 animal in each study. These observations would indicate that sufficiently high doses were used in these studies. In the 2-wk study, an increase in heart rate was reported at all doses. Body weight was reduced (compared to C) at doses ≥0.5 mg/kg in males and at 2.5 mg/kg in females. There was no clear drug-related effect on food consumption, hematology, clinical chemistry, urinalysis, or gross pathology. However, the sponsor considered the decrease in A/G ratio in one male at 2.5 mg/kg to be potentially drug-related, as well as detection of blood in urine of 2 MDM and 1 HDM in the 13-wk study. [It should be noted that the small "n" used made evaluation of many parameters difficult.] ECG was performed in the 13-wk study. The primary findings were increased heart rate (all doses) and inverted T-wave polarity (suggestive of ischemia) at all doses. There were no consistent organ wt findings between studies. An increase in spleen wt MDM and HD animals in the 13-wk study was attributed an Org 5222-induced inhibition of an agonal reflex (spleen contracture). Why this was noted in the 13-wk study, but not the 2-wk study is unclear, unless different methods of euthanasia were used. The completeness of the histopathology data are in question. As noted previously, the sponsor has indicated that not all microscopic findings were included in the summary tables. Based on the summaries provided by the sponsor, the only microscopic finding noted in both studies was thymus atrophy (all doses). In the 2-wk study, vacuolated hepatocytes were detected in 1/3 HDF and reduced sperm count was noted in 1 MDM and 1 HDM. In the 13-wk study, additional findings consisted of (1) spleen congestion (≥0.5 mg/kg), (2) mononuclear cell aggregation (all doses) and granulomatous lesion (1 HDM) in liver, (3) renal tubular cell pigmentation (2.5 mg/kg), (4) prostate acinar cell atrophy (≥0.5 mg/mg), and (5) mammary gland secretion in 1 F at 0.5 and 2.5 mg/kg.

TK data were collected and PK parameters were calculated in the 13-wk study. The $t_{1/2}$ was 2-3 hrs, the Cl and V_d were 5-6 L/hr/kg and 5-7 L/kg, respectively, following multiple dosing. Plasma exposure (AUC) was fairly proportionate with dose, and did not accumulate notably with multiple dosing. Plasma exposure (AUC) at the HD (2.5 mg/kg) was 536.75 ± 130.39 and 574.60 ± 32.29 ng*hr/mL in females and males, respectively. C_{max} was not estimated.

No NOEL was established in either study, based on a number of findings (e.g., CNS) at the LD.

The oral toxicity of Org 5222 in Beagle dogs was tested in 4- and 13-wk studies. Org 5222 was administered at doses of 0, 20, 40, and 80 mg/ kg in the 4-wk study (n = 3/ sex/grp) and at doses of 0, 1.25, 7.5, and 20 mg/kg in the 13-wk study (n = 3-4/sex/grp for main study, 1/sex for C, MD, and HD for recovery period). [It should be noted that, although the 13-wk study incorporated a 5-wk recovery period, the no. of animals examined during this period were to small to make definitive conclusions about reversibility of any findings.]

There were no unscheduled deaths in either study. Drug-related clinical signs were noted primarily at doses ≥7.5 mg/kg (miosis was observed in 1 F at 1.25 mg/kg), including miosis, reduced motor activity, pushing behavior, vocalization, tremors, stereotypies, and restlessness. Blepharospasms, motor incoordination, and vomiting were noted at doses ≥20 mg/kg. Salivation at 80 mg/kg was attributed to the "nasty taste" of the drug by the sponsor. Upon veterinary examination, the following findings were noted: (1) lactation at 80 mg/kg in the 4-wk study, and at 20 mg/kg in the 13-wk study, (2) enlarged liver at ≥ 7.5 mg/kg in the

13-wk study, (3) small/flaccid prostate ≥1.25 mg/ kg in the 13-wk study, (4) generalized body tremors (≥40 mg/kg), and (5) increased heart rate at all doses (i.e., ≥1.25 mg/kg); the sponsor considered this finding to be secondary to the hypotensive effects of Org 5222. The sponsor also noted that "yellowish mucous membranes and sclera" were evident in 1/sex at 20 mg/kg in the 13-wk study. Body weight was reduced in males and females (compared to C) at doses ≥40 mg/kg (maximum: 7-10%). The only notable effect on food consumption was a decrease in females at 80 mg/kg (4-wk) and at 20 (13-wk) mg/kg during the last 2-3 wks of dosing. There were no clear, dose-related changes on hematology parameters in either study. On clinical chemistry parameters, the major findings were as follows: (1) increases in AST and ALT at ≥7.5 mg/kg in males and females, (2) increases in bilirubin at ≥20 mg/kg in males and females, (3) increases in cholesterol at 40 and 80 mg/kg in females, and (4) increases in alkaline phosphatase in the 13-wk study at 7.5 and 20 mg/kg. In the 2-wk study, glucose was reduced at all doses in females, and following a glucose load, was increased in females at 80 mg/kg at 30 min post-load; no changes were noted in serum glucose in the 13-wk study. There were no drug-related changes in urinalysis parameters in the 13-wk study. In the 2-wk study, a number of findings were noted, particularly at 40 and 80 mg/kg: (1) detection of protein, reducing substances, glucose, epithelial cells, and organisms in urine, primarily in females and (2) an increase in urinary volume in males. The primarily gross pathology findings were detected in liver (enlarged, ≥7.5 mg/kg; whitish/depressed foci, ≥20 mg/kg) and mammary gland (secretion, 80 mg/kg in 4-wk, 20 mg/kg in 13-wk). On organ wt, reductions were noted in thymus wt (40, 80 mg/kg) and increases in liver wt were noted at 20-80 mg/kg in the 4-wk study, but not at 20 mg/kg in the 13-wk study. The summary histopathology tables (submitted upon request) did not include all findings, but were based on summary reports by the pathologies. According to the summary tables provided, the following are the major microscopic findings: (1) liver changes characterized by foci of hepatic cell necrosis with hemorrhage and/or siderocytic aggregation in 1 M/grp at 20, 40, and 80 mg/kg and in 2 F at 80 mg/kg (4-wk study), and as hypertrophy with vacuolation and varying degrees of bile duct involvement (e.g., bile thrombi, epithelial cell proliferation) and perivasculitis at 7.5 and 20 mg/kg in males and females, and as sinusoidal ectasia and hepatic cell atrophy at 20 mg/kg (13-wk study). (2) increased secretory activity of PRL and/or ACTH cells in the pituitary at 20-80 mg/kg, (3) increased secretory activity in mammary gland at the HD in each study, (4) kidney changes (characterized as basophilic and dilated cortical tubules with/without acidophilic casts at 40 and 80 mg/kg, and (5) inhibition of spermatogenesis with formation of spermatid giant cells at 40 and 80 mg/kg. The sponsor considered the primary target organ for toxicity to be the liver.

Comparison of i.v. and p.o. toxicity in 13-wk studies: the doses used in these studies were 0, 0.1, 0.5, and 2.5 mg/kg i.v. and 0, 1.25, 7.5, and 20 mg/kg p.o.

In neither study were there any drug-related deaths. Drug-related clinical signs were observed at all i.v. doses and at 7.5 and 20 mg/ kg p.o. Ataxia and vomiting were observed at ≥0.5 mg/kg i.v. and ≥20 mg/kg p.o. Body weight was reduced (compared to C grps) at i.v. doses ≥0.5 mg/ kg in males and 2.5 mg/kg in females, but was not affected with p.o. dosing. Food consumption was affected (decreased) only in females at 20 mg/kg p.o. With neither route were there drug-related effects on hematology or urinalysis parameters. With p.o. dosing, increases were noted in several clinical chemistry parameters: AST, ALT, bilirubin, and/or alkaline phosphatase (≥7.5 mg/kg); no changes were noted in the i.v. study. An increase in heart rate was observed at all i.v. and p.o. doses. Inverted T-wave polarity was detected at all i.v. dosing, but not with p.o. dosing. No gross pathology findings were detected in the i.v. study. In the oral study, liver changes (i.e., enlarged and/or whitish, depressed foci) and mammary gland secretion were noted at 7.5 and/or 20 mg/kg. An increase in spleen wt due, according to the sponsor, to Org 5222-induced inhibition of contracture was noted at doses ≥7.5 mg/kg i.v.; no organ wt changes were observed in the p.o. study. A comparison of microscopic findings indicated the following: (1) thymic atrophy (all doses), spleen congestion (≥0.5 mg/kg), and mammary gland secretion (≥0.5 mg/kg) were only associated with i.v. dosing, [mammary gland secretion was noted in shorter-term p.o. studies at higher doses], (2) increased secretory activity of PRL and/or ACTH in pituitary at 20 mg/kg p.o., (3) liver changes were noted in both i.v. and p.o. studies; however, the type of effects differed between the two routes. With i.v. dosing, mononuclear cell aggregation (all doses) and granulomatous lesions (2.5 mg/kg) were noted, whereas with p.o. dosing, changes consisted of hypertrophy/vacuolation with bile duct involvement (thrombi, epithelial cell proliferation) and perivasculitis at 7.5 and 20 mg/kg and sinusoidal ectasia and hepatic cell atrophy at 20 mg/kg. (4) prostate acinar cell atrophy was noted only in the i.v. study (≥0.5 mg/kg).

The most notable differences between the i.v. and p.o. 13-wk study were (1) the severity of CNS signs at lower doses with the i.v. route, (2) the T-wave inversion noted with i.v. dosing, and (3) the drug-related effects on liver with p.o. dosing, and on thymus and prostate with i.v. dosing. It is unfortunate that TK were not collected in the p.o study. A comparison of plasma exposures achieved in the two studies would have been helpful in interpreting the toxicities noted in these studies.

Chronic: chronic oral toxicity (1-yr) studies were conducted in Wistar rat and Beagle dog.

Rat: to assess chronic toxicity, Org 5222 was administered to 30/sex/grp (20/sex for C, LD, and MD grps and 12-13/sex for HD for main study; 7-10/sex for C and HD for 9-wk recovery phase) at doses of 0, 0.3, 1.8, and 10.8 mg/ kg b.i.d. (total of 0, 0.6, 3.6, and 2 1.6 mg/ kg/day). Doses were given by gavage 5-7 hr apart.

There were 13 unscheduled deaths during the study that were considered drug-related. All occurred at the HD. Of these, 11 were spontaneous (on Days 35-362 of dosing) and 2 were moribund sacrifices due to continuous seizure activity (Days 241-279 of dosing). In the 13-wk subchronic study, drug-related mortality was observed at 22.5 and 75 mg/kg (given as one daily dose); these deaths occurred on Days 1-91, but only 4 of 11 spontaneous deaths occurred within 5 days of the initial dose. The fact that b.i.d. did not reduce the lethal dose and that most deaths occurred after prolonged exposure would suggest that mortality is linked more to AUC than C_{max}. Drug-related clinical signs were similar to those noted in subchronic studies, although stereotypies were not reported in the 1-yr study. Miosis and reduced activity were observed at all doses. Micturition, lacrimation, vocalization upon handling were noted at the MD and HD, and tremors and/or convulsive seizures were observed at the HD. Convulsive episodes were noted as lasting for several minutes. There were no drug-related changes upon ophthalmology examination. Body weight was reduced (compared to C) at all doses in males (5-28%) and at the HD in females (16%). In recovery animals, body weight in HD animals remained lower than in C-recovery grps. Consistent changes in food consumption were not observed. On hematology parameters, decreases were observed in wbc and lymphocytes at all doses in males and females. Similar findings were noted in the 13-wk toxicity study (doses 6.5-75 mg/kg). In males, hgb and pcv were slightly elevated at the MD and HD. In females, there was a decrease in reticulocytes at the HD. Neither of these latter findings were noted in the subchronic studies. In recovery animals, decreases in reticulocytes were noted in both HDM and HDF. There were a number of findings on clinical chemistry parameters. Those findings observed in previous toxicity studies (particularly the 13-wk study) consisted of the following: (1) decreases in glucose (all doses), (2) increased urea nitrogen (MD, HD), (3) reduced cholesterol (all doses in females; HDM), (4) increased creatinine (MD, HD), and (4) decreased TG (MD, HD). In the 13-wk study, increases (<2-fold) were noted in AST at all doses; in the 1-yr study, only small increases in AST and ALT were observed and only in males. In addition to TG, other serum lipids (PL, cholesterol) were reduced at the MD and HD. In recovery animals, changes were still noted in glucose, urea nitrogen, cholesterol, TG and PL. On urinalysis parameters, an increase in urinary volume(3.6 and 21.6 mg/kg) was the primary finding, as noted in the 4- and 13-wk studies. In addition, there was a small, but consistent increase in urinary pH at 3.6 and 21.6 mg/kg, and an increase in epithelial cells at 21.6 mg/kg. In recovery animals, an increase in urinary volume was still evident in HDF. Upon macroscopic analysis, changes were noted in thymus (small, all doses), liver (yellow discoloration, MD, HD), testis (flabby, HD), epididymides(yellow nodule in head, 1 MD, 1 HD), and adrenal gland (enlarged and/or dark, HD). Regarding organ wt. data, the primary findings were an increase in adrenal wt in males and a decrease in uterus wt in females. As noted for previous studies, the summary histopathology tables provided by the Sponsor did not include all findings. [This summary is based on the summary tables provided by the sponsor. Only C and HD animals were examined.] In the subchronic studies, microscopic changes were noted primarily in thymus and liver. In the 1-yr study, these organs were not particularly affected. Only fat deposition (minimal) in liver was noted in the 1-yr study, and no changes were detected in thymus in main study animals. Findings noted in the 1-yr study consisted of the following at the HD (the only drug-treated grp examined): (1) aggregation of foamy alveolar macrophages and/or granulomas in lung, (2) splenic contraction and/or hemosiderin accumulation in spleen, (3) ceroid laden macrophages in lymph node, (4) eosinophilic hyperplasia in bone marrow in males, (5) fat deposition in tubular epithelium of kidney, (6) diestrus phase of vagina, (7) acinar development of mammary gland in females, (8) and cystic degeneration of the adrenal gland in males. In recovery animals, findings were still evident in adrenal gland, spleen, kidney, and mammary gland.

TK data were collected during the 1-yr study and have been summarized in this review (after pg ####). The maximum achieved plasma levels were 110 ± 119 ng/ mL (C_{max}) and 21.3 ± 11.5 pg•min/mL (AUC) for parent compound, 290 ± 72 ng/mL (C_{max}) and 84.4 ± 42.2 pg•min/mL (AUC) for the N(2)-demethyl metabolite, and 146 ± 67 ng/mL (C_{max}) and 28.9 ± 11.8 pg•min/mL (AUC) for the N(2)-oxide metabolites. These plasma levels represent plasma exposure following 1 of 2 daily doses (i.e., ≈50% of total daily exposure for AUC), and were associated with mortality in 13 HD animals.

[Note: In a separate review of the IND 51,641 of January 9, 1998 Dr. Freed indicated that "in response to the Division's request, the Sponsor performed microscopic analysis of tissues for the LD and MD grps for the 52-wk oral toxicity study in rats. In the original report (submitted in the original IND application), histopathology data were provided only for C and HD grps". Dr. Freed concluded that "there were some discrepancies between the original data and the data as summarized in the current submission. These discrepancies were due, in part, to the fact that some tissues listed as examined and/or findings noted in the original tables were not listed in the "updated" tables [e.g., bone marrow, liver (minimal fat deposition), lung (granuloma), kidney (fat deposition in tubular epithelium)]. There were also unexplained discrepancies. For example, in the original review, it was noted that "many aggregates of ceroid laden macrophages" in lymph node occurred in 0/20 CM, 2/13 HDM, 0/18 CF, and 2/10 HDF. In the new data table, the incidences of "pigment laden macrophages" in these grps were 1/20 CM, 0/ 18 HDM, 0/20 CF, and 0/20 HDF. A similar discrepancy between data sets was noted for incidence of alveolar macrophages in lung in CM. Therefore, both sets of summary tables need to be considered.

The new data indicate that drug-related microscopic changes were noted in HDM and at all doses in females".]

Dog: to assess chronic toxicity in Beagle dogs, Org 5222 was administered in tablet form to 4/sex/grp for main study and l/sex for C and 2/sex for HD recovery (6-wk) animals at doses of 0, 0.1, 0.6, and 3.6 mg/kg b.i.d. (total dose: 0, 0.2, 1.2, and 7.2 mg/kg/day).

There were no unscheduled deaths during the study, and no drug-related clinical signs, except in animals accidently dosed with a different drug (Org 9160). There were also no drug related effects on veterinary examination, ophthalmology, body weight (although not statistically significant, body weight was higher in females at 7.2 mg/kg than in CF), or food consumption, on hematology, clinical chemisty, or urinalysis parameters. The only drug related findings upon macroscopic examination were an increase in small thymus and obesity in 2 females at 7.2 mg/kg. Microscopic findings consisted of the following: (1) mild hemosiderin accumulation in spleen of, primarily, HDM, (2) eosinophilic cell infiltration in liver in HDM, (3) gastritis in HDF, with evidence of muscle fiber degeneration (of stomach) in 1 HDM, and (4) mammary gland changes in HDF (intraductular acinar aggregation of macrophages and/or mononuclear cell aggregation). This study was notable in the lack of signs of toxicity as compared with the subchronic oral and i.v. studies.

Toxicokinetic parameters for asenapine and metabolites in rats and dogs are shown below:

Toxicokinetic data (Wistar rat), Org 5222

STUDY	DOSE (mg/kg)	DURATION	ROUTE	SEX	N	T_{max} (min)	C_{max} (ng/mL)	AUC_{0-inf} (ng·h/mL)	$t_{1/2}$ (min)	F (%)
2979	0.3	single	p.o.	M	4	26 ± 5	16 ± 11	2.7 ± 1.7		64
				F		62 ± 61	14 ± 3	2.8 ± 1.4		40
			i.v.	M	3	9 ± 6	47 ± 10	4.2 ± 0.8	212 ± 15	
				F		6 ± 3	73 ± 23	6.7 ± 1.9	370 ± 190	
	1.8		p.o.	M	3	46 ± 43	35 ± 15	5.3 ± 0.8		26
				F		23 ± 6	38 ± 24	6.2 ± 2.8		21
	10.8			M	3	137 ± 108	226 ± 46	70.6 ± 5.7		68
				F		245 ± 162	367 ± 223	96.8 ± 67.9		40
2810/2979	0.3[a]	52-wk	p.o.	M	3	30 ± 13	15 ± 15	2.3 ± 1.6		65
	1.8				4	34 ± 36	26 ± 17	5.6 ± 2.6		26
	10.8	b.i.d.			4	46 ± 37	110 ± 119	21.3 ± 11.8		21

[a] dosing was b.i.d., so total doses were 0.6, 3.6, and 21.6 mg/kg/day. The AUCs for this study were based on samples collected after one of the two daily doses.

