CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-117

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-117**
SERIAL NUMBER:	**0039 (Class 1 Resubmission in response to January 13, 2009 Complete Response Letter)**
DATE RECEIVED BY CENTER:	**Letter date: February 12, 2009** **Stamp date: February 13, 2009**
PRODUCT:	**Asenapine maleate (Saphris)**
INTENDED CLINICAL POPULATION:	**Asenapine is indicated for acute treatment of schizophrenia in adults and acute treatment of manic or mixed episodes associated with bipolar I disorder in adults.**
SPONSOR:	**Organon USA Inc.**
DOCUMENTS REVIEWED:	**Electronic submission**
REVIEW DIVISION:	**Division of Psychiatry Products (HFD-130)**
PHARM/TOX REVIEWER:	**Elzbieta Chalecka-Franaszek, Ph.D.**
PHARM/TOX TEAM LEADER:	**Aisar Atrakchi, Ph.D.**
PHARM/TOX SUPERVISOR:	**Barry Rosloff, Ph.D.**
DIVISION DIRECTOR:	**Thomas Laughren, M.D.**
PROJECT MANAGER:	**Keith Kiedrow, Pharm.D.**

Date of review submission to DARRTS: July 27, 2009

EXECUTIVE SUMMARY

Background:

This submission constitutes a complete response to the comments outlined in the Agency's January 13, 2009 action letter. The following pharmacology/toxicology comment was included in that letter: "*Your submission dated August 29, 2008, included the previously requested information on the rat and mouse carcinogenicity studies. Our review and evaluation of these data will be completed within the next cycle of the NDA"*. In response, the Sponsor acknowleged that the submission dated August 29, 2008 will be reviewed within the next cycle of the NDA. Moreover, the Sponsor submitted a draft labeling text with proposed changes, including changes in sections routinely reviewed by pharmacology/toxicology reviewers.

Therefore, this review contains the following:

1. Review of carcinogenicity studies in rats and mice, including the review of new data submitted on August 29, 2008
2. Review of the relevant labeling sections

I. Recommendations

A. Recommendation on approvability

It is recommended the NDA 22-117 for asenapine maleate be approved. See the original pharmacology/toxicology NDA review dated April 30, 2008 for additional details.

B. Recommendation for nonclinical studies

The Sponsor should perform an embryofetal development study with (b) (4) in the rabbit to qualify this impurity during phase IV or reduce the specification (b) (4) to the ICH Q3A(R) qualification limit of (b) (4). See the original pharmacology/toxicology NDA review dated April 30, 2008 for additional details.

C. Recommendations on labeling

The following labeling is recommended in the ***Carcinogenesis*** section:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: In a lifetime carcinogenicity study in CD-1 mice asenapine was administered subcutaneously at doses up to those resulting in plasma levels (AUC) estimated to be 5 times those in humans receiving the maximum recommended human dose (MRHD) of 10 mg twice daily. The incidence of malignant lymphomas was increased in female mice, with a no-effect dose resulting in plasma levels estimated to be 1.5 times those in humans receiving the MRHD. The mouse strain used has a high and

variable incidence of malignant lymphomas, and the significance of these results to humans is unknown. There were no increases in tumors in male mice.

In a lifetime carcinogenicity study in Sprague-Dawley rats, asenapine did not cause any increases in tumors when administered subcutaneously at doses up to those resulting in plasma levels (AUC) estimated to be 5 times those in humans receiving the MRHD.

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INTRODUCTION AND DRUG HISTORY

NDA number: 22-117
Review number: 2
Review completion date: July 13, 2009
Drug:

Trade name: Saphris
Generic name: Asenapine maleate
Code name: Org 5222
Chemical name: (3aR,12bR)-rel-5-chloro-2,3,3a, 12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole(2Z)-2-butenedioate (1:1)
CAS registry number: 85659-56-2
Molecular formula/molecular weight (g/mol): $C_{17}H_{16}ClNO.C_4H_4O_4$/asenapine maleate: 401.84; active entity: 285.8

Relevant INDs/NDAs/DMFs:
IND 51,641 for asenapine maleate (Org 5222) sublingual tablets for schizophrenia
IND 70,329 for asenapine maleate (Org 5222) sublingual tablets for bipolar I disorder
IND 103,642 for asenapine hemipamoate (Org 5222-2) suspension for IM injection for schizophrenia

Drug class: Asenapine is a potent multi-receptor antagonist with a high affinity for several serotonin, dopamine, noradrenaline, and histamine receptors, indicated for the treatment of schizophrenia or acute manic or mixed episodes associated with bipolar I disorder.

Intended clinical population: Asenapine is indicated for acute treatment of schizophrenia in adults and acute treatment of manic or mixed episodes associated with bipolar I disorder in adults.

Clinical formulation: tablets (5 mg and 10 mg)

Route of administration: sublingual

Purpose of this submission: This is the submission that adresses recommendations described in the Agency's letter dated April 8, 2008 requesting that the Sponsor examine histopathology slides from additional dose groups, the low dose (LD) and medium dose (MD), in the previously performed carcinogenicity studies in rats (males and females) and mice (females). The background of this issue and the rationale for these requests can be found in Dr. Chalecka-Franaszek's NDA review of April 30, 2008 available in DFS or DARRTS. The Executive CAC concurred with the reviewer's recommendations at the ECAC meetings of March 25, 2008, May 27, 2008, and March 3, 2009 (see Attachments II, III, and IV, respectively). The reasons for recommendations were different for the rat and mouse studies and are discussed below.

The reasons for recommendation to conduct a full histopathology examination of the LD and MD male and female rats, and female mice: Based on review of the carcinogenicity studies, entitled "104 week subcutaneous administration oncogenicity study with Org 5222 in the rat" and "104 week subcutaneous administration oncogenicity study with Org 5222 in the mouse" it was concluded that both studies are inadequate and further information from the Sponsor is needed to complete evaluation of the carcinogenic potential of asenapine.

Rat study: In the rat carcinogenicity study, the maximum tolerated dose (MTD) was clearly exceeded 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. The incidence of preneoplastic changes and tumors (total number of tumors and tumor-bearing animals) was decreased at the HD when compared to the vehicle controls. The LD and MD groups were not routinely examined. Since it is known that a significant decrease in body weight can lead to a decrease in tumor development, the Sponsor was asked to conduct a full histopathology examination of the LD and MD males and females. The Executive CAC concluded that the final evaluation of the neoplastic findings will be made after the additional data are received.

Mouse study: In the mouse carcinogenicity study, the MTD was clearly exceeded in males and females at the MD and HD levels based on high mortality. The incidence of pleomorphic malignant lymphomas and all combined lymphomas in the hemolymphoreticular system was statistically significantly increased in the female mice at the HD (20/60) compared to the vehicle control (7/57). However, the incidence of these tumors in the female mice at the HD was similar to that in the untreated control (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. The Sponsor was asked to provide an explanation for the large difference in the incidence of lymphomas between the vehicle and untreated female control groups. Furthermore, full histopathology examination of the LD and MD female groups was requested. The Executive CAC concluded that the final evaluation of the lymphomas will be made after the additional data are received.

Sponsor's position regarding the rat carcinogenicity study (including new LD and MD data): Extended examination of the remaining LD and MD male and female rats yielded 30 additional common tumors, 15 of which were benign pituitary adenomas. Statistical analysis did not show a positive trend for any tumor type, however, the negative trend for some tumors observed in the original report was still present.
The Sponsor's position regarding the rat carcinogenicity study is that the available data indicate the following:
• the completed studies represent a full and complete assessment of the carcinogenic potential of asenapine maleate. There were no effects on survival or food consumption and no clear target organ toxicity contributing to mortality;
• the effects on body weight gain in the rats did not result in an adverse effect on survival over the course of the 2 year study;

• there were no trends indicative of a tumorigenic response to asenapine maleate administration across dose groups in either sex of either species tested;
• the weight of evidence when the results of genetic toxicity studies are included indicate that asenapine maleate administration does not constitute a human carcinogenic hazard;
• the appropriate doses were selected for this study considering that lower doses would not provide any multiples of the clinical dose.

Sponsor's position regarding the mouse carcinogenicity study:
Extended histopathological examination of the remaining LD and MD female groups in the mouse carcinogenicity study did not reveal any additional malignant lymphomas. Furthermore, there was no significant trend shown for other tumor types. Therefore, the following conclusion of the original report remains unchanged:
"There were no treatment-related increases in tumors in the skin/subcutis, or injection sites indicative of local oncogenicity. The tumor incidence did not reveal any evidence of systemic oncogenicity. The incidence of malignant lymphoma in high dose females appeared increased versus vehicle controls, but it was not increased when compared with untreated controls."
Furthermore, the incidence lies within the historical control data for the conducting laboratory. The pronounced difference between untreated and vehicle controls suggests that the increasing trend in lymphomas in treated females was probably a chance event rather than an effect of the test article. Therefore, the Sponsor's position regarding the mouse carcinogenicity study is that the incidence of lymphomas in female CD-1 mice is not suggestive of a tumorigenic effect of asenapine maleate.

REVIEW OF THE CARCINOGENICITY STUDY IN THE RAT

Study title: Org 5222: Additional histopathology evaluation of tissues taken on (b) (4) Study 0082/074 (104 week subcutaneous administration oncogenicity study with Org 5222 in the rat) (present Study No. 080114)

The objective of the study was to further investigate the oncogenic potential of asenapine maleate by the preparation and examination of all tissues from all animals assigned to the LD and MD groups that were not previously examined in the (b) (4) study No. 0082/074. In the previous study, subcutaneous administration of asenapine maleate at 0.3, 1.2, and 3.0/5.0 mg/kg/day (LD, MD, and HD, respectively) 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 (body weight gain was 27, 37, and 52% lower in LD, MD, and HD males, respectively, and 41% lower in HD females than that of the vehicle control group at termination). The reduction in body weight was not consistent with changes in food consumption.

Note: Dose levels in this section and in the histopathology tables are expressed as salt. Dose levels of 0.3 and 1.2 mg/kg/day (expressed as salt) correspond with dose levels of 0.2 and 0.9 mg/kg/day (expressed as active entity) respectively.

Key study findings:
In an additional histopathology examination of the 20 LD and 33 MD male rats as well as 21 LD and 27 MD female rats 30 new tumors (28 benign and 2 malignant) were noted, including 3 LD and 2 MD benign pancreatic islet cell adenomas, 1 LD benign lymph nodes hemangioma, 1 LD and 1 MD benign prostate gland acinar cell adenoma, 6 LD and 8 MD benign pituitary gland pars distalis adenomas, 1 MD malignant lymphoma in males, and 1 MD malignant pancreatic islet cell carcinoma, 1 LD benign ovarian stromal tumor, 1 LD and 2 MD benign endometrial stromal polyp in the uterus, 1 LD benign granular cell tumor of vagina, and 1 LD benign pituitary gland pars distalis adenoma in females.

Histopathology revealed no significant increase in one or more specific tumor-types and no rare tumors were encountered that could be attributed to the asenapine maleate treatment for additionally examined animals and all combined animals.

The incidence of preneoplastic changes and tumors (total number of tumors and tumor-bearing animals) decreased at the HD when compared to the vehicle control. Asenapine maleate decreased the incidence of benign mammary tumors and pituitary pars distalis tumors in females, fatal pituitary pars distalis tumors and injection site fibromas in males, adrenal phaeochromocytomas, squamous cell tumors and histiocytic sarcoma in both sexes. The overall incidence of tumors was significantly reduced in both the MD and HD asenapine maleate treated groups.

Other microscopic findings noted in the animals were similar to the non-neoplastic findings noted for the animals examined earlier, including aggregation of foamy macrophages in the lungs (NOAEL 1.2 mg/kg/day, the MD), hypertrophied follicular epithelium in thyroid glands (NOAEL for males 0.3 mg/kg/day, the LD; for females 1.2 mg/kg/day, the MD), increased incidence of cystic/haemorrhagic (cortical) degeneration in adrenal glands at the MD and HD treated males, and aggravated local reaction comprising dose-dependent increased subcutaneous fibrosis at the injection sites. In contrast to the other non-neoplastic findings, the incidence and severity of diffuse C-cell hyperplasia in the thyroid glands was dose dependently decreased. No increased incidences of other hyperplastic (pre-neoplastic) lesions were observed among treated animals. According to the Sponsor, above mentioned microscopic findings are not suggestive of local or systemic toxicity due to the test article, but more likely represent aggravation of common background findings or are related to changes in hormonal and/or physical status of the animals.

Methods:
The pre-existing slides, pre-existing wax blocks and pots of formalin fixed tissues kept their original Study Number identification (0082/074) were used in this study. Tissues from the following terminally sacrificed animals assigned to the Groups 2 (LD) and 3 (MD) were processed at (b) (4) and examined:

Group number	Group codes	Animals/group	
		Male	Female
2	Green	61, 64, 65, 73, 74, 75, 76, 77, 78, 81, 84, 91, 93, 103, 104, 109, 110, 112, 116, 119 **Total = 20**	362, 363, 367, 369, 372, 376, 377, 379, 380, 382, 384, 390, 396, 397, 403, 406, 408, 409, 414, 416, 419 **Total = 21**
3	Blue	121, 125, 126, 128, 129, 130, 131, 132, 133, 134, 135, 141, 142, 143, 144, 148, 150, 154, 155, 156, 159, 160, 163, 166, 168, 170, 171, 173, 174, 175, 178, 179, 180 **Total = 33**	423, 425, 426, 428, 433, 434, 435, 440, 441, 444, 446, 450, 451, 452, 454, 455, 459, 461, 463, 464, 465, 468, 472, 475, 477, 478, 479 **Total = 27**

All other animals assigned to the LD and MD groups that are not identified above were decedents and were processed and subsequently examined previously (Study 0082/074). Tissues from all animals in the LD and MD groups previously processed and examined in (b) (4) study No. 0082/074 included tissue masses, female mammary glands, adrenals, thyroids, and parathyroids.

Tissues from the LD and MD groups processed and examined in this study included: eyes (x 2), seminal vesicles (x 2), optic nerves (x 2), urinary bladder, skin, prostate, muscle (quadriceps), uterus (x 3), femur with bone marrow and articular surface, vagina, sternum with bone, marrow, salivary glands, sciatic nerves (x 2), mandibular lymph nodes, liver (x 2), thymus, spleen, lungs (x 2), pancreas, heart, mesenteric lymph nodes, aorta, stomach, trachea, duodenum, esophagus, jejunum, pituitary, ileum, brain (x 3), cecum, spinal cord cervical, colon, spinal cord thoracic, rectum, spinal cord lumbar, kidney (x 2), trachea bifurcation, testes and epididymides, injection sites (x 4),

and ovaries (x 2). For tissues previously sampled and processed for study 0082/074 (e.g., gross lesions), new samples were processed where sufficient residual tissue was available. The additional tissues from animals assigned to the LD and MD groups were embedded in paraffin wax BP, sectioned at a nominal 5 µm and stained with haematoxylin and eosin. The sections from all designated animals were examined microscopically by the Sponsor's Principal Investigator for pathology and the Sponsor's contributing Study Pathologist. External Pathology Peer Review was conducted.

The following microsopic pathology data are included in this submission:
• Additionally observed neoplastic lesions in the LD and MD groups from the present study.
• Individual and incidence tables of histopathology (non-neoplastic and neoplastic) from the LD and MD groups from the present study.
• Updated histopathology findings/conclusions (non-neoplastic and neoplastic), for all groups from the original study (0082/074) and the present study combined.
• Updated summary incidence histopathology data (primary neoplastic lesions and merged tumor types) from all animals/all tissues from the original study and present study combined.
• Updated chronological listing of tumor data from original and present study combined.
Microscopic pathology data from treated animals were compared with control data from the original study (0082/074), by the Sponsor's Principal Investigator.

For all other data pertaining to this carcinogenicity study, please refer to the original pharmacology/toxicology review of study 0082/074 dated April 30, 2008 in DFS or DARRTS.

Neoplastic findings:
Extended histopathology evaluation of the LD and MD rats indicated 30 new tumors (28 benign and 2 malignant). Additionally observed neoplastic lesions (the incidence of benign and malignant tumors) are summarized per sex, dose-level, and per organ/tissue(s) in the following table:

Group	**2 (LD)**		**3 (MD)**	
Dose (mg/kg/day)	**0.3**		**1.2**	
Sex/number of animals examined	**males** (N=20)	**females** (N=21)	**males** (N=33)	**females** (N=27)
Benign tumors				
Lymph nodes(s)	1		0	
Ovaries		1		
Pancreas	3		2	
Pituitary gland	6	1	8	
Prostate	1		1	
Uterus		1		2
Vagina		1		
Subtotal	**11**	**4**	**11**	**2**

Malignant tumors				
Hematopoietic system			1	
Pancreas				1
Subtotal			**1**	**1**

These tumors are specified in the following Sponsor's tables:

Males:

Autopsy code: ST	Sex	M	M
	Group number	DS2	DS3
	Animals on study	20	33
Finding(s)	'ST' Animals	20	33
Pancreas	*	3	2
Adenoma, islet cell (B)		3	2
Lymph nodes, NOS	*	1	0
Hemangioma (B)		1	
Prostate gland	*	1	1
Adenoma, acinar cell (B)		1	1
Pituitary gland	*	6	8
Adenoma, pars distalis (B)		6	7
Adenoma, pars distalis, multiple (B)			1
Hematopoietic system	*	0	1
Lymphoma, malignant (S)			1

* = Number of animals where organ/tissue was examined (and suitable)

Females:

Autopsy code: ST	Sex	F	F
	Group number	DS2	DS3
	Animals on study	21	27
Finding(s)	'ST' Animals	21	27
Pancreas	*	0	1
Carcinoma, islet cell (M)			1
Ovaries	*	1	0
Tumor, sex cord stromal, mixed, benign (B)		1	
Uterus	*	1	2
Polyp, endometrial stromal, benign (B)		1	2
Vagina	*	1	0
Tumor, granular cell, benign (B)		1	
Pituitary gland	*	1	0
Adenoma, pars distalis (B)		1	

* = Number of animals where organ/tissue was examined (and suitable)

Statistical analysis of tumor data:

According to the reviewing statistician Dr. Min Min, statistical analyses were done for the original study and the present study combined. For histopathology findings, statistical analysis was performed whenever there are at least 3 tumors, or other lesions, of a given type. The tumor data were analyzed for dose response relationship and pair-wise comparisons of control group with each of the treated groups. The same type and number of additional tumors were found in the statistical reviewer's tumor analysis as the Sponsor's in the LD and MD groups. The following tumor types showed p-values less than or equal to 0.05 either in tests for dose response relationship and/or pair-wise comparisons between control and each of individual treated groups:

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons

(Untreated control, low, medium and high dose groups)

			Untreated_Control	0.3 mg Low	1.2 mg Med	5 mg High	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Organ Name	Tumor Name		N=60	N=60	N=60	N=60				
Male injection site(	histiocytoma, fibrou		1	5	7	2	0.622	0.107	0.041	0.446
Female mammary glands	adenocarcinoma (M)		11	18	23	25	0.017	0.059	0.020	0.005

Based on the criteria of adjustment for multiple testing of trends proposed by Lin and Rahman, the incidence of none of the above or any other tumor types in either sex was considered to have a statistically significant positive dose response relationship. Also based on criteria by Haseman, increased tumor incidences of adenocarcinoma of mammary glands in HD group in female rats were considered to be statistically significant only when compared to the untreated control group because the p-value was less than 0.01. Please see Dr. Min Min's statistical review for additional details.
The incidence of adenocarcinoma of mammary glands was 40/60, 25/60, 39/60, 37/60 and 15/ 59 in the vehicle control, 0.3, 1.2 and 5.0 mg/kg/day, and untreated control, respectively. Therefore, the increased incidence of this tumor in the HD group compared to the untreated control is not considered biologically important.

Sponsor's statistical tumor data analysis:
According to the Sponsor, taking into account the survival differences, there was statistically no evidence that asenapine maleate increased the incidence of any type of tumor significantly. In contrast, there was strong evidence that asenapine decreased the incidence of a number of types of tumors, including benign mammary tumors and pituitary pars distalis tumors in females, fatal pituitary pars distalis tumors and injection site fibromas in males, adrenal phaeochromocytomas, squamous cell tumors and histiocytic sarcoma in both sexes. The overall incidence of tumors was significantly reduced in the 1.2 and 5.0 mg/kg/day asenapine-treated groups. No rare tumors were encountered that could be attributed to asenapine maleate. In conclusion, asenapine given subcutaneously to male and female rats at doses of 0.3, 1.2, and 5.0 mg/kg/day did not exhibit any organ specific, systemic or local tumorigenic potential.
The incidences of all benign and malignant tumors are summarized in the following Sponsor's tables:

Benign tumors[**]

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	2	9	6
Brain	1			1				1		2
Eye(s)				1						
Injection site(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)			3			1	1	1	2	
Mammary gland(s)		47	1	24		32		22		44
Oral cavity & related structures	1								1	
Ovaries				1				1		1
Pancreas	2		6		2	2	1	1	2	1
Parathyroid gland(s)	5	1	2		1		3	1	4	
Pituitary gland	18	42	30	38	31	30	20	27	30	39
Prostate			1		1					
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		3		6		2		4
Vagina		1		5		3		4		7
TOTAL No. OF BENIGN TUMORS	**56**	**108**	**67**	**84**	**63**	**88**	**35**	**73**	**71**	**111**