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Toxicokinetic data (Wistar rat), N(2)-desmethyl

STUDY	DOSE (mg/kg)	DURATION	ROUTE	SEX	N	T_{max} (min)	C_{max} (ng/mL)	AUC (ng·min/mL)
2979	0.3	single	p.o.	M	4	329 ± 180	18 ± 7	5.9 ± 2.2
				F		237 ± 221	12 ± 5	5.5 ± 1.2
			i.v.	M	3	319 ± 173	8 ± 2	2.8 ± 0.4
				F		310 ± 148	18 ± 8	5.9 ± 2.8
	1.8		p.o.	M	3	86 ± 30	66 ± 13	28.7 ± 3.2
				F		300 ± 106	88 ± 47	19.3 ± 14.7
	10.8		p.o.	M	3	462 ± 35	684 ± 56	297.2 ± 24.2
				F		294 ± 174	561 ± 214	158.4 ± 27.4
3210/2979	0.3	52-wk	p.o.	M	3	291 ± 296	12 ± 3	3.6 ± 0.8
	1.8				4	150 ± 112	101 ± 51	38.7 ± 12.4
	10.8				4	307 ± 65	298 ± 72	84.4 ± 42.2

Toxicokinetic data (Wistar rat), N(2)-oxides

STUDY	DOSE (mg/kg)	DURATION	ROUTE	SEX	N	T_{max} (min)	C_{max} (ng/mL)	AUC (ng·min/mL)
2979	0.3	single	p.o.	M	4	48 ± 8	13 ± 3	3.5 ± 0.7
				F		94 ± 74	11 ± 7	2.4 ± 0.4
			i.v.	M	3	16 ± 12	12 ± 2	2.2 ± 0.6
				F		13 ± 14	15 ± 3	3.6 ± 0.6
	1.8		p.o.	M	3	35 ± 38	88 ± 37	14.4 ± 3.5
				F		150 ± 212	60 ± 35	11.0 ± 4.7
	10.8			M	3	41 ± 9	327 ± 94	76.3 ± 16.5
				F		246 ± 165	305 ± 165	[illegible]
3210/2979	0.3	52-wk	p.o.	M	3	241 ± 140	12 ± 6	2.1 ± 0.1
	1.8				4	28 ± 8	66 ± 30	11.5 ± 3.9
	10.8				4	97 ± 107	148 ± 47	[illegible]

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[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3034	0.5	single	i.v.	M	3			[illegible]	[illegible]	[illegible]	[illegible]	
				F				7.9 ± 2.5	100 ± 46	[illegible]	[illegible]	
3034	0.1	single	p.o.	M	3	17 ± 9	[illegible]	[illegible]				[illegible]
				F		[illegible]	[illegible]	[illegible]				[illegible]
	0.6			M		[illegible]	[illegible]	[illegible]				[illegible]
				F		[illegible]	[illegible]	[illegible]				[illegible]
	3.6			M		[illegible]	[illegible]	[illegible]				[illegible]
				F		[illegible]	[illegible]	[illegible]				[illegible]
4417	0.1	[illegible]	i.v.	M	3			[illegible]		[illegible]	[illegible]	
				F				[illegible]		[illegible]	[illegible]	
	0.5			M				[illegible]		[illegible]	[illegible]	
				F				[illegible]		[illegible]	[illegible]	
	2.5			M				[illegible]		[illegible]	[illegible]	
				F				[illegible]		[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	p.o.	M	3	[illegible]	[illegible]	[illegible]				
				F		[illegible]	[illegible]	[illegible]				
	0.6			M	[illegible]	[illegible]	[illegible]	[illegible]				
				F		[illegible]	[illegible]	[illegible]				
	3.6			M	3	[illegible]	[illegible]	[illegible]				
				F		[illegible]	[illegible]	[illegible]				

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	0.1	single	p.o.	M	3	[illegible]	5 ± 2	1.0 ± 0.5	[illegible]
				F		[illegible]	4 ± 1	[illegible]	241 ± 67
	0.6			M		[illegible]	31 ± 13	[illegible]	[illegible]
				F		[illegible]	[illegible]	[illegible]	191 ± 37
	3.6			M		[illegible]	[illegible]	[illegible]	[illegible]
				F		[illegible]	704 ± 181	[illegible]	[illegible]

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	single	p.o.	M	3	[illegible]	3 ± 1	[illegible]
				F		[illegible]	[illegible]	[illegible]
	0.6			M		[illegible]	11 ± 7	[illegible]
				F		[illegible]	[illegible]	[illegible]
	3.6			M		[illegible]	[illegible]	[illegible]
				F		[illegible]	[illegible]	[illegible]

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The 39-week intravenous toxicity study in dogs was a pivotal chronic toxicity study in non rodents because the intravenous route was more adequate to the intended human sublingual route of administration. In addition, the oral chronic toxicity (52-week) study in dogs failed to reach an MTD. This study was reviewed under the IND 70,329 by Dr. Sonia Tabacova. Please see the review of July 20, 2005 for further details. The following is the summary taken directly from her review:

Study title: A 39-week intravenous toxicity and toxicokinetic study with Org 5222 in beagle dogs. (Study No. NL0012511)

Study Release Report No. NL0012511; Date May/29/1999
This is a GLP study performed by a Research and Development Group (N.V.Organon, Oss, the Netherlands and RL-CERM, Riom, France)
Doses: Intravenous doses of 0, 0.1, 0.4, and 1.6 mg/kg/day were administered for 39 consecutive weeks to beagle dogs (4/sex/group).
Assessments: Mortality, clinical signs, physical examination, ECG and ophthalmologic examinations, body weight, food consumption, hematology, blood clotting, blood biochemistry, urine analysis (all animals). ECGs were recorded pre-dose, at the end of the 1st week of dosing and at the end of the entire treatment period (method not indicated; neither the QTc calculation formula). Gross anatomy, organ weights, histopathology and myelograms were examined in all animals. For TK evaluations, blood samples were collected from 3 animals/sex/group on the 1st day of treatment (single dose) and at the end of the dosing period.
Results:

- **No mortality occurred in any group.**
- **Clinical signs: Dose-dependent predominantly CNS signs were observed in all treated groups: hypoactivity and/or hypotonicity [accompanied by episodes of recumbency at the MD (4/4 M and 2/4 F) and HD (all animals)]; excitation and compulsive behavior at LD (all animals), MD (3/4 M and 4/4 F), and HD (all animals), leading to self-inflicted wounds at MD and HD; tremors in 50% of the**

LD animals and in all MD and HD animals; myosis in 1/4 LDM and in all MD and HD animals; hypersalivation in all dose groups (1/4 M and 1/4 F, each) and vomiting at the HD (3/4 M and 1/4 F).
All signs generally occurred within 10 min after administration and lasted up to 2, 4, and 6 h. at the LD, MD, and HD, respectively.

- Increased incidence of sustained body weight loss at MD and HD in comparison to control [this effect was most likely associated with insufficient food supply supposedly due to behavioral modifications (excitation and compulsive behavior); in some animals (1MDM, 1 MDF and 2 HDM) it was reversible upon increasing the food supply].
- Cardiovascular effects were registered in all dose groups, dose-dependently, after 1 and/or 38 weeks of dosing:
  - increased incidence of tachycardia in LD (2/4 M and 3/4 F), MD (3/4 M and 2/4 F), and HD (3/4 M and 1/4 F) vs. control (1/4 M and 1/4 F) (see sponsor's table on this and next page)
  - increased QTc intervals (LD – 2M, 3F; MD - 3M, 1F; HD – 3M, 2F) vs. control (1/4 M and 1/4 F). The QTc prolongation was moderate, but present in all treated groups in a dose-dependent manner: mean increase by 4%, 7.5% and 11.5% and max increase of 15%, 32% and 30% vs. pre-dose values in the males at LD, MD, and HD respectively as compared to a mean and max. increase of 4% and 8% in the control (see sponsor's table on the next page)
  - flattened T-wave in LD (3/4 F), MD (1/4 M and 2/4 F), and HD (2/4 F) and occasionally inverted (negative) T wave polarity in MD (1/4 F), and HD (2/4 F).

Heart rate (females)

Experiment No : XS004

Absolute individual data

Heart rate in beats/min

Gr. 1: Placebo		0 mg/kg
Gr. 2: Org 5222	(low dose)	0.1 mg/kg
Gr. 3: Org 5222	(mid dose)	0.4 mg/kg
Gr. 4: Org 5222	(high dose)	1.6 mg/kg

Group No	Sex	Animal No	Animal Status	Exp. Day	Exp.day -14 to -7	7	266 to 269
1	F	1002	ST	274	60-	120-	144-
1	F	1004	ST	274	77-	95-	98-
1	F	1006	ST	276	106-	81-	115-
1	F	1008	ST	281	101-	98-	107-
Mean					86	98	116
St. Deviation					21	16	20
Number of Data					4	4	4
2	F	2002	ST	281	85-	79-	144-
2	F	2004	ST	274	140-	187-	142-
2	F	2006	ST	276	74-	156-	171-
2	F	2008	ST	276	99-	122-	116-
Mean					99	136	143
St. Deviation					29	46	23
Number of Data					4	4	4
3	F	3002	ST	281	83-	182-	161-
3	F	3004	ST	281	107-	106-	102-
3	F	3006	ST	274	120-	143-	158-
3	F	3008	ST	276	162-	143-	117-
Mean					118	144	134
St. Deviation					33	31	29
Number of Data					4	4	4
4	F	4002	ST	276	117-	105-	126-
4	F	4004	ST	281	101-	129-	128-
4	F	4006	ST	281	120-	140-	139-
4	F	4008	ST	274	69-	187-	145-
Mean					102	140	134
St. Deviation					23	35	9
Number of Data					4	4	4

Heart rate (males)

Experiment No : X8004

Absolute individual data

Heart rate in beats/min

Gr.	Treatment	Dose
Gr. 1:	Placebo	0 mg/kg
Gr. 2:	Org 5222 (low dose)	0.1 mg/kg
Gr. 3:	Org 5222 (mid dose)	0.4 mg/kg
Gr. 4:	Org 5222 (high dose)	1.6 mg/kg

Group No	Sex	Animal No	Animal Status	Exp. Day	Exp.day -14 to -7	7	266 to 269
1	M	1001	ST	274	93-	99-	86-
1	M	1003	ST	274	88-	121-	116-
1	M	1005	ST	276	150-	173-	164-
1	M	1007	ST	281	134-	147-	129-
Mean					116	135	124
St. Deviation					36	32	32
Number of Data					4	4	4
2	M	2001	ST	281	109-	150-	127-
2	M	2003	ST	274	155-	187-	156-
2	M	2005	ST	276	76-	155-	164-
2	M	2007	ST	276	133-	125-	153-
Mean					118	154	150
St. Deviation					34	25	16
Number of Data					4	4	4
3	M	3001	ST	281	55-	128-	129-
3	M	3003	ST	281	90-	140-	113-
3	M	3005	ST	274	128-	116-	130-
3	M	3007	ST	276	110-	135-	152-
Mean					96	130	131
St. Deviation					31	10	16
Number of Data					4	4	4
4	M	4001	ST	281	116-	123-	106-
4	M	4003	ST	281	73-	173-	149-
4	M	4005	ST	276	76-	136-	147-
4	M	4007	ST	274	124-	143-	158-
Mean					97	144	140
St. Deviation					26	21	23
Number of Data					4	4	4

QTc females

Experiment No : XS004

Absolute individual data

Corrected QT interval in s

Gr.	Treatment	Dose
Gr. 1:	Placebo	0 mg/kg
Gr. 2:	Org 5222 (low dose)	0.1 mg/kg
Gr. 3:	Org 5222 (mid dose)	0.4 mg/kg
Gr. 4:	Org 5222 (high dose)	1.6 mg/kg

Group No	Sex	Animal No	Animal Status	Exp. Day	Exp.day -14 to -7	7	266 to 269
1	F	1002	ST	274	0.21-	0.27-	0.27-
1	F	1004	ST	274	~~0.24*~~	~~0.25*~~	~~0.25*~~
1	F	1006	ST	276	0.28-	0.27-	0.26-
1	F	1008	ST	281	0.27-	0.25-	0.24-
Mean					0.25	0.26	0.26
St. Deviation					0.04	0.02	0.01
Number of Data					3	3	3
2	F	2002	ST	281	0.25-	0.25-	0.29-
2	F	2004	ST	274	0.28-	0.30-	0.27-
2	F	2006	ST	276	0.23-	0.26-	0.25-
2	F	2008	ST	276	0.27-	0.28-	0.26-
Mean					0.25	0.27	0.27
St. Deviation					0.02	0.02	0.01
Number of Data					4	4	4
3	F	3002	ST	281	0.23-	0.31-	0.30-
3	F	3004	ST	281	0.26-	0.27-	0.26-
3	F	3006	ST	274	0.31-	0.29-	0.27-
3	F	3008	ST	276	0.30-	0.30-	0.27-
Mean					0.27	0.29	0.27
St. Deviation					0.03	0.02	0.01
Number of Data					4	4	4
4	F	4002	ST	276	0.26-	0.27-	0.29-
4	F	4004	ST	281	0.26-	0.28-	0.28-
4	F	4006	ST	281	0.28-	0.29-	0.28-
4	F	4008	ST	274	0.22-	0.30-	0.28-
Mean					0.26	0.28	0.28
St. Deviation					0.03	0.01	0.01
Number of Data					4	4	4

1004 : 2nd degree AV block with Luciani-Wenckebach periods

QTc males

Experiment No : X8004

Absolute individual data

Corrected QT interval in s

Gr. 1:	Placebo	0 mg/kg
Gr. 2:	Org 5222 (low dose)	0.1 mg/kg
Gr. 3:	Org 5222 (mid dose)	0.4 mg/kg
Gr. 4:	Org 5222 (high dose)	1.6 mg/kg

Group No	Sex	Animal No	Animal Status	Exp. Day	Exp.day -14 to -7	7	266 to 269
1	M	1001	ST	274	0.26-	0.25-	0.25-
1	M	1003	ST	274	0.25-	0.28-	0.27-
1	M	1005	ST	276	0.31-	0.29-	0.28-
1	M	1007	ST	281	0.29-	0.29-	0.26-
Mean					0.27	0.28	0.26
St. Deviation					0.03	0.02	0.02
Number of Data					4	4	4
2	M	2001	ST	281	0.26-	0.30-	0.29-
2	M	2003	ST	274	0.30-	0.30-	0.29-
2	M	2005	ST	276	0.25-	0.29-	0.28-
2	M	2007	ST	276	0.30-	0.29-	0.29-
Mean					0.28	0.29	0.29
St. Deviation					0.03	0.01	0.00
Number of Data					4	4	4
3	M	3001	ST	281	0.22-	0.29-	0.29-
3	M	3003	ST	281	0.26-	0.29-	0.28-
3	M	3005	ST	274	0.30-	0.28-	0.29-
3	M	3007	ST	276	0.29-	0.30-	0.30-
Mean					0.27	0.29	0.29
St. Deviation					0.04	0.01	0.01
Number of Data					4	4	4
4	M	4001	ST	281	0.27-	0.26-	0.25-
4	M	4003	ST	281	0.23-	0.27-	0.30-
4	M	4005	ST	276	0.25-	0.29-	0.30-
4	M	4007	ST	274	0.28-	0.28-	0.30-
Mean					0.26	0.27	0.29
St. Deviation					0.02	0.01	0.02
Number of Data					4	4	4

No notable changes in hematology, blood biochemistry, urine analysis or histopathology were registered. No specific target organs of toxicity were detected; this also applies to the liver (in contrast to oral dosing).

The severe clinical signs and body weight losses induced by the high dose (1.6 mg/kg/day) indicated that the MTD was reached in this study.

TK data (3 dogs/sex/group) showed that systemic exposure, as indicated by AUC and Cmax, increased linearly with the dose, there were no signs of drug accumulation upon multiple i.v. administration, and exposure parameters were similar in males and females (see sponsor's table below).

Mean values of TK parameters in 39-week dog i.v. study*

Dose	0.1 mg/kg				0.4 mg/kg				1.6 mg/kg			
Sex	Males		Females		Males		Females		Males		Females	
Regimen	SD	MD	SD	MD	SD	MD	SD	MD	SD	MD	SD	MD
AUCinf ng.h/mL	33	37	26	33	98	121	114	123	492	594	426	759
NAUCinf ng.h/mL/(mg/kg)	339	367	254	323	245	300	289	310	304	370	266	475
Cmax ng/mL	25	18	27	33	81	58	98	78	388	272	309	351
NCmax ng/mL/(mg/kg)	258	182	265	329	203	145	249	196	240	169	193	220
Cl (L/min)	0.6	0.6	0.7	0.7	0.8	0.7	0.7	0.6	0.7	0.6	0.7	0.4
t1/2 (h)	0.9	1.4	0.7	0.8	0.8	1.4	0.8	1.1	0.9	1.6	1.0	1.5
Vc (L)	47	69	45	51	59	87	46	56	50	79	58	57
Vss (L)	127	297	98	94	106	264	76	117	87	178	98	183

SD = single dose, MD = multiple dose

* Bood samples taken after 1 day (single dose) and the end of the 39-week treatment (multiple dose)

For comparison, Cmax in human plasma upon multiple sublingual therapeutic doses of 5 mg (b.i.d), 10 mg (b.i.d), 15 mg (b.i.d), and 20 mg (b.i.d), were: 4.2 ng/ml, 6.6 ng/ml, 8 ng/ml and 10.6 ng/ml, respectively.

Conclusion:
MTD = 1.6 mg/kg/day (severe CNS clinical signs and body weight losses). However, no notable changes in hematology, blood biochemistry, urine analysis or histopathology were registered and no specific target organs of toxicity were detected.
A NOAEL was not reached in this study. CNS and cardiovascular effects (tachycardia, mild QTc prolongation, T-wave depression) were present in a dose-dependent manner down to the lowest tested dose of 0.1 mg/kg/day (NOAEL < 0.1 mg/kg/day i.v. for 39 weeks). Plasma exposure parameters upon 39-weeks of daily i.v. administration at the lowest tested dose of 0.1 mg/kg/day were as follows: AUCinf: 37 ng.h/ml (M) and 33 ng.h/ml (F); Cmax: 18 ng/ml (M) and 33 ng/ml (F).

Study title: <u>(1) A 13-week s.c. toxicity and toxicokinetic study with Org 5222 in CD-1 mice</u> (Study No. SDG RR 7026) and <u>(2) 13 week subcutaneous administration range-finding study in the mouse</u> (Study No. NL0017870)

In one 13-wk (GLP) study, Org 5222 was administered to CD-1 mice at doses of 0, 0.5, 1, and 2 mg/kg/day sc. The only drug-related effect noted was a transient decrease in activity in males and females at all doses.