Malignant tumors**

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	
Auditory sebaceous gland(s)	1		1						1	
Brain	1	1					1	1	1	1
Haematopoietic system	8	3	3	2	1	1	3		1	1
Injection site(s)	19	2	14	4	13	3	6	2	6	
Jejunum				1						
Liver		1							1	
Mammary gland(s)		45		26		40		38		20
Mandibular salivary gland(s)									1	
Oral cavity & related structures				1		1				1
Pancreas		1		1	1	1	1	1	2	
Pineal gland									1	
Pituitary gland				1		1				1
Prostate gland	1									
Rectum									1	
Skin			1						2	
Soft tissues	2		4	1	1		2		2	
Testes	1									
Thymus				1						
Thyroid gland	2	1	3	2	1	1	1	1	2	
Uterus		1								
Vagina						1				
TOTAL NO. OF MALIGNANT TUMORS	**37**	**57**	**26**	**42**	**19**	**50**	**15**	**44**	**23**	**24**
TOTAL NO. OF BENIGN AND MALIGNANT TUMORS	**93**	**165**	**93**	**126**	**82**	**138**	**50**	**117**	**94**	**135**

** updated figures are underlined

Non-neoplastic findings:

Increased incidence of non-neoplastic findings was observed in the lung, adrenal glands, thyroid glands, and at the injection sites. The following additional non-neoplastic lesions were observed in extended histopathology of the LD and MD groups. New and previous observations were combined:

Lung: A dose-related increase in the incidence of aggregation of foamy macrophages was observed in the lungs of males and females treated at 1.2 and 5.0 mg/kg/day {Note: Previously collected data indicated the increased incidence of this observation in males treated at 1.2 and 5.0 mg/kg/day (11 and 16 animals, respectively), and in females treated at 1.2 and 5.0 mg/kg/day (5 and 11 animals, respectively)}. Updated figures are underlined in the following Sponsor's table:

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	0.3	1.2	5.0	-	0	0.3	1.2	5.0	-
FINDING(S)	No. examined	60	59	60	60	60	60	60	60	60	60
- aggregation of foamy macrophages		10	14	18	24	14	11	9	17	23	7

** updated figures are underlined

Adrenal glands: A dose-related increase in the incidence of cystic/hemorrhagic (cortical) degeneration was observed in male rats at 1.2 and 5.0 mg/kg/day. For females no differences were found among the groups. Histopathology of the adrenal glands of all animals was conducted during original study; therefore the following Sponsor's table shows the same data as those reported previously:

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	0.3	1.2	5.0	-	0	0.3	1.2	5.0	-
FINDING(S)	No. examined	60	59	60	60	60	60	60	55	60	60
- Cystic / haemorrhagic degeneration	Grade: 1				2	1	3	4	2		5
	2	2	9	7	14	1	17	15	13	14	14
	3	7	5	14	12	2	17	16	20	30	22
	4	4	2	4	7	5	15	11	13	3	7
	Total	13	16	25	35	9	52	46	48	47	48

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

Thyroid gland: A dose-related increase in 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 in females administered 5.0 mg/kg/day. In addition, a decreased incidence and severity of diffuse C-cell hyperplasia was observed in males and females treated at 1.2 or 5.0 mg/kg/day. Histopathology of the thyroid glands of all animals was conducted during original study; therefore the following Sponsor's table shows the same data as those reported previously:

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	5.0	-	0	0.3	1.2	5.0	-
FINDING(S)	No. examined	60	60	60	60	60	60	60	59	60	60
- Follicles with hyper-trophied epithelium (and inspissated colloid)	Distribution: A	3	8	11	4	6	3	3	3	15	5
	D	6	8	14	19	4	1	2	6	14	3
	Total	9	16	25	23	10	4	5	9	29	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

Injection sites: The development of a subcutaneous layer of fibrosis was observed at the injection sites, with dose-dependent increase in thickness of this layer among animals administered asenapine, was observed in all groups. Updated figures are underlined in the following Sponsor's table:

Selected non-neoplastic findings at the injection site(s)[**]

	Sex	MALES					FEMALES				
	Group	1	2	3	4	5	1	2	3	4	5
	Dose (mg/kg/day)	0	0.3	1.2	5.0	-	0	0.3	1.2	5.0	-
FINDING(S)	No. examined	60	60	60	60	60	60	60	60	60	60
- Fibrosis (layer of varying thickness)	grade: 1	5	6	3	2		15	3	4		
	2	30	25	11	13		38	43	35	28	
	3	15	21	37	44		7	13	20	32	
	Total	50	52	51	59		60	59	59	60	

Key: grade 1 = variable from no lesion to slight thickness, grade 2 = variable from no lesion to moderate thickness, grade 3 = variable from no lesion to marked thickness.

REVIEW OF THE CARCINOGENICITY STUDY IN THE MOUSE

Study title: Org 5222: Additional histopathology evaluation of tissues taken on Covance Study 0082/075 (104 week subcutaneous administration oncogenicity study with Org 5222 in the mouse) (current Study No. 0082/264)

The objective of the study was to further investigate the oncogenic potential of asenapine maleate by preparation and examination of all tissues from all animals assigned to the LD and MD female groups that were not previously examined in the (b) (4) study No. 0082/075. Based on previous analysis, subcutaneous administration of asenapine maleate at 0.5 (LD), 1.5 (MD), and 5.0/4.0 (HD in males) or 7.5/5.0 mg/kg/day (HD in females) 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 lymphoma in the hemolymphoreticular system and all lymphomas combined 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 (20/60) was similar to that in the untreated controls (22/57). 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.

Key study findings:
In an additional histopathology examination of the 29 LD and 19 MD female mice surviving to scheduled termination, tumors have been identified in two LD animals and two MD animals. In the LD animals, these consisted of a single hepatocellular adenoma and a lung bronchio-alveolar carcinoma. In the MD animals, the additional tumors identified were an adrenal sub-capsular adenoma and a benign mast cell tumor at one of the three injection sites. There were no new lymphomas in the LD and MD animals in the present study. In the original and present data combined, there was a statistically significant higher incidence of pleomorphic malignant lymphoma and combination of all types of lymphomas in the HD females compared with the vehicle control. The incidence of pleomorphic lymphoma in the vehicle control is 2/57 (3.5%), which is below the historical control range for pleomorphic lymphoma of 9.2% to 29.4% (although it is not clear what vehicle(s) were used in these studies). The incidence of pleomorphic lymphoma in the HD female group is 14/60 (23.3%) and, therefore, lies within the historical control range. However, the incidence of this lymphoma in the LD and MD groups (2/57; 3.5% and 6/57; 10.5%, respectively) is similar to that of the vehicle control group, indicating that the number of lymphoma in these groups represents the incidence of spontaneously occurring pleomorphic lymphoma in female mice in this particular study. It is noted that the incidence of pleomorphic lymphoma in the untreated control group (14/57; 24.6%) was similar to that in the HD group and greater than that in the vehicle control group. Moreover, the incidence of all malignant lymphomas combined was 7/57, 4/57, 8/57, 20/60, and 22/57 in the vehicle control, LD, MD, HD, and untreated control groups, respectively. Despite the fact that the lymphoma incidence in the HD

group was similar to that in the untreated control group, the vehicle control group is the only appropriate control group to compare tumor incidence values in the drug treated groups. This conclusion is reinforced by the fact that the incidence values in the LD and MD groups were similar to that in the vehicle control group (in essence providing some replication of the values in the vehicle control group). Therefore, an increased incidence of pleomorphic lymphoma and all lymphomas combined in the HD female group compared to the vehicle control group is considered to be test article related. With the exception of lymphoma tumors in females, there were no tumors of either unusual type or incidence suggestive of systemic oncogenicity in mice.

Methods:
Tissues from the following terminally sacrificed animals assigned to the LD (Group 1) and MD (Group 2) were processed and examined by the Study Pathologist:

Group number	Group codes	Animal numbers Females
2	Green	351, 353, 355, 357, 358, 360, 361, 362, 363, 365, 366, 367, 369, 371, 372, 374, 375, 376, 380, 381, 382, 383, 386, 388, 389, 393, 394, 397, 400
		Total = 29
3	Blue	404, 408, 409, 410, 411, 412, 418, 420, 422, 425, 427, 430, 432, 434, 437, 441, 445, 446, 448
		Total = 19

All other animals assigned to Groups 2 and 3 female that are not identified above were decedents and were processed and subsequently examined by the Study Pathologist on Study 0082/075.

All other animals assigned to the LD and MD groups that are not identified above were decedents and were processed and subsequently examined previously (Study 0082/075). Tissues from all animals in the LD and MD groups previously processed and examined in [redacted] (b) (4) study No. 0082/075 included tissue masses, gross lesions, and hearts.

Tissues from the LD and MD groups processed and examined in this study include: adrenals (x 2), mammary glands, thyroids + parathyroids, eyes (x 2), seminal vesicles (x 2), optic nerves (x 2), urinary bladder, skin, muscle (quadriceps), uterus (x 3), femur with bone marrow and articular surface, vagina, sternum with bone marrow, salivary glands, sciatic nerves (x 2), mandibular lymph nodes, liver (x 2), thymus, spleen, lungs (x 2), pancreas, mesenteric lymph nodes, aorta, stomach, trachea, duodenum, esophagus, jejunum, pituitary, ileum, brain (x 3), cecum, spinal cord cervical, colon, spinal cord thoracic, rectum, spinal cord lumbar, kidney (x 2), trachea bifurcation, testes, gall bladder, and epididymides, injection sites (x 3), and ovaries (x 2). For tissues previously sampled and processed for study 0082/075 (e.g., gross lesions), new samples were processed where sufficient residual tissue was available. The additional tissues from animals assigned to the LD and MD groups were embedded in paraffin wax, sectioned at a nominal 5 µm, and stained with haematoxylin and eosin. The sections from all designated animals were examined microscopically by the Sponsor's Principal

Investigator for pathology and the Sponsor's contributing Study Pathologist. External Pathology Peer Review was conducted.

The following microsopic pathology data are included in this submission:
• The pathology results of the original study (0082/075) are updated to include the additional histopathology findings recorded for LD and MD group females from the present study.
• Group mean microscopic pathology tables for all animals (males and females) from the original study and the present study combined.
• Individual macroscopic/microscopic pathology appendix for all females from the original study and the present study combined.
• Chronological listing of female tumors from the original study and the present study combined.
• Statistical analysis of female tumor data from the original study and present study combined.

For all other information pertaining to this carcinogenicity study, please refer to the original review of study 0082/075 dated April 30, 2008 in DFS or DARRTS.

Neoplastic findings:
Examination of the additional tissues from the LD and MD female groups surviving to scheduled termination resulted in the identification of additional tumors in two LD animals and in two MD animals. In the LD animals, these consisted of a single hepatocellular adenoma and a lung bronchio-alveolar carcinoma. In the MD animals, the additional tumors identified were an adrenal sub-capsular adenoma and a benign mast cell tumor at one of the three injection sites. Additionally, review of the other tumors identified previously resulted in the change of diagnosis of a single ovarian tumor in a LD animal from benign sex cord stromal tumor to benign sertoli cell tumor.

With the exception of lymphoma tumors, there were no tumors of either unusual type or incidence suggestive of systemic oncogenicity. Neoplasms in other tissues were generally infrequent and, according to the Sponsor, consistent with the usual pattern in mice of this strain. There was a statistically significant higher incidence of pleomorphic lymphoma and all combined lymphomas in the HD females compared with the vehicle controls. The incidence of the vehicle control was less than that in untreated control females and lies below the historical control data for the conducting laboratory. Group incidence of all malignant lymphoma in mouse carcinogenicity study (males and females) is shown in the following table:

Tumor type	**Group incidence of malignant lymphomas**									
	Male groups Dose level (mg/kg/day)					**Female groups** Dose level (mg/kg/day)				
	1 (C1) Vehic.	2 (LD) 0.5	3 (MD) 1.5	4 (HD) 5.0/4.0	5 (C2) Untr.	1 (C1) Vehic.	2 (LD) 0.5	3 (MD) 1.5	4 (HD) 7.5/5.0	5 (C2) Untr.
No. of animals examined	**57**	**28**	**42**	**60**	**57**	**57**	**57**	**57**	**60**	**57**
mal. lymph. - lymphocytic	0	2	1	0	1	**3**	1	1	**5**	**2**

mal. lymph. - lymphoblastic	1	1	1	2	1	**2**	0	1	**1**	**3**
mal. lymph. - pleomorphic	2	0	2	5	5	**2**	2	6	**14**	**14**
mal. lymph. - plasmacytic	0	0	0	0	1	**0**	0	0	**0**	**1**
mal. lymph. - NOS	0	0	0	0	0	**0**	1	0	**0**	**2**
mal. lymphomas- all combined	3	3	4	7	8	**7**	4	8	**20**	**22**

According to the Sponsor, this pronounced difference between the untreated and vehicle controls suggests that the increasing trend in lymphoma in treated females was probably a chance event rather than an effect of the test article. The Sponsor provided the following historical data for the incidence of lymphoma in mice:

Lymphoma incidence observed in the historical control Vs lymphoma incidence observed in the high dose female CD-1 mouse in study 82/75

Tumor type	Historical Control: Maximum Lymphoma Incidence (%) in data set	High Dose Female: Lymphoma Incidence in study 82/75	
		Absolute	%
Malignant lymphoma - lymphocytic	**10**	6*/60	**10**
Malignant lymphoma - plasmacytic	**0.8**	0/60	**0**
Malignant lymphoma - pleomorphic	**29.4**	14/60	**23.3**
Malignant lymphoma - NOS	**4.2**	0/60	**0**
Malignant lymphocytic leukemia	**2.0**	0/60	**0**

* including one lymphoblastic lymphoma
NOS: not otherwise specified

(b) (4)

Lymphomas are normally merged for statistical analysis – as detailed in Table 1

Table 1

Mouse: crl cd-1 Incidence by merged tumour categories

Control tumour incidence data – carcinogenicity studies

Weeks: 100 – 104, Diet/Gavage dosed, Multiple housed, Ad-Lib diet

		Males					Females			
Tissue & tumours		Absolute incidence	%	% Min	% Max		Absolute incidence	%	% Min	% Max
HAEM/LYMPH/RETIC	No exam:	683					684			
Lymphoid tumours		58	8.5	4.2	11.8		157	22.9	18.6	30.0
M-granulocytic leukaemia		10	1.5	0.0	3.3		7	1.0	0.0	2.0
M-leukaemia		3	0.4	0.0	2.0		2	0.3	0.0	1.0
M-histiocytic sarcoma		4	0.6	0.0	2.0		5	0.7	0.0	1.0
M-mast cell sarcoma		1	0.1	0.0	1.0		0	0.0	0.0	0.0

The incidence of individual lymphoma types is detailed in Table 2

Table 2

Mouse: crl cd-1 Incidence by individual tumour type

Control tumour incidence data – carcinogenicity studies

Weeks: 100 – 104, Diet/Gavage dosed, Multiple housed, Ad-Lib diet

		Males					Females			
Tissue & tumours		Absolute incidence	%	% Min	% Max		Absolute incidence	%	% Min	% Max
HAEM/LYMPH/RETIC	No exam:	683					684			
M-lymphocytic lymphoma		20	2.9	1.7	4.2		44	6.4	1.0	10.0
M-plasmacytic lymphoma		0	0.0	0.0	0.0		2	0.3	0.0	0.8
M-pleomorphic lymphoma		33	4.8	1.7	6.9		100	14.6	9.2	29.4
M-lymphoma		3	0.4	0.0	1.7		9	1.3	0.0	4.2
M-lymphocytic leukaemia		2	0.3	0.0	2.0		2	0.3	0.0	2.0
M-leukaemia granulocytic		10	1.5	0.0	3.3		7	1.0	0.0	2.0
M-leukaemia		3	0.4	0.0	2.0		2	0.3	0.0	1.0
M-histiocytic sarcoma		4	0.6	0.0	2.0		5	0.7	0.0	1.0
M-malignant mast cell tumour		1	0.1	0.0	1.0		0	0.0	0.0	0.0

Statistical analysis of tumor data:

According to the reviewing statistician Dr. Min Min, statistical analyses were done for the original study and the present study combined. For histopathology findings, statistical analysis was performed whenever there are at least 3 tumors, or other lesions, of a given type. The tumor data were analyzed for dose response relationship and pair-wise comparisons of control group with each of the treated groups. The same type and number of additional tumors were found in this reviewer's tumor analysis as Sponsor's in the LD and MD groups. The following tumor types showed p-values less than or equal to 0.05 either tests for dose response relationship or pair-wise comparisons between either the untreated control or vehicle control and each of individual treated groups, respectively.

Statistical analysis to the untreated control:

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons

(Untreated control, low, medium and high dose groups)

Organ Name	Tumor Name	Untreated_Cont N=57	0.5 mg Low N=57	1.5 mg Med N=57	7.5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Female HAEMOLYMPHORETI	MALIGNANT LYMPHOMA								
	-Pleomorphic	14	2	6	14	0.023	0.999	0.941	0.469
	-Lymphocytic	2	1	1	5	0.029	0.509	0.472	0.186
HAEMOLYMPHORETI	ALL_MALIGNANT_LYMPHO	22	4	8	20	0.014	1.000	0.991	0.480

Based on the criteria of adjustment for multiple testing of trends by Lin and Rahman, the incidence of none of the above or any other tested tumor types in females was considered to have a statistically significant positive dose response relationship. Also based on the criteria of Haseman, the increased tumor incidence of none of treated groups in female mice was considered to be statistically significant when compared to the untreated control group.

Statistical analysis to the vehicle control:

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons

(Vehicle control, low, medium and high dose groups)

Organ Name	Tumor Name	Vehicle Cont N=57	0.5 mg Low N=57	1.5 mg Med N=57	7.5 mg High N=60	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Female HAEMOLYMPHORETI	MALIGNANT LYMPHOMA -								
	-Pleomorphic	2	2	6	14	0.000	0.633	0.074	0.000
	-Lymphocytic	3	1	1	5	0.042	0.624	0.584	0.219
Haemolymphoreti	ALL_MALIGNANT_LYMPHO	7	4	8	20	0.000	0.618	0.306	0.000
PITUITARY	ADENOMA	1	0	0	3	0.036	0.457	0.438	0.213

Based on the criteria of adjustment for multiple testing of trends by Lin and Rahman, the dose response relationship in the incidence of pleomorphic malignant lymphoma and combination of all types of malignant lymphomas of haemolymphoreticular system in female mice were considered to be statistically significant since the p-values were less than 0.005. Also based on the criteria of Haseman, the increased tumor incidence of pleomorphic malignant lymphoma and of combination of all types of malignant lymphomas of haemolymphoreticular system in the HD group in female mice was considered to be statistically significant when compared to the vehicle control group because the p-value is less than 0.01.

Non-neoplastic findings:

The following additional non-neoplastic lesions were observed in extended histopathology of the LD and MD groups. New and previous observations were combined:

Microscopic findings at injection sites comprised mainly subcutaneous fasciitis/fibrosis and, to a lesser extent, subcutaneous haemorrhage and dermatitis. The fasciitis/fibrosis was characterized mainly by an increase in subcutaneous collagen fibers variably infiltrated by inflammatory cells and occasional pigment deposits. The severity of these findings in the HD group was slightly more extensive than in the vehicle controls, indicating increased minor local response due to test article injection.

Group incidence of selected microscopic findings – skin/subcutis and injection sites – data from both 82/75 and 82/264

		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Tissue and finding	Level (mg/kg/day)	0	0.5	1.5	5.0/4.0	0	0	0.5	1.5	7.5/5.0	0
Neck (IJ1)	No. examined:	57	39	42	60	56	57	57	57	60	57
fasciitis/fibrosis	Grade -	8	5	2	7	48	13	3	19	8	51
	1	31	26	26	27	7	28	42	29	37	6
	2	18	6	12	25	0	13	11	8	14	0
	3	0	2	2	1	1	3	1	1	1	0
Right hip (IJ2)	No. examined:	57	36	47	60	56	57	57	57	60	57
fasciitis/fibrosis	Grade -	6	6	6	8	50	7	10	14	5	48
	1	32	12	9	12	4	41	35	28	23	8
	2	15	8	23	33	1	9	12	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 (IJ3)	No. examined:	57	38	45	60	56	57	57	57	60	57
fasciitis/fibrosis	Grade -	4	4	3	5	49	14	8	11	6	51
	1	30	16	13	16	6	34	45	38	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

In systemic tissues, a slightly higher incidence of cardiomyopathy was seen in the LD, MD, and HD animals of both sexes. This lesion, characterized by varying degrees of myocardial degeneration/necrosis with increased interstitial fibrosis, is commonly found as a spontaneous background lesion in older mice. The incidence of cardiomypathy is shown in the following Sponsor's table.

Group incidence of selected microscopic findings – heart - data from 82/75

		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Tissue and finding	Level (mg/kg/day)	0	0.5	1.5	5.0/4.0	0	0	0.5	1.5	7.5/5.0	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

Microscopic findings in other tissues reflected both higher and lower incidences of non-neoplastic background findings in treated animals compared with controls. There were no

unusual findings in the liver, kidney or other major organs suggestive of systemic toxicity.

DISCUSSION:

In our communication of April 8, 2008, we requested that the Sponsor examine histopathology slides from additional dose groups in the previously performed carcinogenicity studies in rats and mice. The background of this issue and the rationale for this request is available in Dr. Chalecka-Franaszek's NDA review of April 30, 2008. The Executive CAC concurred with our recommendations at the ECAC meetings of March 25, 2008, May 27, 2008, and March 3, 2009. The reasons for our recommendations are different for the rat and mouse studies and are discussed below. The sponsor did not believe that any additional exams were warranted.