In a second 13-wk study, Org 5222 was administered to CD-1 mice at doses of 0, 3, 4, and 5/7.5 mg/kg/day [the HD was increased on the first day of Wk 6]. Reduced activity (described as "moderate" at the HD) was observed in males and females at all doses. In males, death occurred at the HD (not otherwise described). Body wt gain was reduced at all doses in males (29-39% during the first 6 wks of dosing; however, by the end of the dosing period, body wt gain was similar among groups. Food intake was increased slightly in males from Wk 4 on. Other findings in males included the following: (a) decreased rbc parameters (MD, HD), (b) reduced wbc ct (MD, HD), (c) increased incidence of "mild cellulitis and mild myositis" at the HD, (d) increased in plasma urea at the HD [not measured at the LD or MD], (e) increased heart weight at all doses (no % given). In females, increases in heart (all doses, not dose-related) and liver (MD, HD) wt, and "increased mammary gland development" (HD, but not examined at the lower doses) were observed. The TK data from this study indicated that plasma exposure (AUC) to the parent compound at 4 mg/kg/day was 25-29 times higher than that expected in humans at the maximum therapeutic dose (i.e., 2 x 2500 µg/day; AUC$_{0-24}$ = 17.74 ng•hr/mL). The sponsor's summary table for the second 13-wk study is provided below:

Study groups:	(2)		(3)		(4)	
Important findings:	m	f	m	f	m	f
Deaths or premature kills						
Week 7 – 8					4	
Week 12 - 13	3		4		5	
Body weight gain						
Start – Week 6	d ***		d ***		d **	
Week 6 – Week 13	i ns	i *			i *	
Food consumption						
Start – Week 5		i *		i ns		i ns
Week 6 – Week 13	i **	i ***	i ns	i **	i ***	i ***
Clinical signs						
Reduced activity	+	+	+	+	++	++
Thin fur (%)	+	+/-	+		++	+
Skin excoriations (%)	+		+/-		++	
Haematology						
Haemoglobin					d ns	
Red blood cells			d ns		d *	
Packed cell volume			d ns		d *	
White blood cells					d *	
Leucocytes					d *	
Clinical chemistry						
AST	i *	i ns	i ns	i ns	i ns	i ns
Urea	i *		i ***		i *	
Glucose		i ***			i *	
Conclusion:						
Explanations:	d = decrease i = increase; p = permanent; t = transitory; ns = not significant; * = p < 0.05; ** = p < 0.01; *** = p < 0.001; +/- = very mild; + = mild; ++ = moderate; n = no. of animals; ( ) = due to abnormal control values					

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The HD of 4 mg/kg/day is expected to result in the following:

> **"--(transient) hypoactivity**
> **--reduced body weight gain (29-39%) in males (1st 6 weeks of dosing)**
> **--increased heart weight**
> **--increased liver weight in females**
> **--reduced white blood cell count in males**
> **--no significant local irritation at the injection sites"**
>
> **As noted above, systemic exposure to the parent compound at this dose is expected to be ≥25 times that at the maximum therapeutic dose.**

Study title: A 13-week s.c. toxicity and toxicokinetic study with Org 5222 in Wistar rats. (Study No. SDG RR 7025)

In one 13-wk sc (GLP) study, Org 5222 was administered to Wistar rats at doses of 0, 0.5, 1, and 2 mg/kg/day. At the HD, body wt gain was reduced (at the end of the dosing period) in males (13%) and increased (5%) in females. [Body wt gain was reduced by 8% in LDM and MDM; in females, body wt gain was increased (not dose-related) at all doses (9, 3, and 5% at LD, MD, and HD, respectively.] The following were observed at all doses: (a) "Transient hypoactivity" in all treated animals, (b) decreased food consumption in males ("mild") and increased food consumption ("mild") in females, (c) "Prolonged diestrus, decreased uterus weight, lobular development and an increased secretory activity of the mammary gland..." in females [with no clear dose-response], (d) dose-related increases in adrenal gland wt in males and decreases in uterus wt in females (no % given). The sponsor's summary table for this study follows:

[illegible]

	Group 2		Group 3		Group 4	
Sex (m/f)	m	f	m	f	m	f
Postdiet determinations						
• mean relative body weights at 13 wks	[illegible]	[illegible]	[illegible]	[illegible] (ns)	[illegible]	[illegible]
• Food consumption	D•	I•	D•	I•	D•	I•
• Reduced activity	•	•	•	•	•	•
• [illegible]		I•			I•	I•
Clinical chemistry						
• GPT		D•		D•		D•
• Bilirubin				D•		D•
• Calcium			D•		D•	
• Potassium					I•	
• Creatinine						
• at 2 wks		D•	D•		D•	D•
• at 13 wks	D•					
• Urea-N at 2 wks			D•		D•	
• Cholesterol		D•	D•	D•		D•
• Albumin		D•				D•
• Albumin/globulin		D•		D•		D•
Haematology and blood clotting						
• Haemoglobin	I•		I•		I•	I•
• Packed cell volume	I•		I•	•	I•	
• Mean corpuscular haemoglobin	I•		I•		I•	
• Mean corpuscular volume			I•		I•	
• Thrombocyte distribution width				I•		I•
• Mean thrombocyte volume		I•		I•		I•
• Thrombocyte large cell ratio		I•		I•		I•

[illegible]

	Group 2		Group 3		Group 4	
Sex (m/f)	m	f	m	f	m	f
Vagina						
• Incidence of diestrus				I•		I•
• Incidence of mucification		I•		I•		I•
Mammary gland						
• Incidence of development		I•		I••		I••
-slight		I•		I•		I•
-moderate		I•		I•		I•
-marked						
• Incidence of secretion						
-slight		I•		I•		I•
-moderate				I•		I•
-marked		I•		I•		I•
Uterus						
• Weight		D••		D••		D••
• Incidence of diestrus		I•		I•		I••
Adrenal gland						
• Weight	I•/-		I•		I••	

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Study title: Org 5222: 13 week subcutaneous administration range-finding study in the rat (Study No. NL0017877)

In a second 13-wk s.c. (GLP) study, Org 5222 was administered to Sprague-Dawley rats at doses of 0, 3, 4, and 5 mg/kg/day. In males (only), the following were observed: (a) reduced body wt gain (22-29% at the end of the dosing period, not dose-related) at all doses, (b) reduced food consumption (not statistically significant) at all doses, (c) dose-related decrease in lymphocyte ct (no % given), (d) a decrease in hyaline droplet accumulation in kidney at the HD, (e) mammary gland atrophy at the HD, (f) dose-related increase in adrenal gland wt (all doses). In females (only), the following were observed: (a) increased body wt gain (12-16%, not dose-related), (b) increased (not statistically significant) food consumption at all doses, (c) decreased uterus wt (all doses), (d) "increased development of mammary glands (HD), prominent corpora lutea (all doses) and prolonged diestrus (MD+HD)". In both males and females, the following were observed: (a) "Adrenal cortical hypertrophy and thyroid follicular cell hypertrophy..." at the HD [however, these tissues were not examined at the LD and MD] and (b) transient reduction in spontaneous motor activity at all doses and throughout the dosing period. The sponsor's summary table for the second 13-wk study is provided below.

Study groups	(2)		(3)		(4)	
Important findings	m	f	m	f	m	f
Palpation	+++	+++	+++	+++	+++	+++
Body weight gain	d***	i ns	d***	i ns	d***	i ns
Food consumption	d ns	i ns	d ns	i ns	d ns	i ns
Clinical chemistry						
Serum proteins	d*	d*	d**	d**	d*	d**
AST	i**	i***	i**	i***	i*	i***
Calcium	d ns	d*	d**	d**	d**	d*
Creatinine	i**		i***		i**	
Glucose		d*	d ns	d*	d ns	d*
Haematology						
WBC/Lymphocytes	d**		d***		d***	
Organ weights						
Adrenals	i ns	i*	i*	i*	i*	i ns
Uterus		d*		d***		d***
Histopathology						
Injection sites (# per animal)						
Haemorrhage (placebo m: 2/40, f 4/40)	7/40	4/40	6/40	7/40	3/40	3/40
Fibrosis (placebo m: 5/40, f 2/40)	2/40	4/40	11/40	4/40	21/40	12/40
Adrenal gland						
Cortical hypertrophy (placebo: 0/20)	ne	ne	ne	ne	6/10	6/10
Thyroid gland						
Follicular hypertrophy (placebo: 0/20)	0/1	ne	ne	ne	4/10	3/10
Conclusions:						
Explanations:	d = decrease i = increase; ns = not significant; * = p < 0.05; + = mild; ++ = moderate; [ ] = due to abnormal control values; ne: not examined	p = permanent; ** = p < 0.01; +++ = severe	t = transitory; *** = p < 0.001; n = no. of animals			
Study groups	(2)		(3)		(4)	

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Important findings	M	f	m	f	m	f
Mammary gland						
Acinar atrophy (placebo m: 0/8, f 0/10)	ne	ne	ne	ne	4/4	0/10
Acinar hypertrophy (placebo m: 0/8, f 0/10)	ne	ne	ne	ne	0/4	10/10
Ovaries						
Prominent corpora lutea (placebo f 0/10)		1/1		1/1		10/10
Uterus						
Dilatation (placebo f 3/10)		0/2		1/1		9/10
Vagina						
Mucification (placebo f 0/10)		ne		ne		7/10
Kidney						
Hyalin droplets (placebo m: 0/10, f 0/10)	ne	ne	ne	ne	3/10	0/10

Conclusions: administration of Org 5222 was associated with clinical signs of prolonged [illegible] after dosing, a reduced white blood cell count and increased incidence of haemorrhage and [illegible] (high dose animals) at injection sites. Endocrine effects were observed microscopically in several tissues in high dose animals (tissues from low and intermediate dose animals were not examined). No microscopic evidence of target organ toxicity was observed. The local effects seen in high dose animals would not preclude the use of this dose level for an oncogenicity study.

Explanations:	
	d = decrease I = increase p = permanent t = transitory
	ns = not significant * = p < 0.05 ** = p < 0.01 *** = p < 0.001
	+ = mild ++ = moderate +++ = severe n = no. of animals
	[ ] = due to abnormal control values
	ne: not examined

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The HD of 3 mg/kg/day is expected to result in the following:

"--more than 10% reduction in body weight gain in male rats.
--hypoactivity
--mild hematological effects
--prolongation of diestrus
--decreased uterus weight
--lobular development with secretory activity of mammary glands (females)
--increased adrenal gland weight (males)
--no significant local irritation at the injection sites"

Also, the sponsor noted that a dose of 3 mg/kg/day would be expected to result in an AUC (for parent) "...21-27 times higher than the human AUC at the maximum anticipated human dose (...2x2500 µg/day i.e. 71 microg/kg/day)".

The MD of 1 mg/kg was selected to be "...the logarithmic mean of the LD and the HD.." It is expected to produce system exposure (AUC) 6-7 times that predicted in humans at the maximum therapeutic dose.

The LD of 0.3 mg/kg/day was selected to "...be a low multiple..." of the maximum therapeutic dose. This dose is expected to produce systemic exposure (AUC) to the parent compound 2-4 times that predicted in humans at the maximum therapeutic dose.

The sponsor's summary PK/TK table is provided below:

ADDENDUM 4: Systemic Exposure in Humans, Rats and Mice

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Maximum anticipated human dose: 2x2400 microg/day, i.e. 71 microg/kg/day
AUC_{0-24}: 17.74 ng·h/mL; data obtained from a multiple dose phase II study in patients at a dose of 2x2400 microg/day.

Toxicokinetic data in rat and mouse:

Study	Dose (mg/kg/day)	Multiple Dose AUC_{0-24} (ng·h/mL)		Approximate multiple of human AUC
		Males	Females	
2-week rat	0.12	nd	18	1
	0.6	73	62	[illegible]
	3	[illegible]	[illegible]	[illegible]
13-week rat	0.5	[illegible]	[illegible]	[illegible]
	1	[illegible]	[illegible]	[illegible]
	2	[illegible]	[illegible]	[illegible]
2nd 13-week rat	3	[illegible]	[illegible]	[illegible]
	4	[illegible]	[illegible]	[illegible]
	6	[illegible]	[illegible]	[illegible]
2-week mouse	0.12	[illegible]	8	[illegible]
	0.6	77	44	[illegible]
	3	[illegible]	271	[illegible]
13-week mouse	0.5	[illegible]	[illegible]	[illegible]
	1	[illegible]	[illegible]	[illegible]
	2	[illegible]	131	[illegible]
2nd 13-week mouse	3	[illegible]	[illegible]	> [illegible] (males) > 21 (females)
	4	[illegible]	[illegible]	> [illegible] (males) > [illegible] (females)
	6	*	*	*
	7.5	[illegible]	[illegible]	> [illegible]

nd: no data (too limited measurable data)

* values of 6 mg/kg/day: only data available obtained after single dose, the dose was increased to 7.5 mg/kg/day from day 1 of week 5 onwards

** AUC_{0-8} values

a calculation based on AUC_{0-24} values from 1 mg/kg/day (first 13-week study), assuming linear kinetics

b calculation based on AUC_{0-24} values from 2 mg/kg/day (first 13-week study), assuming linear kinetics

Dr. Tabacova reviewed the summary of plasma exposure data from several toxicity studies (rodent and nonrodent; all routes, including oral) for parent compound and major circulating metabolites submitted to the IND 51, 641 with respect to a switch in development from oral to sublingual administration of asenapine in humans to determine whether the pivotal 13-week subcutaneous rat study, the 52-oral rat study, and the 39-week dog i.v. study are adequate for safety evaluation of asenapine toxicity upon sublingual administration in humans and to compare human/laboratory animal exposures (review of June 21, 2007). The following is the summary of the exposure data and conclusions taken directly from Dr. Tabacova's review:

Human/Animal exposure comparison:
Human exposure values (AUC 0-24) upon sublingual administration of asenapine at therapeutic doses (5 and 10 mg b.i.d.), or higher (20 mg b.i.d.) are 34, 56, and 112 ng.h/ml for the parent compound, and 24, 52, and 48 ng.h/ml for metabolite demethyl asenapine, respectively. Plasma exposures achieved in the rat at the highest employed repeat (52-week) oral dose level (AUC0-24=796 ng.h/ml for parent compound and 5143 ng.h/ml for demethyl asenapine) and at the highest repeat (13-week) subcutaneous dose (AUC0-2.5= 458 ng.h/m for parent compound) are well above human plasma exposure at the therapeutic and even the maximal tested doses in clinical studies. Plasma exposure (AUC) was not determined in 97-week s.c. rat carcinogenicity study, but the HD employed in that study (3.5 mg/kg/day) was similar to LD in the 13-week s.c. study (3 mg/kg/day) that resulted in plasma exposures of 183 and 279 ng.h/ml in M and F, respectively (values higher than human plasma exposure at therapeutic doses). Org 5222 plasma exposures achieved in dogs at the highest multiple oral dose (3.6 mg/kg/day) in the 52-week oral study (AUC0-7= 60 ng.h/ml and 43 ng.h/ml in M and F, respectively) are insufficient as they are similar or lower than human plasma exposure at therapeutic doses. However, asenapine plasma exposures achieved in dogs upon multiple (39-week) intravenous administration (AUC0-∞ of 594 and 759 ng.h/ml in M and F, respectively at the highest employed i.v. dose of 1.6 mg/kg) are well above human plasma exposure at the therapeutic and maximal tested doses in clinical studies.

Asenapine and demethyl asenapine exposure in humans, rats and dogs upon Org 5222 repeat-dose oral and parenteral administration

Species	Route/Dose/Duration	Plasma exposure	Asenapine	Demethyl asenapine
Human	Oral 10 mg BID	AUC 0-24* ng.h/ml	56	52
	Oral 20 mg BID	AUC 0-24*	112	42
Rat	Oral HD 10.8 mg/kg, 52 wks	AUC 0-24	796	5143
	S.c. HD 2 mg/kg, 13 wks	AUC 0-∞	317	No data
	S.c. HD 5 mg/kg, 13 wks	AUC 0-2.5	458	No data
	S.c. HD 3.5 mg/kg, 93 wks	Mean concentr, ng/ml	354	No data
Dog	Oral HD 3.6 mg/kg, 52 wks	AUC 0-7, ng.h/ml	60 (M) 43 (F)	1182 (M) 1918 (F)**
	I.v. HD 1.6 mg/kg, 39 wks	AUC 0-∞, ng.h/ml	594(M) 759 (F)	No data

*Extrapolated from AUC 0-12 in humans ** Upon a single oral dose of 3.6 mg/kg (no data for multiple dose)

Summary and conclusions:

- Org 5222 metabolism is similar in rats, dogs, and humans (in vitro data);
- Same metabolites [N(2)-demethyl and N(2)-oxide asenapine] were determined in rats upon oral and parenteral (i.v.) administration of Org 5222; in dogs, the main metabolite [N(2)-demethyl asenapine] was determined in plasma upon oral and parenteral (i.v., s.l., i.m.) administration of Org 5222; the N(2) oxide metabolite was determined upon Org oral administration but data for other routes are not available.
- The oral bioavailability of Org 5222 in rats is within the range of 20% – 65%. In dogs, oral bioavailability of Org 5222 is very low (3%) while bioavailability after i.v. and s.l. administration are high and comparable (100% and 122%, respectively).
- Plasma exposure (AUC) to the parent compound upon oral or i.v. administration of one and the same single dose of Org 5222 is about 2 times (rat) and 10 to over 20 times (dog) higher for the i.v. vs. oral route. Exposure to demethyl metabolite is higher after oral vs. i.v. dose in both rat (M) and dog; exposure to N-oxide metabolite in the rat is similar upon oral and i.v. administration (dog data not available).
- There is no increase in exposure (AUC) to parent compound after repeated oral (52 weeks) administration vs. single oral administration to either rats or dogs. Likewise, in rats, there is no increase in exposure to parent compound upon repeated parenteral (13 weeks s.c.) administration vs. single s.c. administration. In dogs, there is some increase in exposure to parent compound (1.2x and 1.8x in M and F, respectively) after repeated i.v. (39 weeks) administration vs. single i.v. administration. (Plasma exposure data for metabolites in either rats or dogs upon repeated parenteral administration of Org 5222 are not available).
- Plasma exposures to Org 5222 achieved in the rat at the highest oral dose used in a pivotal 52-week oral toxicity study (AUC0-24=796 ng.h/ml for parent compound and 5143 ng.h/ml for demethyl asenapine) and at the highest subcutaneous dose level in a pivotal 13-week s.c. study (AUC0-2.5= 458 ng.h/m for parent compound) are well above human plasma exposure at the therapeutic and even the maximal tested sublingual doses in clinical studies.
- Plasma exposures to Org 5222 achieved in the dog at the highest multiple oral dose level (3.6 mg/kg) in the 52-week oral study (AUC0-7= 60 and 43 ng.h/ml in M and F, respectively) are at or below human plasma exposure at therapeutic doses. However, asenapine plasma exposures achieved in dogs upon multiple (39-week) intravenous administration (AUC0-∞= 594 and 759 ng.h/ml in M and F, respectively at the highest employed i.v. dose of 1.6 mg/kg) are well above human plasma exposure at the therapeutic and maximal tested sublingual doses in clinical studies.
- Org 5222 bioavailability in dogs is similar following i.v. or s.l. route of administration; therefore the performed 39-week i.v. toxicology study is valid for assessment of toxicity upon sublingual administration in dogs.
- Plasma exposures to Org 5222 and its N(2)-demethyl metabolite achieved in the 13-week subcutaneous rat study, the 52-week oral rat study, and 52-week dog i.v. study are adequate for assessment of toxicity upon sublingual administration in these animal species and for safety evaluation of Org 5222 toxicity upon sublingual administration in humans.

Conclusion: The 13-week subcutaneous rat study, the 52-week oral rat study, and the 39-week dog i.v. study are adequate for safety evaluation of Org 5222 toxicity upon sublingual administration in humans.

2.6.6.4 Genetic toxicology

1. Study title: A *Salmonella* Microsome Mutagenicity Test (Ames Test) with Org 5222.

Key findings: There were no increases in the number of revertants of the *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 when tested with asenapine (Org 5222) at concentrations of 0, 8, 40, 200, and 1000 µg/plate. Therefore, asenapine was not mutagenic in the Ames test under conditions of this study. However, there are no data demonstrating the increase in the number of revertants following treatment of bacteria with positive controls in the absence of metabolic activation. Therefore, a part of the study conducted in the absence of metabolic activation is inadequate.

Study no.: SDG RR No. 2283
Volume #, and page #: electronic submission
Conducting laboratory and location: Scientific Development Group, Organon, Oss, The Netherlands
Date of study initiation: May 20, 1980
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: asenapine (Org 5222), batch C; purity: 100%

Methods
Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538
Doses used in definitive study: 0, 8, 40, 200, and 1000 µg/plate
Basis of dose selection: cytotoxicity observed at 1000 µg/plate
Negative controls: vehicle (ethanol)
Positive controls: 2-acetylaminofluorene (25 µg/plate) for strains TA98, TA100, and TA1538; 2-aminoanthracene (5 µg/plate) for strain TA1535 and benzo(a)pyrene (7.5 µg/plate) for strain TA1537. These chemicals were dissolved in DMSO.
Incubation and sampling times: The number of colonies was determined after incubation at 37°C for 48 hours.

Results
Study validity: Asenapine was tested in bacterial mutagenesis test for possible mutagenic effects in the absence or presence of metabolic activation (S-9, rat liver, Aroclor 1254-induced). Three plates were tested with each concentration. The criteria for the positive response were as follows: (1) dose-related increase in revertants, or (2) the relative increase in the number of revertants must be ≥2-fold, or (3) the increase must be outside historical control range. There was no precipitation of the test article in this study. Cytotoxicity was observed at 1000 µg/plate as indicated by growth inhibition of the bacterial lawn and/or decrease in number of his+ colonies below the lower limit of the normal control range. Thus, the high concentration of asenapine selected for this study was adequate. The positive controls induced a mutagenic response in all strains in the presence of metabolic activation. However, there are no data demonstrating an increase in the number of revertants following treatment of bacteria with positive controls in the absence of metabolic activation. Therefore, a part of the study conducted in the absence of metabolic activation is inadequate.

Study outcome: The number of revertants remained unchanged following treatment with asenapine or solvent in the presence or absence of metabolic activation. Asenapine did not show mutagenic activity in the Ames test under conditions of this study.

2. Study title: Reverse Mutation Test in *Escherichia Coli* of Org 5222.

Key findings: There were no increases in the number of revertants in the *Escherichia coli* strain when tested with asenapine (Org 5222) at concentration from 46.9 to 1500 µg/plate. Therefore, asenapine was not mutagenic in the Ames test under conditions of this study.