Rat study

The problem with the rat study was that the degree of decreased body weight (relative to control group) in all male groups and the HD female group was excessively high. It has been previously shown, primarily by studies employing dietary restriction, that such restriction and the consequent decrease in body weight gain result in lower incidences of "spontaneous" (background) tumors as well as a decreased sensitivity to chemical-induced tumors. In recognition of this, when reviewing Special Protocols for carcinogenicity studies, the CAC routinely requires that, in studies in which the Sponsor proposes to do routine histopathology only in the control and HD groups, the lower doses be examined if an "excessive" decrease in body weight is seen in the examined dose group. (The protocols for the carcinogenicity studies presently discussed were never concurred with by the CAC, so the Sponsor presumably did not specifically receive this advice from us). An "excessive" decrease in body weight gain has generally been considered that which results in mean final (i.e. at the end of the study) weights of 10% or more below control; in the present study weights were 17, 23, and 31% below control in LD, MD, and HD males, respectively, and 23% below control in HD females. We thus requested full histopathologic exams be done in the LD and MD groups. (Note that since an excessive decrease in weight gain was seen in all male groups, the problem still would not be solved in this gender by examining the lower dose groups. However, we concluded that if the lower dose groups in females were examined, the study would not have to be repeated since the mouse study did not have this problem, and thus 3 of the 4 "cells" [female rats and both male and female mice] would be adequately evaluated).

The Sponsor did not believe the lower doses should be examined for the following reasons *(Note: The following comments were written by Dr. Barry Rosloff. This reviewer agrees with his comments)*:

1. The Sponsor did not agree with our statement that the MTD was exceeded. The Sponsor stated various factors on which the MTD should be based (including "no more than 10% decrease in body weight gain...", target organ toxicity, and significant alterations in clinical pathology) and stated that the MTD should not "simply consider bodyweight gain but consider a combination of factors that could alter the normal life span of the animals".

We did not agree. Any single factor which can adversely affect the general health of the animals should be, and in our experience always has been in the past, sufficient to determine the MTD (and in fact excessive decrease in weight gain has been, in our experience, the most commonly used factor for determining the MTD in carcinogenicity studies). One could argue that excessively decreased weight gain is not necessarily detrimental to the animals; in fact as noted above food-restriction can have lower tumor rates [and increased longevity]. On the other hand a decreased weight gain in a drug study can reflect an [often not clearly defined] toxic effect of the drug. At any rate, the real question here is not whether the decreased weight gain defined or exceeded an MTD, but whether it may have decreased the sensitivity of the assay; in fact the ICH guidance S1C for dose selection for carcinogenicity studies specifically mentions interference with the interpretation of the study as a factor to consider in high dose selection. (Note that although our communication to the sponsor of April 8, 2008 did state that the MTD was exceeded, the main point we were conveying, made quite clear in the communication, was that the decreased weight gain may have interfered with the ability of the study to detect drug-induced tumors).

2. The Sponsor stated that the decreased weight gain "reflected pharmacologic and/or hormonal effects rather than evidence of excessive toxicity". The Sponsor listed various endocrine changes which occurred in the 3-month dose range finding study. As indicated below, these endocrine changes were not dose-dependent. However, no evidence or argument was presented to indicate how these changes may have caused the decreased weight gain, and furthermore even if such a mechanism were shown it is not clear why the decreased weight gain would still not be a problem for assessing carcinogenicity in the study.

3. The sponsor stated that effects seen in a 90 day rat study "would not preclude" the use of the HD which was used in the carcinogenicity study. However, regardless of what was seen in the range finding study, it apparently did not successfully predict effects in the carcinogenicity study.

[The HD for the carcinogenicity study was chosen by the Sponsor based on data derived from a pilot s.c. toxicity study in rats of 13-week duration. 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 13% to 29% in all dose groups. Body weight gain of females was increased by 8 to 16% in all dose groups, in parallel with increased food consumption. These effects were not dose related in females. In addition, a decrease in uterus weight as well as 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 (and was subsequently increased to 5.0 mg/kg after 6 weeks).

4. The Sponsor indicated that had the doses been chosen based on a 10% decrease in body weight gain, the exposure ratio (plasma drug AUC in rats vs humans) in HD males would be less than 1. However, in our opinion the negative of a low exposure ratio would be outweighed by a decrease in sensitivity of the carcinogenicity study. (This is a general problem which often occurs in animal studies, usually when exposure in animals is limited by drug toxicity. Attempts to overcome this include use of a different species or route of administration, divided daily dosing, etc. Often, however, it is just concluded that the animal model, while imperfect, is the best that reasonably can be used).

5. The Sponsor stated that "a large body of literature data" attributes the decreases in tumors and increases in survival in dietary restriction studies to reduced caloric intake rather than to decreased bodyweight *per se* (although only one references is cited: Keenan et. al., Toxicological Sciences 24: (6), 757-768, 1996 [This reference is incorrect. The correct journal is Toxicologic Pathology]). The Sponsor stated that food consumption in the asenapine study was only slightly decreased in males (3-9%, not dose-related) and not affected in females. (According to Dr. Chalecka-Franaszek's review, food consumption was also slightly decreased in the MD females). Furthermore, the Sponsor stated that, unlike what has occurred in dietary restriction studies, there was no increase in survival or reduction in tumors in the asenapine study.

   Regarding the issue of the cause of decreased tumors (and decreased sensitivity to tumor induction), we do not think the issue is settled. Most studies have employed dietary restriction paradigms, which do not distinguish between decreased food consumption and decreased bodyweight as the cause for decreased tumors. A recent paper (Huffman et. al., Cancer Research 67:(1), 417-424, 2007) purports to show a role of decreased body weight, and cites other papers supporting this position. The results of Huffman's study imply that the ability of calorie restriction to inhibit or delay cancer incidence and progression is mediated in part by changes in energy balance, body mass, and/or body composition rather than calorie intake *per se*, suggesting that excess calorie retention, rather than consumption, confers cancer risk. At any rate, it doesn't appear that this question has been conclusively answered. The present study appears to support the role of decreased body weight, since both weight and tumor incidences were decreased despite only a slight and non dose-related decrease in food consumption. There are other examples supporting the role of decreased body weight in a reduction of the overall tumor incidence. In a 104-week mouse carcinogenicity study with selegiline (NDA 20-647), the two main findings were (1) reduction noted in overall body weight gain in female mice (15, 23, and 46% in the LD, MD, and HD groups, respectively) and male mice (27 and 48% in the MD and HD groups, respectively), and (2) and decrease in the overall incidence of tumors in the HD groups (30-35% in HD males, 9-10% in HD females). The drug related effect on body weight gain led to overall body weight reduction of 8 and 16% in MD and HD males, respectively,

and of 7, 9, and 18% in LD, MD, and HD females, respectively. No drug-related effects were noted in food and water consumption.

Regarding survival in the asenapine study, it was increased in all female groups, but not-dose related (survival in drug groups 35-45% vs 25% in vehicle control), and was also increased in medium dose males (55% vs 32%) but not in other male groups. At any rate, even if there were no increases in survival, it is possible that the decreased body weight might have produced a tendency toward an increase in survival which was counteracted in part by other (toxic) effects of the drug.

Regarding tumor incidence, there were drug-related decreases in several tumor types (benign mammary tumors in females, pituitary tumors in females and benign adrenal pheochromocytomas in both genders, and injection site tumors in males, and total tumors in both genders). (The Sponsor states that the decrease in injection site tumors "has an important contribution to the overall decrease in tumors [which is in seeming contradiction to their previous statement that there was "no evidence of…reduced tumor development"] but that these tumors "have no relevance for human risk assessment in as much as the subcutaneous injection differs from the clinical route"; however this misses the point, which is that the decrease in body weight due to drug appears to have a retarding effect on tumor development). (Note that even if a decrease in tumor incidence were not seen in this study, it is still possible that the decrease in body weight reduced the sensitivity to any drug effect, or that the drug had a tumorigenic effect in the study which was counteracted by a decrease due to decreased body weight).

6. The Sponsor stated that the number of animals that remain to be examined in the lower dose groups is small ("about 17% of the total number on study for both the rat and female mice"), presumably since exams were done in animals of these groups which died before the end of the study, and that it is not likely that the additional exams "would change the cancer risk assessment significantly". However, what matters in this case is the number of animals to be examined per each LD and MD group. The distribution of decedents among the treatment groups in the rat study and the percentages alive at the terminal sacrifice and examination was as follows:

	Group and sex									
	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (mg/kg/day)	0 (V)	0.3	1.2	3/5	0 (U)	0 (V)	0.3	1.2	3/5	0 (U)
Number of animals/ group	60	60	60	60	60	60	60	60	60	60
Number of animals alive to scheduled sacrifice	19	20	33	19	24	15	21	27	24	26
Number of all decedents	41	40	27	41	36	45	39	33	36	34
Number of spontaneous deaths	8	8	3	15	9	4	8	2	4	8
Unscheduled sacrifice	33	32	24	26	27	41	31	31	32	26
Percentage of decedents	68%	67%	45%	68%	60%	75%	65%	55%	60%	57%
Percentage of animals alive to terminal sacrifice	32%	33%	55%	32%	40%	25%	35%	45%	40%	43%

(U) untreated control
(V) vehicle control

It appears that exam of some prematurely dying animals in all groups, including LD and MD groups, was inadequate, since autolysis of many organs (occasionally noted in all animals of the group) was observed in many animals that died spontaneously and in a few animals of the unscheduled sacrifice group. It is also important to consider that the animals which died or were sacrificed prior to termination were at lower risk for development of tumors than those animals which survived to termination.

Mouse study
In this study the incidence of lymphomas was greatly increased in the HD females compared to the vehicle controls (7/57 and 20/60 in the vehicle control and HD group, respectively) but not to an untreated control group (22/57). In view of this anomaly, as well as the known greatly variable incidence of these tumors in the mouse strain used, it was decided, with concurrence of the ECAC, to perform histopathologic exams in the LD and MD females to help determine if the finding was drug-related. (The Sponsor was also asked to provide an explanation for the large difference in incidence of lymphoma between the vehicle and untreated control groups).

The Sponsor points out that lymphoma has a high and variable incidence in this strain and that the incidence in the asenapine groups is within the historical range. As noted above, we had already considered these points, but in view of the large difference in incidence between the HD and concurrent vehicle control groups we concluded that the additional data requested would be helpful in determining if this were a true drug effect (e.g. a dose-responsive increase in incidence seen after exam of the LD and MD would support a true drug effect; on the other hand finding incidences in the LD and MD which were similar to that in the HD and untreated control groups might support the notion that the low incidence in the vehicle control was a chance occurrence).