Study no.: SDG RR No. 2963
Volume #, and page #: electronic submission
Conducting laboratory and location: — b(4)

Date of study initiation: February 12, 1991
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine (Org 5222), batch H, purity 99.8%

Methods
Strains/species/cell line: single *Escherichia coli* tester strain WP2 uvrA
Doses used in definitive study: 0, 46.9, 93.8, 188, 375, 750, and 1500 µg/plate
Basis of dose selection: Preliminary toxicity test conducted at 5000, 1580, 500, 158, and 50 µg/plate. On the basis of these results, a high dose level of 1500 µg/plate was selected for the main assay.
Negative controls: dimethylsulphoxide (DMSO)
Positive controls: 2-aminoanthracene (1 µg/plate) in the presence of metabolic activation, methylmethanesulphonate (500 µg/plate) in the absence of metabolic activation.
Incubation and sampling times: The number of colonies was determined after incubation of plates at 37°C for 72 hours.

Results
Study validity: Asenapine was tested in two independent bacterial mutagenesis tests for possible mutagenic effects in the absence or presence of metabolic activation (S9, rat liver, Aroclor 1254-induced) in a single *Escherichia coli* WP2 uvrA strain. Three plates were tested with each concentration. The criteria for the positive response were as follows: (1) the relative increase in the number of revertants must be ≥2-fold, or (2) dose-related increase in revertants, or (3) reproducible. Toxicity was observed at the highest two concentrations of asenapine in the absence of metabolic activation and at the highest concentration level in its presence. The positive control produced increased number of revertants. Therefore, this study is considered to be valid.
Study outcome: Asenapine did not induce significant increases in the number of revertant colonies at any concentration level, either in the presence or absence of

metabolic activation. Therefore, asenapine did not induce reverse mutations in *Escherichia coli* strain under conditions of this study.

3. Study title: A Mammalian/Microsome Mutagenicity Test (Ames Test) with Org 5222 in *Salmonella Typhimurium* and *Escherichia Coli*.

Key findings: There were no increases in the number of revertants at any asenapine concentration tested from 8 to 2500 µg/plate with any *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* strain WP2 uvrA used in this test. Therefore, asenapine was not mutagenic in the Ames test under conditions of this study.

Study no.: SDG RR No. 5097
Volume #, and page #: electronic submission
Conducting laboratory and location: Scientific Development Group, N. V. Organon, Oss, location Schaijk, The Netherlands
Date of study initiation: September 16, 1997
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine (Org 5222), batch K; purity: 99.8%

Methods
Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2 uvrA
Doses used in definitive study: 0, 8, 40, 200, 1000, and 2500 µg/plate
Basis of dose selection: previous Ames test data
Negative controls: solvent (DMSO)
Positive controls: In the presence of metabolic activation (S9 mix): 2-acetylaminofluorene (25 µg/plate) for strains TA98 and TA100; 2-aminoanthracene (2 µg/plate) for strains TA1535 and WP2 uvrA, and benzo(a)pyrene (5 µg/plate) for strain TA1537. In the absence of metabolic activation: 4-nitroquinoline-N-oxide at a dose of 0.3 µg/plate for strains TA98 and TA100, and at a dose of 1 µg/plate for strains TA1535, TA1537, and WP2 uvrA. These chemicals were dissolved in DMSO.
Incubation and sampling times: The number of colonies was determined after incubation of plates at 37°C for 48 hours.

Results
Study validity: Asenapine was tested in the bacterial mutagenesis test for possible mutagenic effects in the absence or presence of metabolic activation (S-9, rat liver, Aroclor 1254-induced). The test was carried out in triplicate for each concentration of asenapine. The two-fold relative increase in the number of revertants was used as criteria for the positive response. Cytotoxicity was observed at 1000 and 2500 µg/plate as indicated by growth inhibition of the bacterial lawn and/or decrease in number of his+ colonies below the lower limit of the normal control range indicating adequate concentration selection for this study. The capacity of the strains to give a mutagenic response with a reference mutagen (positive control) was demonstrated in each test in the

presence or absence of metabolic activation (S-9 mix). Therefore, this study is considered to be valid.

Study outcome: The number of revertants remained unchanged following treatment with asenapine or solvent in the presence or absence of metabolic activation. Asenapine did not show mutagenic activity under conditions of this study.

4. Study title: A Gene Mutation Test in Mouse Lymphoma L5178Y TK +/- Cells *in Vitro* with Org 5222.

Key findings: Asenapine (Org 5222) did not induce biologically relevant increases in the mutant frequency in mouse lymphoma L5178Y TK cells when tested at concentrations from 3.125 to 75 µg/ml in the absence, or from 6.25 to 100 µg/ml in the presence of metabolic activation (S-9 mix), and retested at concentrations from 40 to 76 µg/ml in the presence of metabolic activation. Therefore, asenapine was not mutagenic under conditions of this study.

Study no.: SDG RR 4361
Volume #, and page #: electronic submission
Conducting laboratory and location: Scientific Development Group, N. V. Organon, Oss, location Schaijk, The Netherlands
Date of study initiation: March 24, 1993
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine (Org 5222); batch E; purity 100.4%

Methods
Strains/species/cell line: cultured mouse lymphoma L5178Y TK +/- cells
Doses used in definitive study: Asenapine concentrations from 3.125 to 75 µg/ml or from 6.25 to 100 µg/ml were tested in the absence or presence of metabolic activation (S-9, rat liver, Aroclor 1254-induced), respectively. After an expression period of 2 days, cells were plated for viability and TFT resistance at concentrations ranging from 3.125 to 25 µg/ml in the absence of metabolic activation and from 6.25 to 75 µg/ml in the presence of metabolic activation. In the second experiment, asenapine was retested at concentrations from 40 to 76 µg/ml in the presence of metabolic activation. After an expression period of 3 days, cells were plated for viability and TFT resistance at all concentrations tested.
Basis of dose selection: The cytotoxicity results from a dose range finding study conducted at doses ranging from 15.625 to 500 µg/ml were used for the dose selection. In the absence of metabolic activation, cytotoxicity induced by the test article was observed at doses of 15.625, 31.25, 62.5 µg/ml and higher, as indicated by the relative survival values of 50%, 50% and 0%, respectively. In the presence of metabolic activation (S-9 mix), cytotoxicity was observed at doses of 15.625, 31.25, 62.5, 125 µg/ml and higher, as indicated by the relative survival values of 65%, 65%, 15.9%, and 0%, respectively.
Negative controls: dimethylsulphoxide (DMSO) 1% v/v
Positive controls: 4-Nitroquinoline-oxide (NQO) (without S-9 mix) and benzo(a)pyrene (BP) (with S-9 mix).

Incubation and sampling times: Cells were grown and exposed to asenapine for 3 hours. After exposure cells were rinsed and plated for survival for 10–13 days and for growth through an expression period of two days.

Results

Study validity: The study was conducted using duplicate cultures. In the absence of metabolic activation, concentration-related toxicity was observed at doses of 12.5 µg/mL and higher as indicated by relative survival values of 81.2% - 0%. Cells exposed to concentrations of 50 and 75 µg/mL were not plated for the mutant frequency due to severe cytotoxicity. In the presence of metabolic activation, concentration-related toxicity was present at doses of 75 and 100 µg/mL as indicated by relative survival values of 13.0% and 0%, respectively. Cells exposed to concentration of 100 µg/mL were not plated for the mutant frequency due to severe cytotoxicity. The positive controls caused significant increases in the mutant frequency in the absence and presence of metabolic activation. For the negative and positive controls, the number of small and large colonies was determined but not for asenapine-treated cultures since there was no reproducible and dose-related increases in the mutant frequency. This study is considered to be valid.

Study outcome: A statistically significant increase in the mutant frequency was observed at the top concentration of 75 µg/mL in the presence of metabolic activation. The mutation frequency at this concentration was 244, close to historical mean value for the negative control mutant frequency of 211. At this concentration, the relative survival was only 13%. In the second experiment, asenapine did not induce significant increases in the mutant frequency at any concentrations tested (40-76 µg/mL) in the presence of dose-related toxicity (60.4% to 14.1%). Thus, asenapine did not induce reproducible and concentration-dependent increases in the mutant frequency at any of the concentrations tested in the presence and absence of metabolic activation. Therefore, it can be concluded that asenapine was not mutagenic in the mouse lymphoma assay under conditions of this study.

5. Study title: A sister chromatid exchange test in rabbit lymphocytes *in vitro* with Org 5222.

Key findings: Asenapine (Org 5222) did not induce sister chromatid exchange when tested in the non-mutational DNA damage test in cultured rabbit lymphocytes.

Study no.: SDG RR 2317
Volume #, and page #: electronic submission
Conducting laboratory and location: Drug Safety RDL, Scientific Development Group, Organon Oss, Location Schaijk, The Netherlands.
Date of study initiation: April 5, 1982
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine (Org 5222), batch E, purity: 100.4%

Methods

Strains/species/cell line: Phytohaemagglutinin-stimulated SPF Dutch rabbit lymphocytes cultured from peripheral blood for 24 hours
Doses used in definitive study: 0, 12.5, 25, 50, and 100 µg/ml
Basis of dose selection: dose-range finding studies demonstrating cytotoxicity at 205 µg/ml (± S-9)
Negative controls: vehicle (ethanol)
Positive controls: cyclophosphamide in the presence of S-9; no positive control was used in the absence of S-9
Incubation and sampling times: Cultured lymphocytes were exposed to asenapine for 3 hours in the presence and absence of a metabolic activation system (S9, rat liver, Aroclor 1254-induced). Culturing was continued in the presence of 5-bromodeoxyuridine (BrdU) for 25 hours.

Results
Study validity: Lymphocytes were arrested at metaphase by colchicine treatment for the final 3 hours. Then cells were harvested and slides were prepared. The Fluorescent Plus Giemsa technique was used to differentially stain the chromatids. The number of sister chromatid exchanges (SCE's) per metaphase was assessed. 30 differentially stained metaphases were examined per concentration (10 cells per culture). Appropriate controls were included and resulted in expected results. This study was considered to be valid.
Study outcome: Asenapine (Org 5222) did not induce sister chromatid exchange in cultured rabbit lymphocytes.

6. Study title: A chromosome aberration test in peripheral human lymphocytes *in vitro* with Org 5222.

Key findings: There was a small, statistically significant increase in the number of human peripheral lymphocytes with structural chromosomal aberrations following treatment with asenapine for 20 hours in the absence of metabolic activation. This effect was not concentration-dependent and not observed at 44 hours at the concentration tested in the absence of metabolic activation. Moreover, the mean number of cells with structural chromosomal aberrations either in the presence or absence of metabolic activation was within the normal historical range. Therefore, the effect of asenapine on chromosomal aberrations is equivocal in this study.

Study no.: SGS RR 4413
Volume #, and page #: electronic submission
Conducting laboratory and location: b(4)

Date of study initiation: March 16, 1993
GLP compliance: yes (OECD)
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine (Org 222), batch E, purity 100.4%

Methods
Strains/species/cell line: cultured human peripheral lymphocytes from a single female donor

Doses used in definitive study: 12 concentrations of asenapine ranging from 1.0 to 100.0 µg/mL in the absence of metabolic activation and 15 asenapine concentrations ranging from 1.0 to 350.0 µg/mL in the presence of metabolic activation were used in this study. In the absence of metabolic activation, cytotoxicity was observed at doses of 55, 64, 73, and >82 µg/ml following 20 hours of exposure, as indicated by inhibition of mitotic index by 44%, 68%, 95%, and 100%, respectively. In the presence of metabolic activation, following 3 hours of exposure and at a sampling time of 20 hours, cytotoxicity was present at doses of 75, 100, 125, 150, 175, and ≥200 µg/ml, as indicated by inhibition of the mitotic index by 26%, 33%, 64%, 76%, 65%, and 95%, respectively. Based on these data, the following asenapine concentrations were selected for analysis:

Time of sampling	Metabolic activation	Asenapine concentration (µg/ml)	Mitotic inhibition
20 hours	- S9	46, 55, 64	68%[+]
20 hours (3h + 17h recovery)	+ S9	75, 100, 125	64%[+]
44 hours	- S9	46	71%
44 hours	+ S9	125	3%

[+] considered close enough to 50% to justify selection as the top dose for analysis

Basis of dose selection: The selection of concentrations for the main study was based on mitotic index data obtained in a dose range finding study with treatments covering a broad range of doses (5.250-600.0 µg/ml) performed in the presence or absence of metabolic activation. The top concentration for chromosome analysis in the main study was the one at which a 50% reduction in mitotic index has occurred.
Negative controls: dimethylsulphoxide (DMSO)
Positive controls: 4-nitroquinoline-1-oxide (NQO) in the absence of metabolic activation and cyclophosphamide (CPA) in the presence of metabolic activation.
Incubation and sampling times: Lymphocytes in culture were stimulated to divide with phytohaemagglutinin for 48 hours before exposure to asenapine. In the presence of metabolic activation cells were exposed to asenapine for 3 hours followed by a period of recovery (washing and resuspension) prior to sampling at 20 and 44 hours. In the absence of metabolic activation cells were exposed to asenapine for 20 and 44 hours. Colchicine was added to cultures for the last 1.5 hours prior to sampling to arrest dividing cells in metaphase.

Results

Study validity: Asenapine was tested for the induction of chromosomal aberrations in the presence or absence of a metabolic activation system (S9 mix) in human peripheral lymphocyte cultures. The highest dose level used (600 µg/ml) was close to the solubility limit of asenapine in culture medium. Duplicate cultures were used. Two hundred (100 per culture) metaphases per test concentrations were examined. The numbers of aberrant cells observed in negative control in the presence or absence of metabolic activation were within the normal range. Exposure to the positive controls caused a statistically significant increase in the frequency of cells with aberrations. This study was considered to be valid.

Study outcome: Following treatment with asenapine for 20 h in the absence of metabolic activation, a statistically significant increase in the number of cells with aberrations (excluding gaps) was present at concentrations of 55 and 64 µg/mL when compared with concurrent controls. At 55 µg/mL, the number of cells with aberrations was 5 in one culture just outside the normal range of 0-3/100 cells (historical controls) and inside the normal control range in the duplicate culture. Following examination of additional cells at 55 µg/mL, the numbers of cells with aberrations were 4 and 0 in two cultures. Overall number of cells with aberrations was 11 in 376 cells examined (mean value was 2.9 cells with aberrations per 100 cells) following treatment at 55 µg/mL. At 64 µg/mL, both numbers of cells with aberrations (3 and 1) were within the normal historical control range. The increase was not concentration dependent. There was no increase at one concentration (46 µg/mL) tested at 44 hours in the absence of metabolic activation. In the presence of metabolic activation, the number of cells with aberrations was similar to that in concurrent and historical controls. In addition, the number of cells with numerical aberrations increased following treatment in both the absence and presence of metabolic activation. However, the biological significance of increases in polyploidy in cultured cells in chromosomal aberrations assay is unknown. In conclusion, the effect of asenapine on the induction of structural aberrations in cultured human lymphocytes when treated to the limit of toxicity in both the absence of metabolic activation was equivocal under conditions of this study. The "weak" positive results of this study are shown in the Sponsor's table below:

Metaphase Analysis in vitro

TABLE 1

Cells with chromosomal aberrations

20 hour treatment - S-9, 0 hour recovery Test chemical: Org 5222
Donor sex: female

Treatment (µg/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance $	Mitotic index (mean)
Solvent	A	200	2	0		4.3
	B	200	0	0		5.0
	Totals	400	2	0		(4.7)
44	A	100	2	1		3.9
	B	100	1	0		4.3
	Totals	200	3	1	NS	(4.1)
55	A	100	5	2		2.9
	A*	100	[illegible]	4		
	B	100	5	[illegible]		2.0
	B*	76	0	0		
	Totals	376	16	11	p ≤0.001	(2.5)
64	A	100	4	3		1.0
	B	100	2	1		1.0
	Totals	200	6	4	p ≤0.01	(1.4)
NQO 2.5	A	25	[illegible]	[illegible]		
	B	25	[illegible]	[illegible]		
	Totals	50	21	18	p ≤0.001	

* Additional analysis
$ Statistical significance (Appendix 5e)

NS = not significant

Summary of numerical aberrations observed

20 hour treatment - S-9, 0 hour recovery   Test chemical: Org 5222
Donor sex: female

Treatment (µg/ml)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A B	200 201	0 0	0 0	0 1	0 1	0 0.5
	A+B	401	0	0	1	1	0.25
46	A B	103 100	0 0	0 0	3 0	3 0	2.9 0
	A+B	203	0	0	3	3	1.5
58	A B	206 178	1 1	0 0	5 1	6 2	2.9 1.1
	A+B	384	2	0	6	8	2.1
64	A B	109 107	0 0	0 0	9 7	9 7	[illegible]
	A+B	216	0	0	16	16	7.4
NQO 2.5	A B	25 25	0 0	0 0	0 0	0 0	0 0
	A+B	50	0	0	0	0	0

** = Total cells examined for numerical aberrations

Numbers highlighted exceed historical negative control ranges (Appendix 6)

Summary of numerical aberrations observed

3 hour treatment + S-9, 17 hour recovery   Test chemical: Org 5222
Donor sex: female

Treatment (µg/ml)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A B	101 100	1 0	0 0	0 0	1 0	1.0 0
	A+B	201	1	0	0	1	0.5
75	A B	100 102	0 1	0 0	0 1	0 2	0 2.0
	A+B	202	1	0	1	2	1.0
100	A B	108 103	1 0	1 0	6 3	8 3	[illegible] 2.9
	A+B	211	1	1	9	11	5.2
120	A B	103 113	2 0	0 2	1 11	3 13	2.9 [illegible]
	A+B	216	2	2	12	16	7.4
CPA 25	A B	27 25	2 0	0 0	0 0	2 0	[illegible] 0
	A+B	52	2	0	0	2	3.8

** = Total cells examined for numerical aberrations

Numbers highlighted exceed historical negative control ranges (Appendix 6)

Note: The results of this test were considered positive when reviewed by Dr. Lois Freed under the IND 51,641. Please see Dr. Freed's reviews in the Division's file for further information.

7. Study title: A Micronucleus Test in Rats with Org 5222

Key findings: Asenapine did not produce chromosomal or spindle damage leading to the formation of micronuclei in the immature erythrocytes of the rat in the *in vivo* micronucleus assay. Therefore, asenapine did not cause cytogenetic damage under conditions of this study. It is not clear whether a test target for genotoxic activity i.e. the bone marrow erythroblasts have been exposed to asenapine in this study as indicated by the ratio polichromatic/normochromatic erythrocytes that remained within the normal control range in all groups. However, the systemic exposure to the test article was demonstrated by dose-related clinical signs of hyper-irritability and ataxia, body weight loss, increase urine volume, and proteinuria.

Study no.: SGD RR 2323
Volume #, and page #: electronic submission
Conducting laboratory and location: Department of Toxicology and Drug Disposition, N.V. Organon, Oss, Schaijk, The Netherlands
Date of study initiation: May 17, 1982
GLP compliance: yes (OECD 1982)
QA reports: yes (x) no ( ); The study was not inspected. Final report was audited.
Drug, lot #, and % purity: asenapine (Org 5222), batch E (suspension), purity 100.4%

Methods
Strains/species/cell line: Wistar rats
Doses used in definitive study: 0, 25, 50, and 75 mg/kg/day
Basis of dose selection: Oral administration of asenapine for 2 weeks to rats at dose levels of 19, 38, 75, and 150 mg/kg/day caused mortality at 150 mg/kg/day and reduced weight gain and food consumption at all dose levels. In view of these findings, the HD of 75 mg/kg/day was chosen for the rat micronucleus study.
Negative controls: Group of rats receiving aqueous gelatin (0.5%) containing 5% mannitol (vehicle) for 4 weeks served as a negative control.
Positive controls: Methylmethanesulphonate (MMS) administered intraperitoneally at 25 mg/kg/day for 2 days served as a positive control
Incubation and sampling times: Asenapine was administered by gavage to rats for 4 consecutive weeks.

Results
Study validity: Asenapine was administered to rats (8/sex/group) by gavage at a volume of 2 mL/kg body weight for 4 weeks. The bone marrow smears from the femur were examined microscopically for the presence of micronuclei in 1000 polychromatic erythrocytes (PCE) per animal. In addition, the ratios of PCE to normochromatic erythrocytes (NCE) in 500 cells per animal were examined. Additional scoring of slides was performed in 1997 to meet the requirements of updated OECD Guidelines (in

response to the Division's recommendation). At that time, 2000 PCE's were examined for the presence of micronuclei. For the PCE/NCE ratio 1000 erythrocytes were examined. The ratio PCE/NCE was within the normal control range in all vehicle- and asenapine-treated groups and showed no dose-effect relationship indicating that drug treatment had no effect on erythrocyte formation. No other methods for verifying that the test substance reached the general circulation and/or target tissues were employed. However, concentration-related signs of toxicity (hyper-irritability and ataxia, body weight loss and reduced food consumption, increase urine volume and proteinuria) were observed following treatment with asenapine indicating that the systemic exposure to the test article was achieved. It is noted that historical negative and positive control data were not provided. This study was considered to be valid.

Study outcome: Group mean and individual animal values for the frequency of micronucleated PCE's in the vehicle and asenapine-treated groups were similar and within the normal range. A clear increase in the frequency of micronuclei was noted in the positive control group demonstrating mutagenic response.

2.6.6.5 Carcinogenicity

1. Study title: Org 5222: 104 week subcutaneous administration oncogenicity study with Org 5222 in the rat.

Key study findings: Subcutaneous administration of asenapine maleate at 0.3, 1.2, and 3.0/5.0 mg/kg/day to female (F) rats for 99 weeks and to male (M) rats for 105 weeks did not demonstrate any organ specific, systemic, or local tumorigenic potential of the test article. The MTD was clearly exceeded in this study in males at all dose levels and in females at the HD based on significant and dose-dependent decreases in body weight gain and body weight observed in animals of both genders administered the HD, as well as in male rats administered the LD and MD. The reduction in body weight was not consistent with changes in food consumption. The incidence of preneoplastic changes and tumors (total number of tumors and tumor-bearing animals) decreased at the HD when compared to the vehicle controls.

When an excessive decrease in body weight (or survival) is observed in the examined dose group, the Agency recommends conducting a histopathologic examination of the lower dose groups. However, the Sponsor evaluated microscopically a few tissues of the LD and MD groups. Full histological evaluation was conducted only on tissues from the two control groups and the HD group in both genders. Since it is known that a significant decrease in body weight leads to a decrease in tumor development and the microscopic evaluation of the LD and MD groups has not been conducted, the rat carcinogenicity study is inadequate for both genders. Moreover, based on limited toxicokinetic data, margins of safety based on exposure cannot be reliably estimated.

Adequacy of the carcinogenicity study and appropriateness of the test model:
This study was designed to assess the carcinogenic potential of asenapine following subcutaneous administration to male and female Sprague-Dawley rats for 104 weeks. Rats were selected based on recommendations of applicable guidelines and the available

background data for this species. Subcutaneous injection has been chosen as an appropriate alternative for the intended sublingual clinical route because the sublingual route of administration is not feasible in rodents. A sufficient number of animals (60/sex/group) was used in the study. Rats were treated with asenapine at dosages that were not previously concurred with by the Executive CAC due to the lack of sufficient information (see Attachment 1).

The study procedures were modified when compared to the original study protocol. The HD of 3 mg/kg/day was increased to 5 mg/kg/day in both sexes after six weeks of dosing. Due to high morbidity/mortality in the female vehicle control group, the female study was terminated in Weeks 100 to 102. The males were terminated in Weeks 106 to 107 as originally planned. There were more than 50% of animals alive in Week 90 in both sexes except the female vehicle control group, in which the survival rate was 33% (20/60). Although mortality in this study was high, it can be concluded that enough rats were exposed to the HD for a sufficient amount of time, and the high mortality did not have a negative influence on the validity of the study.

Treatment produced evidence of toxicity based on significantly decreased body weight gain and body weight in both sexes:

Males gained 73%, 63%, or 48% of the vehicle control body weight gain at the LD, MD, and HD, respectively. Body weight in males was 83%, 77%, and 69% of the vehicle control weight at the LD, MD. and HD, respectively, at termination in Week 104. The body weights were decreased throughout most of the study at all dose levels (for example, the weights were 90%, 84%, and 78% of the vehicle control in Week 52 of dosing at the LD, MD, and HD, respectively). The overall body weight gain and body weight of the male untreated control group were 19% and 11% higher, respectively, than that of the vehicle control group at termination. In conclusion, the MTD was exceeded in this study in males at all dose levels.

Females gained 91%, 79%, or 59% of the vehicle control weight gain at the LD, MD, and HD, respectively. Body weight in females was 96%, 89%, and 77% of the vehicle control group at the LD, MD, and HD, respectively, at termination in Week 96. The body weights were decreased throughout most of the study for the HD group (for example, the weights were 100%, 99%, and 90% of the vehicle control at the LD, MD, and HD, respectively, in Week 52 of dosing). The overall body weight gain and body weight of the untreated control group were 6% and 5% higher, respectively, than that of the vehicle control group at termination. In conclusion, the MTD was exceeded in this study in females at the HD.

Food consumption was decreased in LD and MD males, and in MD females, when compared to the vehicle control group.

According to the Sponsor, all protocol specified tissues were retained. However, the slides were prepared and histopathological examinations were performed only on a complete set of tissues of all animals of the vehicle, untreated control and HD groups, and on all tissues from decedents. Of terminally sacrificed LD and MD animals, only slides of the thyroid glands, adrenal glands and female mammary glands, and all other grossly abnormal organs and tissues (lesions, masses) were prepared and examined.

It has been demonstrated that dietary (caloric) restriction results in decreased body weight, increased life span, and reduced incidences of spontaneous and chemically-induced tumors when compared to *ad libitum* feeding. Clearly, in this study reduced body weight gain was associated with a reduction in the overall incidence of tumors at the HD in both males and (to a lesser extent) females. The reduced body weight associated with lower incidence of tumors at the HD and the lack or limited tumor data in the lower dose groups make the study potentially less sensitive to detect the carcinogenic potential of asenapine. In conclusion, asenapine carcinogenicity study in rats is considered inadequate.

Statistical review: Statistical review and evaluation was conducted by the statistical reviewer Roswitha Kelly, M.S. According to her review, pair-wise comparisons between the control and HD groups were employed for the majority of tissues. The trend tests, although more powerful, could be employed only for a few tissues examined of the terminally sacrificed LD and MD animals (adrenal glands, thyroid glands, female mammary glands). The statistical reviewer also performed approximate trend tests on tumors arising from tissues which were not microscopically examined in all groups using the software that treated unexamined tissues the same as tissues with no tumor findings. Two control groups (a vehicle control and untreated control) were not pooled in the statistical review. In conclusion, in general, findings between the statistical reviewer and the Sponsor were consistent even though the Sponsor presented grouped tumor results per tissue, whereas the reviewer presented the standard individual tumor per tissue results [please see the statistical review and evaluation for further details].

Evaluation of tumor findings: Assessments included mortality, clinical observations, gross pathology, histopathology, body weights, food consumption, and selected hematology parameters (red blood cell count and leukocyte total and differential counts). Blood samples collected from main study animals demonstrated exposure to the test article. All protocol specified tissues were retained. However, the slides were prepared and histopathological examinations were performed only on a complete set of tissues of all animals of the vehicle, untreated control and HD groups, and on all tissues from decedents. Of terminally sacrificed LD and MD animals, only slides of the thyroid glands, adrenal glands, and mammary glands (females only), and all other grossly abnormal organs and tissues (lesions, masses) were examined.

According to reviewing statistician, "taking intercurrent mortality into account, there were no statistically significant increased tumor findings among the female rats when the vehicle controls were used. When the untreated controls were employed, there were increases in adenocarcinoma in the mammary glands and in benign hair follicle tumors at the injection sites, neither one reaching the standard criteria for significance for common or rare tumors, respectively, much less any more stringent criteria had the added multiplicity been taken into account. Using the same methods of analysis, there were no statistically significant increases in tumors among the male rats, whether employing the vehicle controls or the untreated controls".

The statistical review did not include evaluation of the tumor incidences decreasing with dose. Based on available data, there is strong evidence that asenapine dose-dependently decreased the incidence of preneoplastic lesions and several types of tumors, including the diffuse C-cell hyperplasia in the thyroid gland of both genders, benign mammary tumors and pituitary pars distalis tumors in females, injection site fibromas in males, adrenal pheochromocytomas, and hystiocytic sarcomas in both sexes. The Sponsor also, based on statistical analysis, concluded that "The overall incidence of tumors was significantly reduced in both the 1.2 mg/kg/day and 5.0 mg/kg/day asenapine-treated groups", and suggested a reason: "A plausible explanation for his phenomenon is the lower body weight gain in the treated animals".

Study no.: INT00039803
Volume #, and page #: electronic submission
Conducting laboratory and location b(4)

Date of study initiation: August 30, 2000
GLP compliance: yes, excluding the statistical analysis of tumor data
QA report: yes (x) no ( ) excluding toxicokinetic & formulations analyses, and statistical analysis of tumor data
Drug, lot #, and % purity: asenapine (Org 5222), batches number: IU016-2-2 (purity 99.8%), IU016-2-3 (purity 99.8%), IU016-2-1 (purity 99.8%), and IPAI015-2 (purity 100%). Note: The batches listed above were listed in the study report. Certificates of analysis for three additional batches (IV015-2, S, and K) were attached to the report. According to the certificates, "related substances" were present at <1%. No further information regarding impurities was provided except for batches K and S, in which the impurity ______ was not tested or present at <0.05%, respectively.
CAC concurrence: No (see Appendix 1)
Methods

Doses: Dosing groups are presented in a table below:

group no.	group description	dose level (mg/kg/day) expressed as salt	dose level (mg/kg/day) expressed as active entity
1	vehicle control 1 (C1)	0	0
2	Low (LD)	0.3	0.2
3	Intermediate (MD)	1.2	0.9
4	High (HD)	3.0/5.0*	2.1/3.5*
5	untreated control 2 (C2)	0	0

*After six weeks the HD was increased to 5 mg/kg/day by increasing the volume-dose from 3 to 5 mL/kg (Note: dose levels were expressed as salt; a factor of 0.7097 should be used to express the doses as active entity).

Basis of dose selection: The HD for the carcinogenicity study was chosen based on data derived from a pilot s.c. toxicity study in rats of 13-week duration. According to the

Sponsor, asenapine was administered at dose levels of 3.0, 4.0, and 5.0 mg/kg/day in this study. Body weight gain of males was reduced by 22% to 29% in all dose groups. Body weight gain of females was increased by 12% to 16% in all dose groups, in parallel to the increased food consumption. These effects were not dose related in males and females. In addition, a decrease in the uterus weight and prominent corpora lutea were observed in females at all dose levels, and an increased development of mammary glands, prolonged diestrus, adrenal cortical hypertrophy, and thyroid follicular cell hypertrophy were observed in females at the HD. The Sponsor concluded that the endocrine organ changes and effects on body weight indicate a physiological disturbance at all dose levels. Based on these observations, the Sponsor determined that the dose of 3.0 mg/kg/day was the MTD in the 13-week study. Therefore, this dose was selected as the HD for the carcinogenicity study. The LD of 0.3 mg/kg/day was 10% of the HD. The MD of 1.2 mg/kg/day was selected as the geometric mean between the LD and MD. Long lasting (4-6 hours after dosing) prostration was observed in all asenapine-treated animals during the first six weeks of dosing. This clinical sign decreased after six weeks to reduced activity lasting also 4-6 hours after dosing. Therefore, the HD was increased from 3.0 to 5.0 mg/kg/day after six weeks of dosing in the carcinogenicity study.

Species/strain: rat/Sprague-Dawley Crl:CD®(SD)IGSBR
Number/sex/group (main study): 60/sex/group
Route, formulation, volume: subcutaneous; solution in the vehicle [a sterile, isotonic, non-pyrogenic solution of cytric acid monohydrate (9.4 mg/mL), sodium hydrogen phosphate (14.5 mg/mL) and NaOH, for pH adjustment]; volume: at initiation of treatment, the asenapine groups each received dose preparations at a volume-dose of 3 mL/kg. After six weeks the dose of 3 mg/kg was increased to 5 mg/kg/day by increasing the volume-dose from 3 to 5 mL/kg.
Frequency of dosing: Asenapine was administered daily subcutaneously via four sites (left and right shoulder, left and right flank); Due to thickening of the injection sites, particularly in the HD animals, the injection sites were redefined as the left and right front dorsal quadrant, and the left and right rear dorsal quadrant.
Satellite groups used for toxicokinetics or special groups: none
Age: approximately 7 weeks old at the initiation of treatment
Animal housing: The protocol indicated that the animals will be housed in groups of five in stainless steel mesh cages. There was no further information in the study report.
Restriction paradigm for dietary restriction studies: N/A
Drug stability/homogeneity: Drug stability data were provided only for few of several drug substance batches. Pretest, interim and final analysis of the active preparations was conducted. According to the Sponsor, the formulations complied with the pharmaceutical standards and the parameters tested were within specifications. Deviations were not thought to have adversely affected the integrity of the study.
Dual controls employed: Group 1 animals received the vehicle (see above); Group 5 animals remained untreated.
Interim sacrifices: none
Deviations from original study protocol: The study was designed to administer asenapine to the rat for at least 104 weeks. However, due to high mortality rate in the female control group, the females were terminated in Weeks 100 to 102. After six weeks

the HD was increased from 3.0 to 5.0 mg/kg/day by increasing the volume-dose from 3 to 5 mL/kg. According to the Sponsor, none of the additional minor deviations (for example, all dose preparations were below range during Week 24; very low level of asenapine was detected in the pump and vacuum filtered placebo preparations) affected the integrity or outcome of the study.

Observation times

Mortality: Animals were observed twice daily for mortality/morbidity.
Clinical signs: Routine health checks were performed twice daily. Postdose observations were performed on 20 animals/sex/group (in rotation) daily for the first 8 weeks of dosing and weekly thereafter immediately after dosing and at 0.5, 2 and 4 hours after dosing. Physical examinations, including palpation, were conducted weekly.
Body weights: Body weights were examined before treatment on the first day of dosing, at weekly intervals for 16 weeks, once every 4 weeks thereafter and before necropsy in Week 96 for females and Week 104 for males.
Food consumption: Food consumption was determined weekly for the first 16 weeks and once every 4 weeks thereafter.
Hematology: Blood samples were collected from all animals at termination and where possible from decedents for determination of red blood cell counts, and leukocyte total and differential counts.
Gross pathology: Necropsy was performed on females in Weeks 100-102 and in males in Weeks 106-107. The following protocol specified tissues were preserved: blood sample, bone marrow smear (femur), eyes, optic nerves, Harderian glands, skin, mammary, muscle, femur with bone marrow, sternum with bone marrow, sciatic nerve, liver, spleen, pancreas, mesenteric lymph nodes, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, gall bladder, adrenals, kidney, testes, epididymides, ovaries, seminal vesicles, urinary bladder, prostate, uterus, vagina, salivary gland, mandibular lymph nodes, thymus, lungs, heart, aorta, trachea, esophagus, tongue, thyroids, parathyroids, larynx, pituitary, brain, spinal cord cervical, spinal cord thoracic, spinal cord lumbar, lacrimal glands, zymbal glands, bronchial lymph nodes, trachea bifurcation, head, nasal turbinates, nasopharynx, injection sites, tissue masses, and gross lesions.
Histopathology: Peer review: yes (x), no ( ). A consensus was obtained in all cases. Hematoxilin and eosin-stained paraffin slides of the tissues/organs listed above of all animals of all groups were prepared. Histopathological examinations were performed on a complete set of these tissues of all animals of the vehicle, untreated control and HD groups, and on all decedents in the LD and MD groups. Of terminally sacrificed animals of the LD and MD groups, slides of all adrenal glands, thyroid glands, mammary glands (females only), and all other grossly abnormal organs and tissues were also prepared and examined. Bone marrow smears were prepared at necropsy but not examined.
Toxicokinetics: Blood samples were collected from the lateral caudal vein from the LD, MD and HD animals using 3 animals/group/sex on day 1 and in Weeks 26, 85 and 97 at the following time points: 0.25, 0.5, and 1 hour after dosing from the lateral caudal vein.

Results

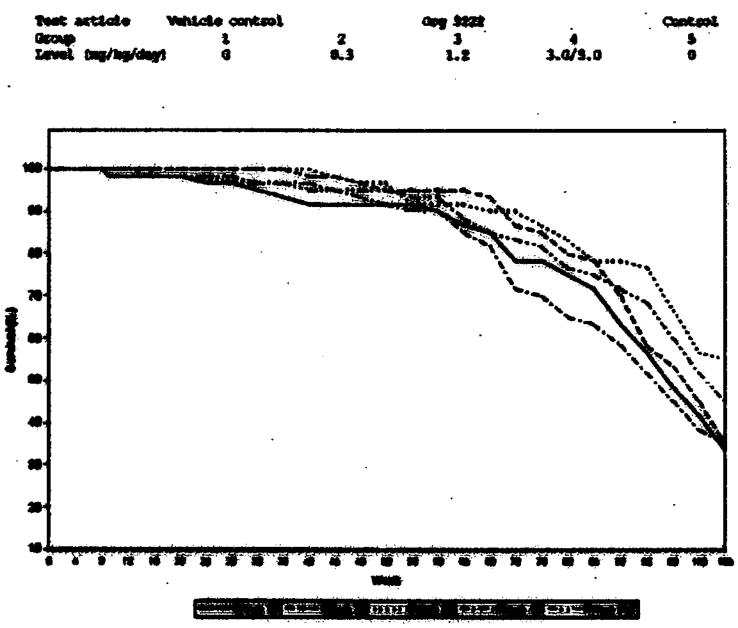
Mortality: A total of 185 males and 187 females died or were killed early during the study. Due to high mortality rate in the vehicle control group, the females were terminated in weeks 100 to 102. In females, the survival in all treatment groups was significantly better than that in the vehicle control group without relation to the dose level. The males were terminated in weeks 106 to 107. In males, the survival of the vehicle control and the HD group was the same. Mortality of males in the MD group was significantly lower than that in the vehicle control group. There were more than 50% of animals alive in Week 90 in both sexes except the females vehicle control group, in which the survival rate was 33% [20/60]. The distribution of decedents among the treatment groups at termination is shown in the following Sponsor's table:

	Group and sex									
	1M	2M	3M	4M*	5M	1F	2F	3F	4F*	5F
Dose level (mg/kg/day):	0	0.3	1.2	3/5	0	0	0.3	1.2	3/5	0
Number animals/group:	60	60	60	60	60	60	60	60	60	60
Number of decedents:	41	40	27	41	36	45	39	33	36	34

* The high dose group was started with 3.0 mg/kg/day and increased to 5.0 mg/kg/day after 6 weeks.

Therefore, 19, 20, 33, 19, and 24 males in the vehicle control, LD, MD, HD, and untreated control groups, respectively, survived to the study termination in Weeks 106-107, and 15, 21, 27, 24, and 26 females in the vehicle control, LD, MD, HD, and untreated control groups, respectively, survived to the study termination in Weeks 100-102. The Sponsor's figures illustrating the percent survival of the animals over the course of the carcinogenicity study are shown below:

Group mean survival - males



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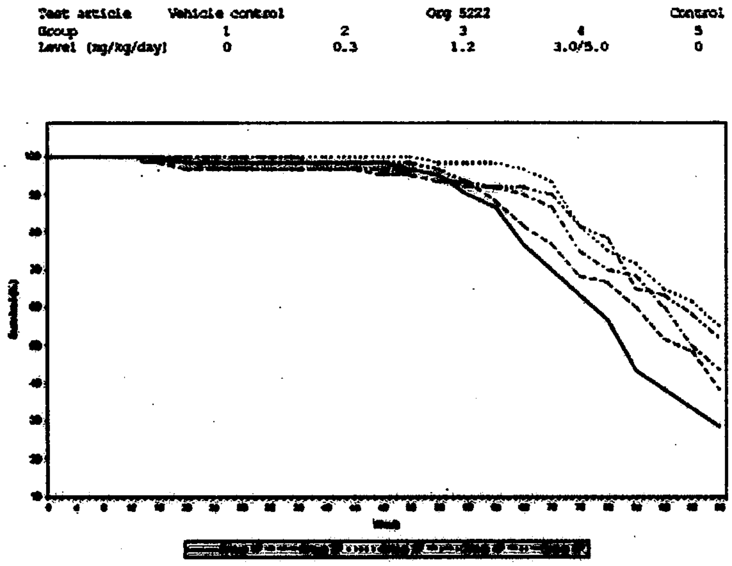
Group mean survival - females



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The incidence of deaths due to neoplastic lesions was clearly decreased with the increasing dose in both genders. Factors contributing to mortality during this study are summarized in the following Sponsor's table.