The Sponsor pointed out that the female vehicle control group had higher survival and lower food consumption and weight gain than the female untreated control group, but it is

not clear how this can be used to explain the large difference in incidence of lymphomas between the two groups.

CONCLUSIONS:

Rat study:
The Sponsor conducted additional histopathology examination of the following animals: 20 LD and 33 MD male rats as well as 21 LD and 27 MD female rats. This examination indicated 30 new tumors, 28 benign and 2 malignant, including 3 LD and 2 MD benign pancreatic islet cell adenomas, 1 LD benign lymph nodes hemangioma, 1 LD and 1 MD benign prostate gland acinar cell adenoma, 6 LD and 8 MD benign pituitary gland pars distalis adenomas, 1 MD malignant lymphoma in males, and 1 MD malignant pancreatic islet cell carcinoma, 1 LD benign ovarian stromal tumor, 1 LD and 2 MD benign endometrial stromal polyp in the uterus, 1 LD benign granular cell tumor of vagina, and 1 LD benign pituitary gland pars distalis adenoma in females.

Histopathology revealed no significant increase in one or more specific tumor-types and no rare tumors were encountered that could be attributed to asenapine treatment for additionally examined animals and all combined animals. The incidence of preneoplastic changes and tumors (total number of tumors and tumor-bearing animals) decreased at the HD when compared to the vehicle control. Asenapine decreased the incidence of benign mammary tumors and pituitary pars distalis tumors in females, fatal pituitary pars distalis tumors and injection site fibromas in males, adrenal phaeochromocytomas, squamous cell tumors and histiocytic sarcoma in both sexes. The overall incidence of tumors was significantly reduced in both the MD and HD asenapine-treated groups.

Mouse study:
The Sponsor conducted additional histopathology examination of the following animals: 29 LD and 19 MD female mice. Examination of the additional tissues from the LD and MD female groups surviving to scheduled termination resulted in the identification of additional tumors in two LD animals and in two MD animals. In LD animals, these consisted of a single hepatocellular adenoma and a lung bronchio-alveolar carcinoma. In MD animals, the additional tumors identified were an adrenal sub-capsular adenoma and a benign mast cell tumor at one of the three injection sites. There were no new lymphomas in the LD and MD animals.

In the original and present data combined, there was a statistically significant higher incidence of pleomorphic malignant lymphoma and combination of all types of lymphomas in the HD females compared with the vehicle control. The incidence of pleomorphic lymphoma in the vehicle control was 2/57 (3.5%), which is below the historical control range for pleomorphic lymphoma of 9.2% to 29.4% (although it is not clear what vehicle(s) were used in these studies). The incidence of pleomorphic lymphoma in the HD female group is 14/60 (23.3%) and, therefore, lies within the historical control range. However, the incidence of this lymphoma in the LD and MD groups (2/57; 3.5% and 6/57; 10.5%, respectively) is similar to that of the vehicle control group, indicating that the number of lymphomas in these groups represents the incidence

of spontaneously occurring pleomorphic lymphoma in female mice in this particular study. It is noted that the incidence of pleomorphic lymphoma in the untreated control group (14/57; 24.6%) was similar to that in the HD group and greater than that in the vehicle control group. Moreover, the incidence of all malignant lymphomas combined was 7/57, 4/57, 8/57, 20/60, and 22/57 in the vehicle control, LD, MD, HD, and untreated control groups, respectively. Despite the fact that the incidence of lymphoma in the HD group was similar to that in the untreated control group, the vehicle control group is the only appropriate control group to compare tumor incidence values in the drug treated groups. This conclusion is reinforced by the fact that the incidence values in the LD and MD groups were similar to that in the vehicle control group (in essence providing some replication of the values in the vehicle control group). Therefore, an increased incidence of pleomorphic lymphoma and all lymphomas combined in the HD female group compared to the vehicle control group is considered to be test article related.

With the exception of lymphoma tumors in females, there were no tumors of either unusual type or of increased incidence suggestive of systemic oncogenicity in mice.

Elzbieta Chalecka-Franaszek, Ph.D., Pharmacologist *{see appended electronic signature page}*

Barry Rosloff, Ph.D., Supervisor *{see appended electronic signature page}*

Aisar Atrakchi, Ph.D., Team Leader *{see appended electronic signature page}*

APPENDIX/ATTACHMENTS

Attachment I:
Executive Carcinogenicity Assessment Committee Meeting Minutes April 4, 2000
Attachment II:
Executive Carcinogenicity Assessment Committee Meeting Minutes March 25, 2008
Attachment III:
Executive Carcinogenicity Assessment Committee Meeting Minutes May 27, 2008
Attachment IV:
Executive Carcinogenicity Assessment Committee Meeting Minutes March 3, 2009

Attachment I

Executive CAC
Date: 4/4/00

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Charles Resnick, HFD-110, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Team Leader
Lois M. Freed, Ph.D., HFD-120, Presenting Reviewer

Author of Draft: Lois M. Freed, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND #51,641
Drug Name: Org 5222
Sponsor: Organon Inc.

Mouse Dose Selection: the doses selected by the sponsor for the mouse carcinogenicity study were 0, 0.5, 1.4, and 4 mg/kg s.c. in males and females. No study protocol was submitted. Doses were based on the results of previous studies (one 2-wk, two 13-wk s.c.) in CD-1 mouse. The strain to be used in the carcinogenicity study was not specified, but was assumed to be the CD-1 mouse. The HD selected for the carcinogenicity study is expected to result in the following (as described by the sponsor): (1) transient hypoactivity, (2) reduced body weight gain (males), (3) increased heart weight, (4) reduced wbc count (males), and (5) no significant injection site effects. The sponsor also pointed out that the plasma exposure (to the parent compound) at the HD is expected to be ≥25 times that predicted in humans at the maximum therapeutic dose.

The reviewer could not concur with the doses selected by the sponsor for the following reasons: (1) there was a discrepancy between the text and the summary table for one of the 13-week studies making it impossible to determine the doses associated with death in males and (2) there were no clear dose-limiting toxicities identified in females. In addition, the sponsor did not provide sufficient information to allow for selection of a HD based on the 25-fold exposure (i.e., PK) criterion. There was no discussion of the metabolic profile in mouse or human, no data on major metabolites, no serum protein binding data for mouse, or any discussion of the genotoxicity data for Org 5222.

Rat Dose Selection: the doses selected by the sponsor for the rat carcinogenicity study were 0, 0.3, 1, and 3 mg/kg s.c. in males and females. No study protocol was submitted. Doses were based on the results of previous studies (one 2-wk, two 13-wk s.c.). The 2-wk and one of the 13-wk studies were conducted in Wistar rat; the second 13-wk study was conducted in Sprague-Dawley rat. The strain to be used in the carcinogenicity study was not specified. The HD selected for the carcinogenicity study is expected to result in the following (as described by the sponsor): (1) a >10% reduction in body weight gain (males), (2) hypoactivity, (3) mild hematological effects, (4) prolongation of diestrus, (5) decreased uterus weight, (6) lobular development/secretory activity of mammary glands (females), (7) increased adrenal gland weight (males), (8) no significant local injection site effects. The sponsor also pointed out that the plasma exposure (to the parent compound) at the HD is expected to be 21-27 times that predicted in humans at the maximum therapeutic dose.

The reviewer could not concur with the doses selected by the sponsor for the following reasons: (1) the strain of rat to be used in the carcinogenicity study was not specified [the studies used to support the doses were conducted in Wistar and Sprague-Dawley rat] and (2) there were no clear dose-limiting

toxicities identified in females. In addition, the sponsor did not provide sufficient information to allow for selection of a HD based on the 25-fold exposure (i.e., PK) criterion. There was no discussion of the metabolic profile in rat or human, no data on major metabolites, no discussion of the genotoxicity data for Org 5222. Most importantly, the plasma exposure data provided for the proposed HD were extrapolated from data at lower doses but were not assessed in the 13-wk study in which the proposed HD was tested.

For both the mouse and rat dose-selections, the summary tables did not provide information sufficient to evaluate drug-related effects. Actual data (and/or percentages) should have been given instead of designations such as "d", "i", etc.

Executive CAC Recommendations and Conclusions:

The Exe-CAC could not concur with the doses selected by the sponsor for the 2-yr carcinogenicity studies in mouse or rat for the reasons given by the reviewer. The sponsor should provide full study reports for those studies that have not previously been submitted to the Division or protocols and summary data tables and line listings. The data specifically used to support dose-selection should be provided in summary tables as actual data, not as designations such as "d", "i", etc.

The Committee expressed concern that daily s.c. dosing would not be well tolerated in either mouse or rat for a 2 year period and suggested consideration of a different route or less frequent dosing (e.g., 3-4 dose/wk) for the carcinogenicity studies. Appropriate dose ranging data would be needed for any change in dosing regimen.

04/07/00

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\
HFD-120
/Division File
/GFitzgerald
/LMFreed
/SHardeman
HFD-024
/ASeifried

Attachment II

Executive CAC
Date of Meeting: March 25, 2008

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
William Taylor, Ph.D., DSPTP, Alternate Member
Barry Rosloff, Ph.D., DPP, Team Leader
Elzbieta Chalecka-Franaszek, Ph.D., DPP, Presenting Reviewer

Author of Draft: Elzbieta Chalecka-Franaszek, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #22-117
Drug Name: Asenapine maleate
Sponsor: Organon USA Inc.

Background: Asenapine maleate (Org 5222) is a psychopharmacologic agent belonging to the group of dibenzoxepinopyrrolidine compounds with high affinity for blocking serotonin, dopamine, adrenergic and histamine receptors. Asenapine is being developed for the treatment of schizophrenia and treatment of acute manic or mixed episodes associated with Bipolar I Disorder.

Rat Carcinogenicity Study: 104 week subcutaneous administration oncogenicity study with Org 5222 in the rat

Subcutaneous administration of asenapine maleate at 0.3 (LD), 1.2 (MD) and 3.0/5.0 (HD) mg/kg/day in pH-adjusted citric acid monohydrate to Sprague-Dawley female rats for 99 weeks and to male rats for 105 weeks did not demonstrate any organ specific, systemic or local tumorigenic potential of the test article. The dosages selected by the sponsor were not previously concurred with by the Executive CAC due to the lack of sufficient information at the time. Due to high morbidity/mortality in the female vehicle control group, the female study was terminated in weeks 100 to 102 after obtaining the Agency's concurrence. 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. The reduction in body weight did not correlate 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. The LD and MD groups were not routinely examined.