Group Incidences of Causes of Morbidity and Mortality (provided by Sponsor):

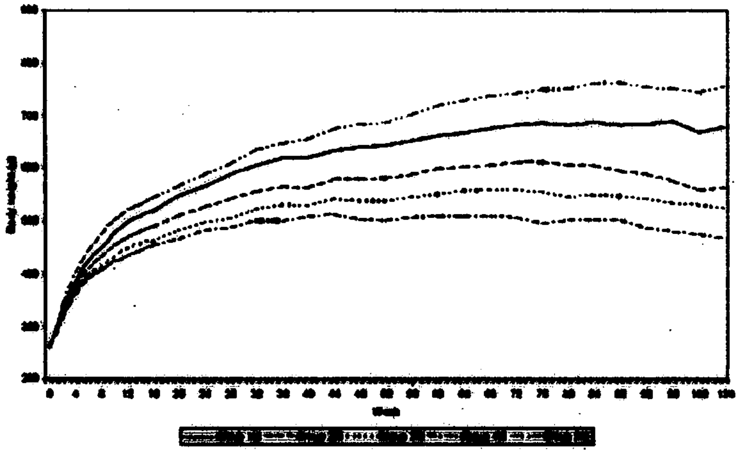
Sex	MALES					FEMALES				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	0.3	1.2	3.0	-	0	0.3	1.2	3.0	-
Number of animals	60	60	60	60	60	60	60	60	60	60
Factors contributing to death/morbidity — Number of deaths	41	40	27	48	34	45	39	35	36	34
- Clinical observation(s) (killed for humane reasons)	23	21	16	28	13	22	12	17	23	13
- Traumatic lesion(s)				1				1		
- Haematological disease (anaemia)						1				
- Non-neoplastic lesions in respiratory system		1	1	1						
- Non-neoplastic lesions in digestive system							1			
- Non-neoplastic lesions in urinary system			1	2	1		1			
- Non-neoplastic lesions in cardiovascular system	1			1		1	1			
- Non-neoplastic lesions in nervous system						1	1		2	
- Combined non-neoplastic lesions							2			
- Neoplastic lesion(s)	14	13	5	4	16	20	19	15	10	17
- Combined neoplastic & non-neoplastic lesions							1			1
- Not determined	2	3	4	12	4		1		1	3

Note: In the case a clear macroscopic factor contributing to death/morbidity was found, this factor overruled the clinical- and/or macroscopical findings

Clinical signs: The incidence of clinical signs of fur staining increased slightly in MDM and HDM between Weeks 37 and 76 of the study. Beginning approximately at Week 44 until the end of the study, the incidence of fur thinning was consistently slightly to moderately increased in MD and HD animals. Sores and lesions were observed more frequently in HDM, mainly in the second year of the study. Hypoactivity was observed in all groups administered asenapine. Partially closed eye lids (eye squinting) were noted in all MD and HD animals. These signs were observed at 30 min, 2, and 4 hours after dosing starting from day 2. The duration of hypoactivity in LD animals became less after the first 25 days of the treatment until it was not longer observed from Day 552 of the treatment. According to the Sponsor, there was no pattern to the incidence of detection of palpable masses to indicate an effect of treatment with asenapine. However, the reviewer notes a slight decrease in the incidence of small movable masses in HDM and HDF, and a decrease in small stationary masses in MDM, HDM, and HDF. The incidence of large stationary masses was also slightly decreased in HDF in the second year of the study.
Body weights: Group mean body weight gain was decreased in all groups administered asenapine when compared to that of each control group. Male rats gained 73%, 63%, or 48% and female rats gained 91%, 79%, or 59% of the vehicle control mean group weight at the LD, MD, and HD, respectively. The overall body weight gain of the untreated control group was 19% higher for males and 6% higher for females than that of the vehicle control group. Group mean body weight at termination was decreased in all asenapine-treated groups. In males, body weight was decreased by 17%, 23%, and 31% at the LD, MD, and HD, respectively, when compared to the vehicle control group in Week 104 (for comparison, these decreases were 10%, 16%, and 22% in Week 52 at the LD, MD, and HD, respectively). In females, body weight was decreased by 4%, 11%, and 23% at the LD, MD, and HD, respectively, when compared to the vehicle control group in Week 96 (for comparison, these decreases were 0%, 1%, and 10% in Week 52 at the LD, MD, and HD, respectively). The overall body weight of the untreated control group was 11% higher for males and 5% higher for females than that of the vehicle control group.
The Sponsor's figures illustrating changes in body weight of the animals over the course of the carcinogenicity study are shown below:

Group mean body weights - males

Test article	Vehicle control		Org 5222		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	0.3	1.2	3.0/5.0	0



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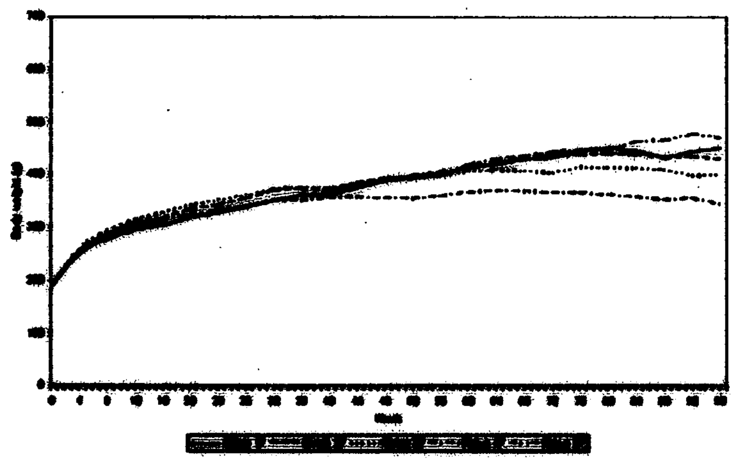
Group mean body weights - females

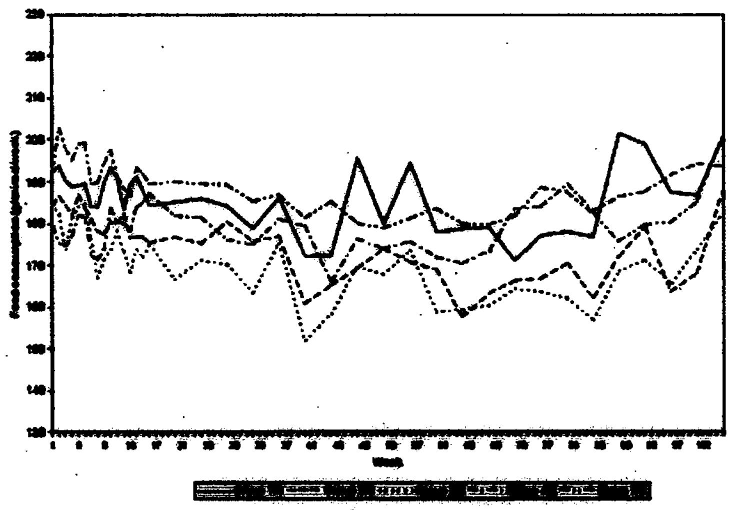
Test article	Vehicle control		Org 5222		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	0.3	1.2	3.0/5.0	0



Food consumption: Food consumption was decreased in LD and MD males, and in MD females, when compared to the vehicle control group. This decrease was most obvious in the second year of the study. The Sponsor's figures illustrating changes in food consumption over the course of the carcinogenicity study in male and female rats are shown below:

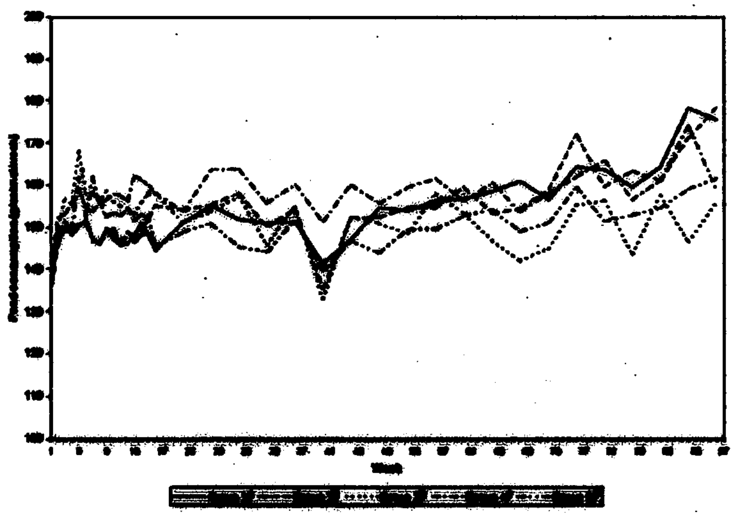
Group mean food consumption - males

Test article	Vehicle control		Org 3222		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	0.3	1.2	3.0/5.0	0



Group mean food consumption - females

Test article	Vehicle control		Org 3222		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	0.3	1.2	3.0/5.0	0



Hematology: Red blood cell count was slightly lower in HD females when compared with those in the control groups. There were no other test article-related changes.
Gross pathology: The incidence of the following observations was increased: pale focus/area in the lungs of MD males, HD males, and HD females, thickening of the

injection sites in HD females, enlarged pituitary in both sexes at all dose levels and tail sores in HD females. The decreased incidence of large and pale liver in both sexes at all dose levels and mottled liver in both sexes at the HD was observed. Other tissues were histologically unremarkable.

Histopathology:

Non-neoplastic findings: Test article-related increased incidence of non-neoplastic findings was noted in the lungs, adrenal glands, thyroid glands and at the injection site. An increased incidence of mild aggregation of foamy macrophages was observed in the lungs of MD and HD males, and in HD females. Since in the LD and MD only 41 to 50 animals/group that died/were terminated early had been examined, therefore, the NOAEL could not be determined. This finding correlates with pale areas and/or foci noted on the lungs of animals in these groups at necropsy. The data are shown in the Sponsor's table below:

Selected non-neoplastic findings in the lungs

	Sex	MALES					FEMALES				
	Group	1	2	3	4	5	1	2	3	4	5
	Dose (mg/kg/day)	0	0.5	1.2	3.0	-	0	0.5	1.2	3.0	-
FINDING(S)	No. examined	60	50	44	60	60	60	46	41	60	60
- aggregation of foamy macrophages		10	11	16	24	14	11	5	11	23	7

An increased incidence of cystic/hemorrhagic (cortical) degeneration of the adrenal glands was observed in MD and HD males. There were no clear changes in females. These findings are shown in the Sponsor's table below:

Selected non-neoplastic findings in the adrenal glands

	Sex	MALES					FEMALES				
	Group	1	2	3	4	5	1	2	3	4	5
	Dose (mg/kg/day)	0	0.5	1.2	3.0	-	0	0.5	1.2	3.0	-
FINDING(S)	No. examined	60	50	60	60	60	60	60	55	60	60
- Cystic / hemorrhagic degeneration	Grade 1				2	1	3	4	2		5
	2	2	9	7	16	1	17	15	13	24	16
	3	7	5	14	13	2	17	16	20	30	22
	4	4	2	4	7	3	15	11	13	3	7
	Total	13	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Key: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked

An increased incidence of follicles with hypertrophied epithelium (and inspissated colloid within the follicular lumen) was observed in the thyroid glands from all asenapine-treated males and from HD females. The stimulation of the thyroid gland was considered by the Sponsor to be mild. There was no increase in follicular hyperplasia or thyroid follicular tumors in this study. A decreased incidence and severity of diffuse and focal C-cell hyperplasia was noted in the thyroid glands of the MD and HD animals. These findings are shown in the Sponsor's table below:

Selected non-neoplastic findings in the thyroid gland

	Sex	MALES					FEMALES				
	Group	1	2	3	4	5	1	2	3	4	5
	Dose (mg/kg/day)	0	0.3	1.2	3.6	-	0	0.3	1.2	3.6	-
FINDING(S)	No. examined	60	60	60	60	60	60	60	59	60	60
- Follicles with hypertrophied epithelium (and inspissated colloid)	Distribution: A	3	8	11	4	6	3	1	3	13	5
	D	6	8	14	19	4	1	2	6	14	3
	Total	9	16	25	23	10	4	3	9	27	8
- Hyperplasia, C-cell, diffuse	Grade: 2	18	17	10	4	16	26	24	14	12	19
	3	4	9	3		5	14	7	2	3	15
	4					1	4				3
	Total	22	26	13	4	22	44	31	16	15	37

Key: A= areas of, D= diffuse, grade1 = minimal, 2 = slight, 3 = moderate, 4 = marked

A dose-dependent increase in severity of the thickness of the subcutaneous layer or fibrosis (that was often accompanied with hemorrhages and/or hematomas and aggregation of pigmented macrophages) as a reaction to the repeated injection procedure was observed in asenapine-treated animals at the injection sites.

Neoplastic findings: There were no statistically significant increased tumor findings among the female rats when the vehicle controls were used. When the untreated controls were employed, there were increases in adenocarcinoma in the mammary glands and in benign hair follicle tumors at the injection sites, neither one reaching the standard criteria for significance for common or rare tumors, respectively. The incidence of the mammary gland adenocarcinoma in female rats was 23, 18, 23, 25, and 11 in the vehicle control, LD, MD, HD, and untreated control groups, respectively. There was a positive trend for mammary adenocarcinomas in females based on the Sponsor's analysis involving the combined controls. According to the Sponsor, this finding "is considered not to be suggestive of a true effect" based on the lack of dose relationship within the treated groups and the evidence of a negative trend of asenapine effects on the benign mammary gland tumors. Using the same methods of analysis, there were no statistically significant increases in tumors among the male rats, whether employing the vehicle controls or the untreated controls.

The incidence of several benign and malignant tumors was decreased at the MD and HD when compared to the control groups, including benign mammary tumors (mainly fibroadenomas) in female groups, pituitary pars distalis tumors and adrenal phaeochromocytomas in both sexes, and fibromas and histiocytic sarcomas at the injection sites in male rats. The decrease in the incidence reached a statistical significance in tests conducted by the Sponsor using combined control groups (as indicated in the statistical review, based on the standard approach in the FDA Office of Biostatistics, only increased tumor incidences were statistically evaluated by the reviewing statistician). The incidences of all benign and malignant tumors are summarized in the following Sponsor's table:

Benign tumours

Group	1		2		3		4		5	
Dose (mg/kg/day)	0		0.3		1.2		5.0		-	
Sex	M	F	M	F	M	F	M	F	M	F
Adrenal gland(s)	8	4	11		8	1	3	3	9	6
Brain	1			1				1		2
Eye(s)				1						
Harderian gland(s)	5	1	5	1	3	1	1	4	5	1
Kidney(s)	1					1				
Liver		1		2		1		1		
Lungs & bronchi	1									
Lymph node(s)			2			1	1	1	2	
Mammary gland(s)		47	1	24		32		22		44
Oral cavity & related structures	1								1	
Ovaries								1		1
Pancreas	2		3		1	2	1	1	2	1
Parathyroid gland(s)	5	1	2		1		3	1	4	
Pituitary gland	18	42	24	37	23	38	28	27	30	39
Skin	2	2	2		1		1		7	2
Soft tissues		1	2	1	5	3	2		3	2
Testes	3								2	
Thymus		1				2		2		
Thyroid gland(s)	9	4	4	7	10	4	3	4	6	2
Urinary bladder						1				
Uterus		3		2		4		2		4
Vagina		1		4		3		4		7
TOTAL No. OF BENIGN TUMOURS	56	108	56	80	52	86	35	73	71	111

Malignant tumours

Group	1		2		3		4		5	
Dose (mg/kg/day)	0		0.3		1.2		5.0		-	
Sex	M	F	M	F	M	F	M	F	M	F
Adrenal gland(s)	2	2		2	2	1	1	1	2	
[illegible]	1		1						1	
Brain	1	1					1	1	1	1
[illegible]	8	3	3	2		1	3		1	1
[illegible]	10	2	14	4	11	3	6	2	4	
[illegible]				1						
Liver		1							1	
Mammary gland(s)		45		22		40		31		20
[illegible]									1	
Oral cavity & related structures				1		1				1
[illegible]		1		1	1		1	1	2	
[illegible]									1	
[illegible]				1		1				1
[illegible]	1									
[illegible]									1	
Skin			1						2	
Soft tissues	3		4	1	1		2		2	
[illegible]	1									
[illegible]				1						
Thyroid gland	2	1	3	2	1	1	1	1	2	
[illegible]		1								
[illegible]						1				
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TOTAL No. OF BENIGN AND MALIGNANT TUMOURS	93	165	82	122	70	135	50	117	94	135

A summary of the incidence of all, benign and malignant tumors in rats is shown in the following table:

Tumor type	Group incidence of tumors in rats									
	Male groups					Female groups				
	1 (C1)	2 (LD)	3 (MD)	4 (HD)	5 (C2)	1 (C1)	2 (LD)	3 (MD)	4 (HD)	5 (C2)
all	93	82	70	50	94	165	122	135	117	135
benign	56	56	52	35	71	108	80	86	73	111
malignant	37	26	18	15	23	57	42	49	44	24

Moreover, according to the reviewing statistician, there is a statistically significant lower number of tumor bearing animals in the HD female rats (46/60) compared to the vehicle controls (57/60) (p=0.0044, two-sided, significant against alpha=0.05) and in the HD male rats (29/60) compared to the vehicle controls (42/60) (p=0.0164, two-sided, significant compared to alpha=0.05). [test was performed two-sided because a priori we would not know which group would have higher number of tumor-bearing animals].
Toxicokinetics: Asenapine was detected in the plasma of animals receiving all dose levels. Generally, plasma levels increased in proportion to the dose. The maximum concentrations were observed within 0.5 hours of dosing. When compared with single dosing, plasma levels were slightly higher (or similar) after multiple dosing for 26 or 85 weeks; these levels were up to 2-fold higher in week 97. There were no toxicologically important differences in plasma concentrations between males and females.
Mean plasma levels of asenapine in rats in the carcinogenicity study are shown in the following Sponsor's table:

	Mean concentration ($ng.mL^{-1}$) [n=3]							
	Low dose*: 0.2 $mg.kg^{-1}$ once daily							
	Single dose		Week 26		Week 85		Week 97	
Protocol time (h)	M	F	M	F	M	F	M	F
0.25	16.7	11.3	21.1	23.0	21.4[a]	23.3[b]	34.0	25.2
0.5	14.9	22.4	17.6	16.1	19.3	19.8	24.6	20.1
1	9.71	8.37	14.0	14.2	14.5[b]	-[c]	22.6	19.2
	[illegible]							
	Single dose		Week 26		Week 85		Week 97	
	M	F	M	F	M	F	M	F
0.25	65.1	46.1	89.9	65.2	73.5	73.1	132	103
0.5	61.4	50.9	89.9	52.8	72.6	51.1	130[a]	80.1[a]
1	37.5	32.9	59.0	31.7	44.0	37.3	68.2	64.4
	High Dose*: [illegible] $mg.kg^{-1}$ once daily							
	Single dose		Week 26		Week 85		Week 97	
	M	F	M	F	M	F	M	F
0.25	135	119	199	198	182	138	298	282
0.5	129	105	215	215	204	136	317	354
1	86.9	86.9	148	123	136	120	235	218

key: F=female, M=male
[a] n=2; [b] n=1; [c] all values below LLOQ
* expressed as active entity

According to the Sponsor, sparse toxicokinetic sampling applied to demonstrate exposure did not allow an estimation of the AUC and C_{max} in the rat carcinogenicity study. Based on the highest concentrations of asenapine achieved at 0.5 h in Week 97 in males and females administered the high dose of 3.5 mg/kg/day (AE) (317 ng/mL and 354 ng/mL, respectively), safety margin calculated relative to the C_{max} of 6.56 ng/mL obtained at the maximum recommended dose of 10 mg b.i.d. in humans is 51. However, the reported animal plasma concentrations may not correspond to the C_{max} values. Therefore, toxicokinetic data from the 13-week s.c. study in rats were used to estimate multiple dose AUCs in this study. Safety margins were calculated by the Sponsor based on "the average dose-normalized AUC and C_{max}" values in animals relative to the maximum recommended human dose of 10 mg b.i.d. and the associated AUC_{0-24} of 86.8 ng•h/mL (twice the AUC_{0-12}) and C_{max} of 6.56 ng/mL. The following safety margins were provided by the Sponsor:

Species	Dose (mg/kg)	Safety margin			
		mg/kg-based	AUC-based	C_{max}-based	mg/m^2-based
Rat (M and F)	5 (3.5 AE)	11	8	47 (38)*	1.7

* estimated from the carcinogenicity study

2. Study title: Org 5222: 104 week subcutaneous administration oncogenicity study with Org 5222 in the mouse.

Key study findings: Subcutaneous administration of asenapine maleate at 0.5, 1.5, and 5.0/4.0 (HD in males) or 7.5/5.0 (HD in females) mg/kg/day to male (M) mice for up to 89 weeks and to female (F) mice for up to 98 weeks demonstrated equivocal evidence of carcinogenicity. Although statistically significant evidence of tumorigenic potential was noted in animals of both genders, the significance depended on which control group was employed. The incidence of pleomorphic malignant lymphomas in the hemolymphoreticular system was statistically significantly increased in the female mice at the HD compared to the vehicle control. However, the incidence of this tumor at the HD was similar to that in the untreated controls (20 versus 22 animals). The incidence of interstitial cell adenomas in the testes in males was statistically significantly increased in the HD group when compared to the untreated controls. However, the incidence of this tumor was no longer statistically significant compared to the vehicle controls.

Adequacy of the carcinogenicity study and appropriateness of the test model:
This study was designed to assess the carcinogenic potential of asenapine following subcutaneous administration to male and female CD-1 mice for 104 weeks. Mice were selected based on recommendations of applicable guidelines and the available background data for this species. Subcutaneous injection was chosen as an appropriate alternative for the intended sublingual clinical route because this route of administration is not feasible in rodents. A sufficient number of animals (57-60/sex/group) was used in the study. Mice were treated with asenapine at dosages that were not previously concurred with by the Executive CAC due to the lack of sufficient information (see Attachment 1).

The study was modified when compared to the original protocol. The original HD levels had to be reduced during Week 25 to 5.0 and 4.0 mg/kg/day for females and males, respectively, due to high morbidity and mortality. For the same reason, dosing was stopped in MD and HD males in Week 88, and in MD and HD females in Weeks 95 and 97, respectively. All these animals were maintained treatment-free until termination. All groups were terminated early due to increased mortality among all asenapine-treated groups. The males were terminated in Weeks 89/90 and the females in Weeks 98/99. In general, a 50% survival rate to Weeks 80 to 90 of the 50 initial animals (or 20-30 surviving animals) in any treatment group is considered adequate. The lowest number of surviving males (16) was observed in the MD male group. However, 27 MD males entered Week 81 of the study. The lowest number of surviving females (15) was observed in the HD female group. However, 21 HD females entered Week 81 of the study. Therefore, the number of animals of both sexes surviving in this study to Week 80 was sufficient for an adequate study.

The Sponsor concluded that a variety of findings including skin/appendage lesions, uro-genital tract lesions, gastro-intestinal lesions and hemolymphoreticular tumors contributed to morbidity and death. However, the treatment-related increase in mortality could not be attributed to a single factor. Hemolymphoreticular tumors had been found on the average to be the most frequent cause of deaths or ill-being of male and, to a larger extent, in female mice in carcinogenicity studies reaching 31.5% and 52.6% of the cases with clear cause of deaths in male and female mice, respectively (Ettlin et al., Toxicologic Pathology, Vol. 22, No 2, 1994).

Statistical review: Statistical review and evaluation was conducted by Roswitha Kelly, M.S. The reviewing statistician used the same approach of analysis as was employed for the rat study. According to her review, pair-wise comparisons between the control and HD groups were employed for the majority of tissues. The trend tests, although more powerful, could be employed only for a few tissues (e.g. heart) examined in the terminally sacrificed LD and MD animals. The reviewing statistician also performed approximate trend tests on tumors arising from tissues which were not microscopically examined in all animals using the software that treated unexamined tissues the same as tissues with no tumor findings. The two control groups (a vehicle control and untreated control) were not pooled by the reviewer for statistical purposes. In conclusion, in general findings between the statistical reviewer and the Sponsor were consistent even though the Sponsor presented grouped tumor results per tissue, whereas the reviewer presented the standard individual tumor per tissue results [please see the statistical review and evaluation for further details].

Evaluation of tumor findings: Assessments included the incidence and morphology of tumors, mortality, clinical observations, gross pathology, and histopathology. Body weights, food consumption and selected hematology parameters (red blood cell count and leukocyte total and differential counts) were also evaluated. Blood samples collected from satellite and main study animals demonstrated exposure to the test article. According to the Sponsor, all protocol specified tissues were retained. However, the slides were prepared and histopathological examinations were performed only on a complete set of tissues of all animals of the vehicle, untreated control and HD groups, and on all tissues from decedents. Of terminally sacrificed LD and MD animals, only slides of the heart and all other grossly abnormal organs and tissues (lesions, masses) were examined.

There were no macroscopic or microscopic findings indicating local or systemic carcinogenicity due to asenapine in female mice except for pleomorphic malignant lymphomas in the hemolymphoreticular system. The incidence of these tumors was 2, 2, 6, 14, and 14 in the vehicle control, LD, MD, HD, and untreated control group, respectively. According to the statistical review, the pair-wise comparison showed statistically higher incidences for these malignant lymphomas among the HD female group versus the vehicle control with a p-value of 0.000. The reviewing statistician also indicated that the trend test for this tumor was significant at p=0.0002. Since not all tissues for the LD and MD were microscopically examined, trend test results are approximate. In addition to pleomorphic lymphomas, lymphoblastic, lymphocytic and NOS lymphomas were also observed. The incidence of combined malignant lymphomas in female mice was 7, 4, 8, 20, and 22 in the vehicle control, LD, MD, HD, and untreated control group, respectively. The incidence of combined lymphomas in HD females increased by 186% when compared to the vehicle control group (20 at the HD versus 7 in the vehicle control). The Sponsor's statistical comparison was based on all combined malignant lymphomas in the hemolymphoreticular system and resulted in similar finding (p-value of 0.001). Although the incidence of hemolymphoreticular tumors (and the incidence of deaths due to these tumors) increased at the HD in female mice, it is unclear whether this finding is test article-related because similar incidence (22) was observed in the combined untreated control group. Moreover, according to the Sponsor, the incidence of lymphomas was within the historical control data for this laboratory (the historical control data was provided by the Sponsor upon the reviewer's request). A minimal increase in malignant lymphomas in males did not reach statistical significance.

Based on the data sets available to the reviewing statistician for female mice, there were a total of 45 tumors of any kind among the vehicle controls and a total of 54 tumors of any kind among the untreated controls. To compare tumor rates between these two control groups, the reviewer used the number of tumor bearing animals. In the number of tumor bearing animals, there is no statistical difference between the two control groups of the female mice, indicating that a vehicle effect on total number of tumors is unlikely.

The incidence of interstitial cell adenomas in the testes in males was statistically significantly increased using the untreated controls. However, the incidence of this tumor was no longer statistically significant using the vehicle controls (1, 0, 0, 3, and 0 in the vehicle control, LD, MD, HD, and untreated control group, respectively). Historical control data have not been provided by the Sponsor.

Study no.: NL0050405
Volume #, and page #: electronic submission
Conducting laboratory and location:

Date of study initiation: August 15, 2000 b(4)
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: asenapine (Org 5222), batches number: IU016-2-2 (purity 99.8%), IU016-2-3 (purity 99.8%), IU016-2-1 (purity 99.8%), and IPAI015-2 (purity 100%). Note: The batches listed above were listed in the study report. Certificates of analysis for three additional batches (IV015-2, S, and K) were attached to the report. According to the certificates, "related substances" were present at <1%. No further information regarding impurities was provided except for batches K and S, in which the impurity was not tested or present at <0.05%, respectively.
CAC concurrence: No (see Appendix 1)
Methods

Doses:

group number	group description	dose level (mg/kg/day) expressed as salt	dose level (mg/kg/day) expressed as active entity
1	vehicle control (C1)	0	0
2	low (LD)	0.5	0.35
3	intermediate (MD)	1.5	1.06
4	High (HD)	Males: 5.0/4.0* Females: 7.5/5.0*	Males: 3.5/2.8* Females: 5.3/3.5*
5	untreated control (C2)	0	0

*From Week 25 the HD was reduced from 5.0 mg/kg to 4.0 mg/kg for males and from 7.5 mg/kg to 5.0 mg/kg/day for females (Note: dose levels were expressed as salt; a factor of 0.7097 should be used to express the doses as active entity).

Basis of dose selection: The HD for male mice was chosen based on data derived from a pilot s.c. toxicity study of 13-week duration. According to the Sponsor, data obtained in this study indicated a risk of death in males treated at 7.5 mg/kg/day. Therefore, a HD of 5 mg/kg/day was chosen for a carcinogenicity study as the MTD. In females, the dose limiting toxicity was not determined. However, the maximum feasible concentration (1 mg/ml) and the maximum feasible injection volume of 7.5 mL/kg limited the dose level selected for females to a HD of 7.5 mg/kg. The LD of 0.5 mg/kg/day was 10% (males) and 6.7% (females) of the HD. The MD of 1.5 mg/kg/day was selected as the geometric mean between the LD and HD.
Species/strain: mouse/Crl:CD-1(ICR)BR (males: 24.2 - 37.5 g; females: 18.4 - 31.2 g)
Number/sex/group (main study): 57/sex/group in groups 1, 2, 3, and 5; 60/sex in the HD group
Route, formulation, volume: subcutaneous; solution in the vehicle [a sterile, isotonic, non-pyrogenic solution of cytric acid monohydrate (9.4 mg/mL), sodium hydrogen phosphate (14.5 mg/mL), NaOH for pH adjustment]; volume: 7.5 mL/kg
Frequency of dosing: Animals were dosed by subcutaneous injection daily, rotating 3 sites (neck, left hip, and right hip). Due to high morbidity/mortality, dosing ceased in MD and HD males at Week 88, in MD females at Week 95, and in HD females at Week 97. All these animals were maintained treatment-free until termination.
Satellite groups used for toxicokinetics or special groups: 18 animals/sex/group administered the LD, MD, and HD (additional toxicokinetic data were obtained from the main study animals before their scheduled necropsy).
Age: approximately 7 weeks old at the initiation of treatment
Animal housing: The animals were housed in groups of three in cages.
Restriction paradigm for dietary restriction studies: N/A
Drug stability/homogeneity: Stability and homogeneity analysis was not performed for each batch of test article used on the study as formulation analysis samples were only taken from lots 1, 2, and 3. The analysis of the test article was conducted prior to start.
Dual controls employed: Initially, two control groups (57 animals/sex/group) received the vehicle. However, during Week 2 the treatment of group 5 was stopped. Group 1 animals received the vehicle (see above); Group 5 animals remained untreated.
Interim sacrifices: no

Deviations from original study protocol: Due to high morbidity/mortality, dosing ceased in MD and HD males at Week 88, in MD females at Week 95, and in HD females at Week 97. All these animals were maintained treatment-free until termination. Both the male and female studies were terminated early due to increased mortality among all asenapine-treated groups. The males were terminated in Weeks 89/90 and the females in Weeks 98/99. According to the Sponsor, none of other deviations from the original study protocol affected the integrity or outcome of the study.

Observation times

Mortality: Animals were observed twice daily for morbidity and mortality.

Clinical signs: Postdose observations were performed immediately after dosing and at 0.5, 2, and 4 hours after dosing. Initially all animals were observed daily. From Week 9, only 20 animals/sex/group (in rotation) were observed weekly. Physical examinations including palpation were conducted weekly.

Body weights: Body weights were examined once before treatment on the first day of dosing, at weekly intervals for 16 weeks, once every 4 weeks thereafter and before necropsy.

Food consumption: Food consumption was determined weekly for the first 16 weeks and once every 4 weeks thereafter.

Hematology: Blood samples were collected from all animals at termination for determination of the red blood cell count and leukocyte total and differential counts.

Gross pathology: Necropsies was performed on toxicokinetic animals in Week 27, on all main study males in Week 89, and on all main study females in Weeks 98/99. The following protocol specified tissues were preserved: blood sample, bone marrow smear (femur), eyes, optic nerves, Harderian glands, skin, mammary, muscle, femur with bone marrow, sternum with bone marrow, sciatic nerve, liver, spleen, pancreas, mesenteric lymph nodes, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, gall bladder, adrenals, kidney, testes, epididymides, ovaries, seminal vesicles, urinary bladder, prostate, uterus, vagina, salivary gland, mandibular lymph nodes, thymus, lungs, heart, aorta, trachea, esophagus, tongue, thyroids, parathyroids, larynx, pituitary, brain, spinal cord cervical, spinal cord thoracic, spinal cord lumbar, lacrimal glands, zymbal glands, bronchial lymph nodes, trachea bifurcation, head, nasal turbinates, nasopharynx, injection sites, tissue masses, and gross lesions.

Histopathology: Peer review: yes (x), no ( ). A consensus was obtained in all cases. The following tissues were embedded in a paraffin wax, sectioned and stained with hematoxilin and eosin: all protocol listed tissues (see above) from the control groups and HD animals of both sexes (Groups 1, 4, and 5), the heart from all control and treated animals, all protocol tissues from decedents and gross lesions and tissue masses seen in all animals at necropsy.

Toxicokinetics: Blood was collected for toxicokinetic assessments from the satellite animals (18 animals/sex/group administered the LD, MD, and HD) on Day 1 and in Week 27. Necropsies were performed in Week 27 on the satellite animals. Further toxicokinetic data were obtained from the main study animals before their scheduled necropsy.

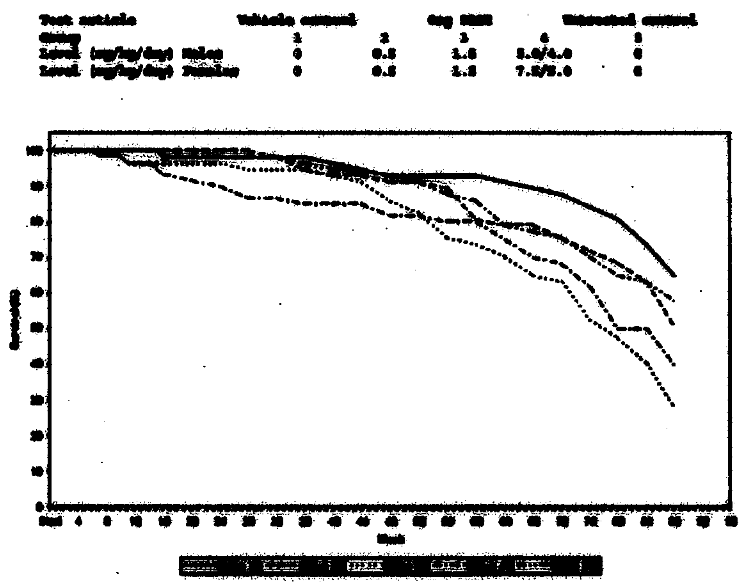
Results

Mortality: A total of 149 males and 160 females died or were killed during the study. The incidence of morbidity/mortality in general increased with the increasing dose in animals of both sexes (except for HD males). These findings are reflected in statistically significant test for linear trend. The distribution of animals that died early among the treatment groups is shown in the following Sponsor's table:

	Group and sex									
	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Number animals/group:	57	57	57	60	57	57	57	57	60	57
Number of decedents:	20	28	41	36	24	16	28	38	45	33

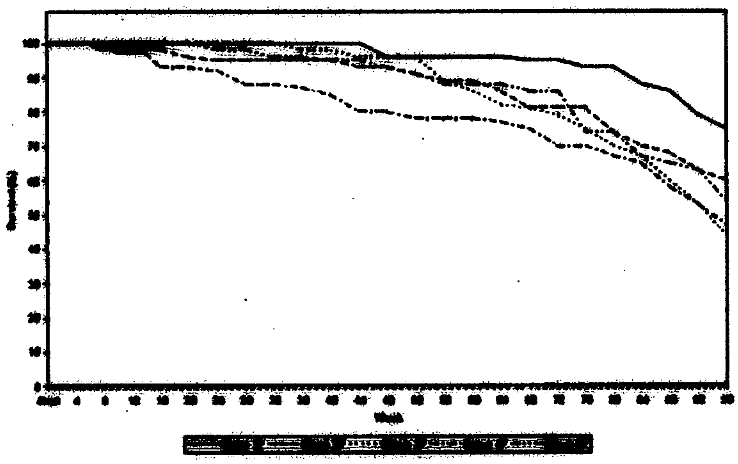
Therefore, 37, 29, 16, 24, and 33 males, and 41, 29, 19, 15, and 24 females in groups 1, 2, 3, 4, and 5, respectively, survived to the study termination. Both the male and female studies were terminated early due to increased mortality among all asenapine-treated groups. The males were terminated in Weeks 89/90 and the females in Weeks 98/99. The highest survival rate was observed in the vehicle control group. The survival of the untreated control group was slightly lower for males and markedly lower for females. The Sponsor's figures illustrating the percent survival of the animals over the course of the carcinogenicity study are shown below:

Group survival - males



Group survival - females

Test article	Vehicle control		[illegible]		Untreated control
Group	1	2	3	4	5
Level (mg/kg/day) Males	0	0.5	1.5	5.0/4.0	0
Level (mg/kg/day) Females	0	0.5	1.5	7.5/5.0	0



In both sexes in this study, the number of animals surviving to Week 80 was greater than 50% of the initial number or at least 20. The lowest number of surviving males was observed in the MD male group (16). However, 27 MD males were alive in Week 81 of the study. The lowest number of surviving females (15) was observed in the HD female group. However, 21 HD females were alive in Week 81 of the study. A variety of findings including skin/appendage lesions, uro-genital tract lesions, gastro-intestinal lesions and hemolymphoreticular tumors contributed to morbidity and death. However, the treatment-related increase in mortality could not be attributed to a single factor. The incidence of deaths due to hemolymphoreticular tumors increased with the increasing dose in female mice. According to the Sponsor, this finding was unlikely test-article related because similar incidence was observed in the untreated control group. These results are shown in the Sponsor's table below:

Group incidence of selected factors contributing to morbidity and mortality		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
	Level (mg/kg/day)	0	0.5	1.5	5.0/4.0	0	0	0.5	1.5	7.5/5.0	0
Decedents	Weeks 1-52	4	5	10	11	5	2	6	6	13	7
	Weeks 53-term	16	23	31	25	19	14	22	32	32	26
	total	20	28	41	36	24	16	28	38	45	33
Cause of demise											
skin/appendage lesion	Weeks 1-52	0	1	4	4	2	0	0	0	0	0
	Weeks 53-term	3	0	0	2	2	4	2	3	3	2
	total	3	1	4	6	4	4	2	3	3	2
uro-genital tract lesion	Weeks 53-term	5	9	4	7	4	0	1	0	1	0
gastro-intestinal tract lesion	Weeks 53-term	0	2	3	3	0	0	1	5	4	0
haemolymphoreticular tumours	total	3	4	5	3	6	5	4	10	15	17
not determined	Weeks 1-52	0	2	1	2	2	0	0	0	8	0
	Weeks 53-term	1	2	7	3	2	1	1	6	2	1
	total	1	4	8	5	4	1	1	6	10	1

Clinical signs: Hypoactivity and partially closed eyelids (eye squinting) were observed in all MD and HD animals, and occasionally in some LD animals. These effects were observed at 30 min and 2 hours after dosing, and occasionally at 4 hours after dosing. There were no test article-related changes in the incidence of palpable masses.