Mouse Carcinogenicity Study: 104 week subcutaneous administration oncogenicity study with Org 5222 in the mouse

1

Asenapine maleate was administered subcutaneously at 0.5 (LD), 1.5 (MD) and 5.0/4.0 (HD in males) or 7.5/5.0 (HD in females) mg/kg/day in pH-adjusted citric acid monohydrate to CD-1 mice. The dosages selected by the sponsor were not previously concurred with by the Executive CAC due to the lack of sufficient information at the time. 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. The study was terminated early (males in Weeks 89/90; females in Weeks 98/99) due to increased, generally dose-dependent mortality among all asenapine-treated groups.
The incidence of pleomorphic malignant lymphomas and all combined lymphomas in the hemolymphoreticular system was statistically significantly increased in the female mice at the HD compared to the vehicle control (7/57 and 22/60 in the vehicle control and HD group, respectively). However, the incidence of this tumor in the female mice at the HD 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. The LD and MD groups were not routinely examined.

Executive CAC Recommendations and Conclusions:

Rat:

Although the exec-CAC did not concur with the doses, the reason for non-concurrence was that it had been thought that the doses were not high enough. However, the doses turned out to be sufficiently high, and it was noted that the MTD was exceeded.

Since it is known that a significant decrease in body weight can lead to a decrease in tumor development, the sponsor should conduct a full histopathologic examination of the lower dose groups when an excessive decrease in body weight (or survival) is observed in the examined dose group. Because full histological evaluation was conducted only on tissues from the two control groups (vehicle and untreated) and the high dose group in both genders, the rat carcinogenicity study cannot yet be evaluated for either male or female rats.

- The sponsor should be asked to perform a full histopathology evaluation of the low dose and mid dose males and females and submit the findings to the Division, as soon as possible.

Mouse:

- Although the exec-CAC did not concur with the doses, the reason for non-concurrence was that it had been thought that the doses were not high enough. However, the doses turned out to be sufficiently high, and it was noted that the MTD was exceeded.

2

- The Committee concurred that the incidence of malignant lymphoma in high dose females was significantly increased compared to the vehicle control but not to the untreated control.

- The Sponsor should be asked for an explanation for the large difference in the incidence of lymphomas between vehicle and untreated female controls. Furthermore, full histopathology of the low-dose and mid-dose female groups should be performed.

- The final evaluation of the lymphomas will be made after the additional data are received.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DPP
/Barry Rosloff, Ph.D., Team Leader, DPP
/Elzbieta Chalecka-Franaszek, Ph.D., Reviewer, DPP
/Keith Kiedrow, Pharm. D., Project Manager, DPP
/ASeifried, OND IO

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/s/

David Jacobson-Kram
3/31/2008 01:50:39 PM

Attachment III

Executive CAC
Date of Meeting: May 27, 2008

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Dan Mellon, Ph.D., DAARP, Alternate Member
Barry Rosloff, Ph.D., DPP, Team Leader
Elzbieta Chalecka-Franaszek, Ph.D., DPP, Presenting Reviewer

Author of Draft: Elzbieta Chalecka-Franaszek, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #22-117
Drug Name: Asenapine maleate
Sponsor: Organon USA Inc.

Background:

- On March 25, 2008, the Executive CAC made the following recommendations regarding carcinogenicity studies in rats and mice:

RAT:

- The sponsor should be asked to perform a full histopathology evaluation of the low dose and mid dose males and females and submit the findings to the Division, as soon as possible. This recommendation was based on excessive decreases in body weight.

MOUSE:

- The Committee concurred that the incidence of malignant lymphoma in high dose females was significantly increased compared to the vehicle control but not to the untreated control.

- The Sponsor should be asked for an explanation for the large difference in the incidence of lymphomas between vehicle and untreated female controls. Furthermore, full histopathology of the low-dose and mid-dose female groups should be performed.

- On April 8, 2008, the Division informed the Sponsor that we consider the rat and mouse carcinogenicity studies to be inadequate until all histopathology slides from the low and medium dose groups of the rat study, and the low and medium dose female

groups from the mouse study, have been examined and the results submitted for review. The Division provided the rationale for this decision. Briefly, the Division stated that the degree of decreased weight gain in the rat study, particularly at the high dose, was of a magnitude which may have decreased the sensitivity of the animals to drug-induced tumors. In the mouse study, a large increase in malignant lymphomas compared to the vehicle control group, but not to an untreated control group, was seen in high dose females and therefore examination of the lower dose groups is necessary to determine if this was a true drug effect and if so, if there is a no-effect dose.

- On April 29, 2008, the Sponsor addressed these concerns; however we still believe that examination of the additional groups is necessary. The sponsor's primary arguments for the rat study were as follows:

1. The Sponsor stated that the literature indicates that in dietary restriction studies, it is the decrease in food consumption, and not the consequent decrease in bodyweight gain, which is responsible for the decrease in tumors seen, and that food consumption was only slightly decreased in the asenapine study. However, it is our opinion that the available evidence is not sufficient to rule out a significant (or even a primary) effect of decreased bodyweight gain. There is also evidence for a role of decreased weight gain in drug studies. e.g. methylphenidate, and in fact a decrease in tumors was seen in the asenapine study. (We noted that the Sponsor stated that the decrease in injection site tumors in the asenapine study had an "important contribution to the overall decrease in tumors" and that this site is not relevant to the proposed [sublingual] clinical route; however these data demonstrates that the decrease in drug-induced bodyweight appeared to have a retarding effect on tumor development in general. Furthermore, other tumors [e.g. benign mammary and pituitary tumors in females, and pheochromocytomas] were also decreased in this study.

2. The Sponsor stated that the number of animals that remain to be examined in the lower dose groups is small, presumably since animals which died or were prematurely sacrificed in these groups were examined. The Sponsor stated that "the number of animals that remain to be fully examined in these groups is... about 17% of the total number on study for both the rat and the female mouse'; however we find the number to be much greater for the low and medium dose groups in the rat study, e.g. the % alive at termination (and thus presumably not fully evaluated) ranged from 33 to 55%. Furthermore, some of the tissues from premature decedents could not be adequately evaluated due to autolysis. Additionally, animals dying or sacrificed prematurely are at lower risk for development of tumors than those which survived to termination (an effect which may be exaggerated in the face of dietary restriction/decreased weight gain- Keenan et. al., Toxicologic Pathology 24:6, 757-768, 1996).

3. The Sponsor stated that the use of doses which would have caused a smaller degree (10%) of weight gain reduction would result in drug exposures in high dose males which are less than those in humans. However, we believe that this is

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less crucial to an assessment of carcinogenic potential than is a decrease in the sensitivity of the assay due to an excessive decrease in weight gain.

The Sponsor's primary argument regarding the mouse study is that there is a high and variable incidence of malignant lymphoma in this strain and that the incidence in the asenapine study is within the historical range; furthermore, the incidence in the untreated control group was similar to that in the high dose females. We agree with the above; however we are still concerned with the much higher incidence in the high dose female group compared to the vehicle control group (which is the most appropriate comparator group). Examination of the low and medium dose female groups would help determine if there was a true drug effect (e.g. if there were a dose-response in incidence) and if there is a no-effect dose; alternatively if the incidences in the low and medium dose female groups were similar to those in the high dose and untreated control groups, it might be concluded that the vehicle control group was an outlier and that there was no drug effect on the incidence of this tumor.

Executive CAC Recommendations and Conclusions:

The Committee concurred that the carcinogenicity studies filed to the NDA are considered "unacceptable" without completion of the full histopathological examination of the low and mid-dose dose male and female groups in the rat carcinogenicity study and the full histopathological examination of the low and mid-dose dose females in the mouse carcinogenicity study.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DPP
/Barry Rosloff, Ph.D., Team Leader, DPP
/Elzbieta Chalecka-Franaszek, Ph.D., Reviewer, DPP
/Keith Kiedrow, Pharm. D., Project Manager, DPP
/ASeifried, OND IO

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/s/

David Jacobson-Kram
6/16/2008 07:56:55 AM

Attachment IV

Executive CAC
Date of Meeting: March 3, 2009

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Barry Rosloff, Ph.D., DPP, Alternate Member
Aisar Atrakchi, Ph.D., DPP, Team Leader
Elzbieta Chalecka-Franaszek, Ph.D., DPP, Presenting Reviewer

Author of Draft: Elzbieta Chalecka-Franaszek, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #22-117
Drug Name: Asenapine maleate
Sponsor: Organon USA Inc.

Background:

On March 25, 2008, the Executive CAC made the following recommendations regarding the completed carcinogenicity studies in rats and mice:

Rat:
Although the exec-CAC did not concur with the doses, the reason for non-concurrence was that it had been thought that the doses were not high enough. However, the doses turned out to be sufficiently high, and it was noted that the MTD was exceeded.

Since it is known that a significant decrease in body weight can lead to a decrease in tumor development, the sponsor should conduct a full histopathologic examination of the lower dose groups when an excessive decrease in body weight (or survival) is observed in the examined dose group. Because full histological evaluation was conducted only on tissues from the two control groups (vehicle and untreated) and the high dose group in both genders, the rat carcinogenicity study cannot yet be evaluated for either male or female rats.

The sponsor should be asked to perform a full histopathology evaluation of the low dose and mid dose males and females and submit the findings to the Division, as soon as possible.

Mouse:
Although the exec-CAC did not concur with the doses, the reason for nonconcurrence was that it had been thought that the doses were not high enough. However, the doses turned out to be sufficiently high, and it was noted that the MTD was exceeded.

The Committee concurred that the incidence of malignant lymphoma in high dose females was significantly increased compared to the vehicle control but not to the untreated control.

The Sponsor should be asked for an explanation for the large difference in the incidence of lymphomas between vehicle and untreated female controls. Furthermore, full histopathology of the low-dose and mid-dose female groups should be performed.

The final evaluation of the lymphomas will be made after the additional data are received.

On May 27, 2008, in response to the Sponsor's contention that additional groups did not have to be examined, the Executive CAC made the following recommendations:

The Committee concurred that the carcinogenicity studies filed to the NDA are considered "unacceptable" without completion of the full histopathological examination of the low and mid-dose dose male and female groups in the rat carcinogenicity study and the full histopathological examination of the low and mid-dose dose females in the mouse carcinogenicity study.

On August 29, 2008, results of the requested extended histopathological examination of the remaining low and mid-dose male and female dose groups in the rat carcinogenicity study and low and mid-dose female dose groups in the mouse carcinogenicity study were submitted for review.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee found that the study was adequate.
- The Committee concluded that there were no drug related neoplasms found in the study.