Body weights: Body weight gain decreased in all groups of male mice receiving asenapine when compared to that of the vehicle control group in Week 88. Males at the LD, MD, or HD gained 79%, 75%, or 73% of the mean vehicle control weight gain, respectively. Body weight decreased in male mice by 9%, 7%, and 9% in the LD, MD, and HD groups, respectively, compared to the mean vehicle control weight. Therefore, it can be concluded that the MTD was achieved (and not exceeded) in male mice.

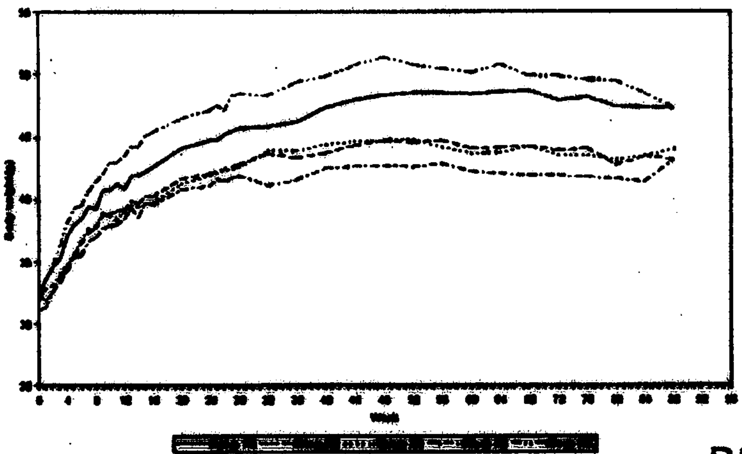
Body weight gain was minimally increased in female mice in Week 96 (109% and 112% of the vehicle control group at the MD and HD, respectively). However, body weight gain increased in the final weeks of the study and the increase was not observed earlier. Body weight in female mice was similar to that of the vehicle control group (95%, 99% and 104% of the vehicle control in the LD, MD, and HD groups, respectively).

Among all groups, the highest body weight was observed in the untreated male and female control groups (107% of vehicle control). Some animals (up to 13) in these groups were classified as obese.

The Sponsor's figures illustrating the group mean body weights of the animals over the course of the carcinogenicity study are shown below:

Group mean body weights - males

Test article	Vehicle control		Org 5222		Untreated control
Group	1	2	3	4	5
Level (mg/kg/day) Males	0	0.5	1.5	5.0/4.0	0
Level (mg/kg/day) Females	0	0.5	1.5	7.5/5.0	0



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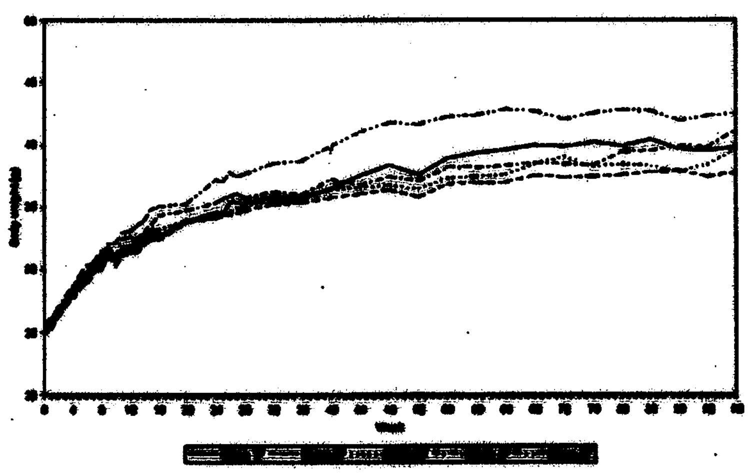
Group mean body weights - females

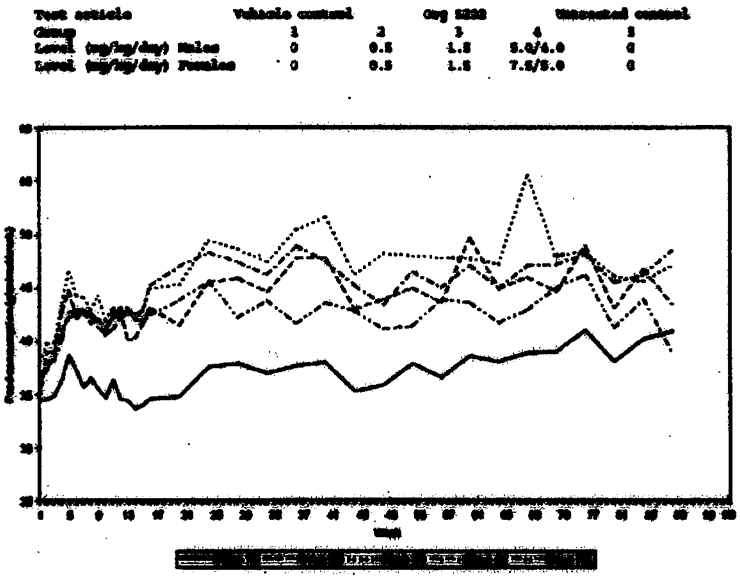
Test article	Vehicle control		Org 5222		Untreated control
Group	1	2	3	4	5
Level (mg/kg/day) Males	0	0.5	1.5	5.0/4.0	0
Level (mg/kg/day) Females	0	0.5	1.5	7.5/5.0	0



Food consumption: The overall food consumption was statistically significantly increased in mice administered asenapine when compared to the vehicle control, but was similar to that of the untreated control. Males given the LD, MD, and HD were eating 16%, 22%, or 22% more food, respectively, when compared to the vehicle control group.

Females given the LD, MD, and HD were eating 22%, 35%, or 30% more food, respectively, when compared to the vehicle control group.

Group mean food consumption - males



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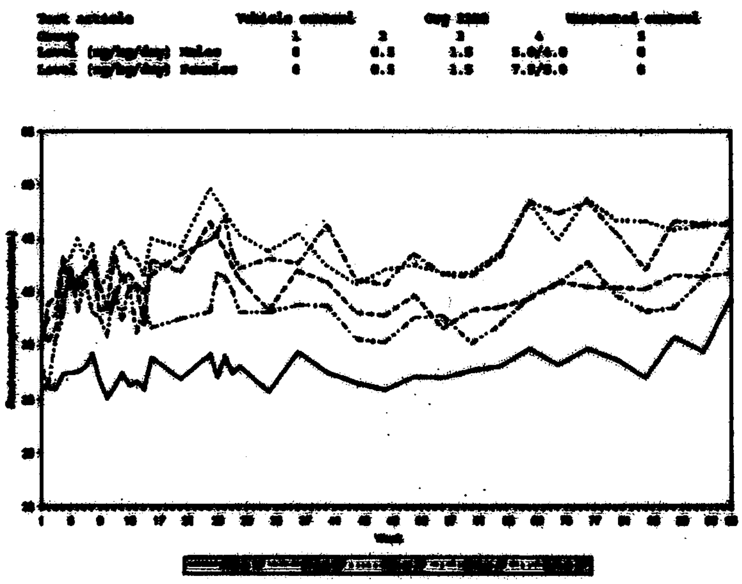
Group mean food consumption - females



Hematology: Total white blood cell count (WBC) and neutrophils count were increased in HD males and HD females. These increases were statistically significant except for the increase in total WBC in females. There were no other treatment- related effects on the erythrocyte and leukocyte counts when examined at necropsy.
Gross pathology: There were no macroscopic findings indicating local or systemic carcinogenicity due to asenapine.
Histopathology:
Non-neoplastic: Microscopic non-neoplastic findings related to asenapine administration included slightly increased severity of fascitis/fibrosis at the injection sites in HD animals of both sexes. These data are shown in the following Sponsor's table:

Group incidence of selected microscopic findings – skin/subcutis and injection sites

Tissue and finding	Level (mg/kg/day)		Males 1M 0	2M 0.5	3M 1.5	4M 5.0/4.0	5M 0	Females 1F 0	2F 0.5	3F 1.5	4F 7.5/5.0	5F 0
Neck (IS1)	No. examined:		57	39	43	60	56	57	39	38	60	57
fasciitis/fibrosis	Grade	-	8	5	2	7	48	13	3	10	8	51
		1	31	26	26	27	7	28	26	20	37	6
		2	18	6	13	25	0	13	9	7	14	0
		3	0	2	2	1	1	3	1	1	1	0
Right hip (IS2)	No. examined:		57	36	47	60	56	57	36	39	60	57
fasciitis/fibrosis	Grade	-	6	6	6	8	50	7	6	4	5	48
		1	32	12	9	12	4	41	21	20	23	8
		2	15	8	23	33	1	9	9	15	28	1
		3	4	9	9	7	1	0	0	0	4	0
		4	0	1	0	0	0	0	0	0	0	0
Left hip (IS3)	No. examined:		57	38	45	60	56	57	34	38	60	57
fasciitis/fibrosis	Grade	-	4	4	3	5	49	14	6	3	6	51
		1	30	16	13	16	6	34	25	27	33	5
		2	19	13	23	31	1	9	3	8	21	1
		3	4	3	5	8	0	0	0	0	0	0
		4	0	2	1	0	0	0	0	0	0	0

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe

Moreover, slightly increased incidence of cardiomyopathy (varying degrees of myocardial degeneration/necrosis with increased interstitial fibrosis) was observed in MD and HD animals of both sexes. This lesion is commonly seen as a spontaneous background lesion in older mice. The data is shown in the following Sponsor's table:

Group incidence of selected microscopic findings – heart

Tissue and finding	Level (mg/kg/day)		Males 1M 0	2M 0.5	3M 1.5	4M 5.0/4.0	5M 0	Females 1F 0	2F 0.5	3F 1.5	4F 7.5/5.0	5F 0
Heart	No. examined:		57	57	57	60	57	57	57	57	60	57
cardiomyopathy	Grade	-	42	43	37	34	48	55	50	43	47	53
		1	13	10	10	12	6	2	5	13	10	2
		2	2	2	8	10	3	0	2	1	2	2
		3	0	2	2	4	0	0	0	0	1	0

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate

Neoplastic: There were no statistically significant increases in any tumors in female mice except for the pleomorphic malignant lymphomas in the hemolymphoreticular system in HD females when compared to the vehicle control group. However, the incidence of

these lymphomas was similar to that of the untreated control group (2, 2, 6, 14, and 14 in the vehicle control, LD, MD, HD, and untreated control group, respectively).
In addition to pleomorphic lymphomas, lymphoblastic, lymphocytic and NOS types were also observed. The incidence of combined malignant lymphomas in female mice was increased compared to the vehicle control and untreated control group, respectively. These results are shown in the following Sponsor's table:

Group incidence of selected neoplastic findings		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Tissue and finding	Level (mg/kg/day)	0	0.5	1.5	3.0/4.0	0	0	0.5	1.5	7.5/5.0	0
Skin/subcutis & Injection sites 1-3	No. examined*:	57	39	47	60	57	57	41	39	60	57
fibrosarcoma	Incidence:	0	3	2	1	0	0	0	0	1	0
malignant fibrous histiocytoma		0	0	0	0	0	1	1	2	2	2
sarcoma NOS		0	4	0	0	0	0	0	0	0	0
Haemolymphoreticular system	No. examined:	57	28	43	60	57	57	28	39	60	57
malignant lymphoma (total)	Incidence:	3	3	4	7	8	7	4	8	20	22

* = number of animals from which at least one sample examined.

The incidence of interstitial cell adenomas in the testes in males was statistically significantly increased compared to the untreated controls (1, 0, 0, 3, and 0 in Groups 1, 2, 3, 4, and 5, respectively). However, the incidence of this tumor was no longer considered statistically significant compared to the vehicle controls. The incidence of all, benign and malignant tumors in male and female mice is shown below:

Tumor type	Group incidence of tumors									
	Male groups					Female groups				
	1 (C1)	2 (LD)	3 (MD)	4 (HD)	5 (C2)	1 (C1)	2 (LD)	3 (MD)	4 (HD)	5 (C2)
all	49	38	26	36	48	47	35	36	42	56
benign	35	23	14	25	30	26	21	15	16	21
malignant	14	15	12	11	18	21	14	21	26	35

The incidence of all tumors was slightly decreased in HD males. This finding is consistent with the decrease in body weight gain among male mice. However, according to the reviewing statistician, there is no statistically significant difference in the number of tumor bearing animals between the HD female mice (9/60) and the vehicle controls (7/57) or between the HD male mice (16/60) and the vehicle controls (22/57). There were no other findings suggestive of local or systemic carcinogenic potential of asenapine.
Toxicokinetics: Asenapine was detected in the plasma of treated animals. According to the Sponsor, toxicokinetic evaluation was not possible due to the composite sampling design, the limited number of samples and the limited time points for sampling. Plasma levels in males and females were comparable. There were no clear differences in plasma levels between single and multiple dosing. Mean plasma levels after subcutaneously administered asenapine in mice in the carcinogenicity study are shown in the following Sponsor's table:

	Mean concentration (ng mL⁻¹) (n=3)					
	0.25 mg kg⁻¹ once daily*					
	Single dose		Week 27		Week 90/99**	
Protocol time (h)	M	F	M	F	M	F
0.25	24.1	24.2	31.2	26.2	33.3	<7.09
0.5	17.2	28.3	29.4	22.7	25.0	40.6
1	16.9 [a]	15.2	22.7 [a]	15.3	14.5	17.2 [b]
	1.06 mg kg⁻¹ once daily*					
	Single dose		Week 27			
	M	F	M	F		
0.25	92.4	93.9	88.4	70.7		
0.5	83.7	70.9	68.4	50.6		
1	61.2	42.6	52.7	80.4 [a]		
	3.55 mg kg⁻¹ once daily*					
	Single dose		Week 27			
	M	F	M	F		
0.25	228	332	201 [a]	24.6 [b]		
0.5	217	273	113	109 [a]		
1	194	203	156 [a]	106 [b]		

key: F=female, M=male
[a] n=2 [b] n=1
* expressed as active entity
** samples in week 90 and 99 were only taken from low dose males and females, respectively.

Based on the highest concentrations of asenapine achieved at 0.25 h after single dose in females administered the high dose of 3.5 mg/kg/day (AE) (332 ng/mL), safety margin calculated relative to the C_{max} of 6.56 ng/mL obtained at the maximum recommended dose of 10 mg b.i.d. in humans is 51. However, the reported animal plasma concentrations may not correspond to the C_{max} values. The AUC values were not calculated for this study.

2.6.6.6 Reproductive and developmental toxicology

Note: Asenapine (Org 5222) refers to asenapine maleate. Active entity is approximately 71% of asenapine maleate.

Fertility and early embryonic development

PILOT STUDIES

1. Study title: A pilot fertility study with Org 5222 in male and female rats. (Study No. SDG 2315). Test facility: Organon Inc; Study initiation date: November 23, 1981; GLP: no

Key study findings: Oral administration of asenapine at 30 mg/kg/day to male Sprague-Dawley rats for two weeks before and during the mating period with vehicle treated females did not affect mating behavior and pregnancy performance. The same dose administered to female rats for one week before and during mating period with vehicle treated males and up to day 6 of pregnancy resulted in slight increase in pre-coital interval, decrease in mating rate and decrease in pregnancy rate. Treatment with asenapine resulted also in a decreased body weight in males and females on day 7 of pregnancy, when compared to the untreated group. These findings justified the dosages selected for a subsequent definitive fertility study in rats.

Methods: Asenapine (Org 5222; batch D; purity: 99.9%) was administered orally by gavage to Sprague-Dawley rats (12/sex/group) at 30 mg/kg/day (21.3 mg/kg/day active entity) in a volume of 2 mL/kg. The males were treated for two weeks before mating. The females were treated for one week before mating, during the mating period, and up to day 6 of pregnancy. Asenapine-treated males were paired with vehicle-treated females. Asenapine-treated females were paired with vehicle-treated males. A group of vehicle treated males and females served as a control. Animals were observed for mortality, clinical signs, body weight and mating performance (i.e., pre-coital interval, mating rate). Female rats were sacrificed on day 14 of pregnancy and examined for gross pathology and pregnancy performance (i.e., pregnancy rate, number of implantation sites and corpora lutea, number and distribution of live and dead embryos). Males were sacrificed at the end of mating.

Results: There was no drug-related mortality and clinical signs. Treatment of female rats with asenapine resulted in slightly increased pre-coital interval (4.3 days compared to 2.0 days in the control group), decreased mating rate (58% compared to 92% in the control group) and decreased pregnancy rate (72% compared to 91% in the control group). Administration of asenapine to male rats did not affect mating behavior and pregnancy performance. Body weight was decreased by 10% in males and by 7% in females on day 7 of pregnancy, respectively, when compared to the untreated group. There were no statistically significant effects on litter parameters and no gross pathology findings attributable to the drug. (Note: This study has been also reviewed by Dr. L. Freed; please see her review of the IND 51,641 of January 9, 1998 for additional details).

PIVOTAL STUDIES

2. Study title: An oral fertility and general reproductive (Segment I) study with Org 5222 in rats administered twice daily.

Key study findings: In the definitive oral study in rats designed for the assessment of fertility, teratogenicity, and F1 reproductive performance asenapine was administered at 0.5, 2.5 and 15.0 mg/kg b.i.d. Marked dose-related effects observed at the MD and HD included clinical signs of piloerection, sedation, and abnormal posture, effects on food consumption (increase before mating, decrease during gestation and lactation), and body weight loss in males and females. There were also effects on reproductive performance including increased pre-coital time at all dose levels, embryotoxic effects including increased pre-implantation loss at all dose levels and increased post-implantation loss at the MD and HD, increased neonatal mortality at all dose levels, and delays in the development of the surviving offspring (decreased body weight gain of the F1 pups associated with a delayed skeletal development of the fetuses most likely related to the reduced body weights). In animals administered the LD treatment-related effects were less marked than in the other two groups and included mild general toxicity (clinical signs of piloerection and lethargy), minimal reduction of reproductive performance of F0 animals and slight retardation of skeletal development of fetuses. There were no statistically significant effects on fertility index, conception rate or gestation index in dosed females (although decreased mating and pregnancy ratios were seen in the pilot study). There were no teratogenic effects observed in this study. However, it is unclear whether the external and visceral malformations were properly examined. Visceral examination demonstrated one MD fetus with a heart defect. Two abnormal fetuses were reported at the LD upon external examination. Therefore, only 3 malformed fetuses were reported upon external or visceral examinations. It appears extremely unlikely that no spontaneous external or visceral findings were detected in any fetus in all other groups. Therefore, evaluation of teratogenic effects in this study is considered inadequate.

Study no.: SDG RR 3115
Volume #, and page #: electronic submission
Conducting laboratory and location: b(4)

Date of study initiation: April 5, 1990
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine maleate (Org 5222); batch G; purity 100.6%

Methods

Doses: 0, 0.5, 2.5 and 15.0 mg/kg b.i.d (dosages refer to the maleate); (0.3, 1.8, and 10.6 mg/kg/day b.i.d. active entity)
Species/strain: rat/Wistar
Number/sex/group: 24 (12 females in Part A, 12 females in Part B)
Route, formulation, volume: oral (gavage), solution (or suspension for the high dose only) in gelatin/mannitol/water, final concentration 0.5%/5%), volume of 2 ml/kg body weight
Satellite groups used for toxicokinetics: none

Study design: Asenapine was administered twice daily to the F0 males for 60 days before mating and during mating to termination at delivery of F1 pups. F0 females were administered asenapine twice daily for 14 days before mating and during mating to termination either on day 21 post coitum or after weaning of the F1 pups on day 21 post partum. The dosages were selected based on the results of dose-range-finding study. Control rats were dosed with the vehicle alone. One half of the F0 parent females in each group was sacrificed on day 21 post coitum, the fetuses were removed, weighed, examined externally and allocated to either visceral or skeletal examination (Part A). The remaining half of the females was allowed to litter and rear their offspring to day 21 post partum (Part B). Developmental and behavioral parameters were monitored in the F1 offspring. Selected F1 animals were reared to maturity, mated at 12 weeks of age, and their reproductive performance was assessed. These F1 and F2 animals were not dosed.

Parameters and endpoints evaluated: Part A: Adult F0 animals were observed for clinical signs, body weight, food consumption, and effects on their reproductive performance (mating behavior). Post mortem examinations of females included gross macroscopic examination of all internal organs with emphasis on the uterus, uterine