Mouse:

- The Committee found that the study was adequate.
- The incidence of pleomorphic malignant lymphomas in the hemolymphoreticular system and all combined malignant lymphomas was statistically significantly increased in the female mice at the high dose compared to the vehicle control. However, the incidence of this tumor at the high dose was similar to that in the untreated controls.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DPP
/Barry Rosloff, Ph.D., Pharm/Tox Supervisor, DPP
/Aisar Atrakchi, Ph.D., Team Leader, DPP
/Elzbieta Chalecka-Franaszek, Ph.D., Reviewer, DPP
/Keith Kiedrow, Pharm. D., Project Manager, DPP
/Adele Seifried, OND IO

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/s/

Abby Jacobs
3/30/2009 02:02:23 PM

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22117	ORIG 1	ORGANON USA INC	SYCREST (ASENAPINE) TABLETS

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/s/

ELZBIETA CHALECKA FRANASZ
07/27/2009

BARRY N ROSLOFF
07/27/2009
I agree with conclusions and recommendations

AISAR H ATRAKCHI
07/27/2009

Barry N. Rosloff, Ph.D.
6/24/08

P/T SUPERVISORY COMMENTS ON OCP REVIEW AMENDMENT #1

In his review amendment #1, filed electronically on 6/18/08, Dr. Kavanagh makes numerous comments on various aspects of the non-clinical studies submitted for asenapine, with the apparent intent of indicating that such studies showed effects which predict serious adverse reactions in humans. I do not find his arguments convincing. Many involve speculation regarding the adverse consequences of serotonergic stimulation. Aside from the fact that data indicate that asenapine itself is a serotonergic *antagonist* (although of course it is possible that its metabolites are not), the range of adverse effects which Dr. Kavanagh is speculating to be due to serotonergic agonism (as well as the wide range of drug classes he implicates) is so broad as to be useless for informing the direction of any future clinical monitoring.

As to the actual data, Dr. Kavanagh discusses the animal reproduction studies which were performed and concludes that asenapine had "dose-dependent embryo-fetal toxicity in all species and strains", caused "an increase in the postnatal loss of pups", and had effects on "skeletal muscle formation, and remodeling, including poor ossification…consequently asenapine is expected to effect bone and connective tissue especially during development, growth, and in the elderly or other populations at risk, e.g. renal failure patients".

An increase in embryofetal toxicity and postnatal loss were indeed seen, and are discussed in Dr. Chalecka-Franaszek's review and are described in our proposed labeling. (However, it should be noted that overall, the animal reproduction findings were not particularly alarming. The drug did not induce malformations. Embryofetal and pup deaths are often seen at the higher doses in these types of studies and can often be attributed to toxicity to the dams resulting in lack of maternal care, although there was some evidence that at least some of the effects of asenapine were due to prenatal drug exposure). Dr. Kavanagh stresses the "poor ossification" seen in these studies; however this is a common finding at higher doses in animal reproduction studies which may be secondary to maternal toxicity but even if not merely represents a transient, reversible delay in development, and not a direct or toxic effect on bone. Finally, I am not aware of any effects on skeletal muscle or connective tissue in these studies.

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/s/

Barry Rosloff
6/24/2008 02:03:01 PM
PHARMACOLOGIST

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO
NDA: 22-117
Date of submission receipt: August 30, 2007
Drug: asenapine maleate (Sycrest)
Sponsor: Organon USA
Indication: adults with schizophrenia or adults with acute manic or mixed episodes associated with bipolar I disorder

Reviewing Division: Division of Psychiatry Products

Comments:
The pharmacology/toxicology reviewer recommended that this NDA not be approved based on inadequate carcinogenicity studies. Two year studies in rats and mice were conducted; however, they are not yet considered adequate.

Carcinogenicity:
Asenapine maleate was administered to rats at doses of 0.3, 1.2 and 3.0/5.0 mg/kg/day by subcutaneous injection. The sponsor increased the high dose from 3.0 to 5.0 mg/kg after six weeks of dosing. Significant decreases in body weight gain were observed in all drug-treated groups. In addition, the overall tumor incidence in the high dose group was significantly lower than control. Complete histopathology of the mid and low dose groups was not conducted.

Asenapine maleate was administered to mice at doses of 0.5, 1.5 and 5.0/4.0 mg/kg/day in males or 7.5/5.0 mg/kg/day in females by subcutaneous injection. The sponsor decreased the high doses during week 25 because of high morbidity and mortality. Dosing was stopped in the mid dose and high dose males in week 88 and in mid dose and high dose females in week 95 and 97. These animals were maintained treatment-free until termination. The males were terminated in weeks 89/90 and the females in weeks 98/99. Body weight gain was decreased in males at all doses. A significant increase in malignant lymphoma was observed when high dose females were compared to the vehicle control but not to the untreated control. There was a large difference in the incidence of lymphoma between the vehicle control and the untreated control. Complete histopathology of the mid and low dose groups was not conducted.

These studies were presented to the CDER executive carcinogenicity assessment committee. The committee could not fully evaluate the rat study because of the significant decrease in body weight and the lack of full histopathology examinations in all dose groups. Therefore, the committee recommended that the sponsor perform a full histopathology evaluation of the low and mid dose groups in the rat study.

The committee could also not fully evaluate the mouse study because of the large variability in the lymphoma incidence in the groups evaluated. Therefore, the committee

recommended that the sponsor perform a full histopathology evaluation of the low and mid dose female groups in the mouse study.

Developmental and reproductive toxicity:
The pharmacology/toxicology reviewer recommended pregnancy category C. Asenapine maleate was not teratogenic in studies in rat and rabbits although the maximum exposures tested did not greatly exceed the anticipated maximum human exposure (approximately 2 fold). Some fetal and neonatal toxicity was observed in a rat study at doses that did not exceed the human exposures. Pregnancy category C is appropriate in spite of these observed adverse effects because of the potential utility of the product in the proposed indication.

Impurities:
The sponsor proposed a (b) (4) specification for an impurity (b) (4) that exceeds the ICH threshold for qualification. The impurity was qualified in genotoxicity studies and 4 week studies in rats and dogs. A non-GLP pilot segment II study was conducted with the impurity in rabbits; however, the reviewer found this study to be inadequate for several reasons. The reviewer recommends that the sponsor conduct a rabbit embryofetal toxicity study with the impurity post-approval or reduce the impurity specification to (b) (4), which is the qualification threshold. I concur with these recommendations.

Conclusion:
Asenapine maleate could be used in a chronic manner in the intended indication; therefore, it is appropriate to have adequate carcinogenicity data prior to approval. I concur with the pharmacology/toxicology recommendation of the Division that this NDA not be approved until the complete information from the carcinogenicity studies is submitted, reviewed and found to support the approval.

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/s/

Paul Brown
6/23/2008 03:05:20 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-117**
SERIAL NUMBER:	**0000**
DATE RECEIVED BY CENTER:	**08/30/2007**
PRODUCT:	**asenapine maleate**
INTENDED CLINICAL POPULATION:	**adults with schizophrenia or adults with acute manic or mixed episodes associated with bipolar I disorder**
SPONSOR:	**Organon USA Inc.**
DOCUMENTS REVIEWED:	**electronic submission**
REVIEW DIVISION:	**Division of Psychiatry Products (HFD-130)**
PHARM/TOX REVIEWER:	**Elzbieta Chalecka-Franaszek, Ph.D.**
PHARM/TOX SUPERVISOR:	**Barry Rosloff, Ph.D.**
DIVISION DIRECTOR:	**Thomas Laughren, M.D.**
PROJECT MANAGER:	**Keith Kiedrow, Pharm.D.**

Date of review submission to Division File System (DFS): April 30, 2008

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

It is recommended the NDA 22-117 for asenapine maleate not be approved until the issues concerning the rat and mouse carcinogenicity studies described below have been resolved.

Note: The reviewing pharmacologist has not received requested information regarding major circulating asenapine metabolites in humans from the Clinical Pharmacology and Biopharmaceutics Review Team. There is a possibility that additional major human metabolites are identified that have not been qualified to date in preclinical toxicity studies. Therefore, the pharmacology/toxicology final recommendations may require modification depending on resolution of this issue.

B. Recommendation for nonclinical studies

1. Carcinogenicity studies: Based on review of the carcinogenicity studies, entitled "104 week subcutaneous administration oncogenicity study with Org 5222 in the rat" and "104 week subcutaneous administration oncogenicity study with Org 5222 in the mouse" it is concluded that both studies are inadequate and further information from the Sponsor is needed to complete evaluation of the carcinogenic potential of asenapine.

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, the Sponsor should conduct a full histopathologic examination of the low and mid dose males and females.

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. The Sponsor should provide an explanation for the large difference in the incidence of lymphomas between vehicle and untreated female controls. 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.

2. Qualification of impurity Org 5033: Drug substance impurity (b) (4) has been present in the drug substance commercial size clinical/stability batches at (b) (4). However, the Sponsor proposed to set a specification limit for this impurity in asenapine drug substance at (b) (4), thus above the ICH Q3A(R) qualification limit of (b) (4). The content of (b) (4) 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 of (b) (4) 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 (b) (4) 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) (b) (4) 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 (b) (4) 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 (b) (4) in the rabbit to qualify this impurity during phase IV or reduce the specification of (b) (4) to the ICH Q3A(R) qualification limit of (b) (4).

C. Recommendations on labeling

Mechanism of Action: "The mechanism of action of asenapine, as with other drugs having efficacy in schizophrenia and bipolar disorder, is unknown. Nonetheless, it has been proposed that the efficacy of asenapine is mediated through a combination of antagonist activity at D_2 and 5-HT_{2A} receptors. Actions at other receptors e.g., 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, 5-HT_6, 5-HT_7, D_3, and α_2-adrenergic receptors, may contribute to the clinical effects of asenapine".

Pharmacodynamics: "Asenapine exhibits high affinity for dopamine D_2, D_3, D_4, and D_1 receptors (Ki values of 1.3, 0.42, 1.1, and 1.4 nM), serotonin 5-HT_{1A}, 5-HT_{1B} , 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_5, 5-HT_6 and 5-HT_7 receptors (Ki values of 2.5, 4.0, 0.06, 0.16, 0.03, 1.6, 0.25, and 0.13 nM), α_1 and α_2-adrenergic receptors (Ki values of 1.2 and 1.2 nM), and H_1 receptors (Ki value 1.0 nM), and moderate affinity for H_2 receptors (Ki value of 6.2 nM). In *in vitro* assays asenapine acts as an antagonist at these receptors. Asenapine has no appreciable affinity for muscarinic cholinergic receptors (e.g., Ki value of 8128 nM for M_1)".

Carcinogenesis: No recommendations can be provided at this time due to the lack of adequate carcinogenicity studies.

Mutagenesis: "The genotoxic potential of asenapine was tested in the *in vitro* bacterial reverse mutation assay, the *in vitro* forward gene mutation assay in mouse lymphoma cells, the *in vitro* chromosomal aberration assay in human lymphocytes, the *in vitro* sister chromatid exchange assay in rabbit lymphocytes, and the *in vivo* micronucleus assay in rats. Asenapine was negative in these assays, except for an equivocal response in the

chromosomal aberration assay. The weight of evidence suggests that asenapine lacks genotoxic potential".
Impairment of Fertility: "Asenapine did not impair fertility in rats when tested at doses up to 15 mg/kg b.i.d. given orally. This dose is 15 times the maximum recommended human dose of 10 mg b.i.d. given sublingually".
Pregnancy: Pregnancy Category C
"Asenapine was not teratogenic in reproduction studies in rats and rabbits at i.v. doses up to 1.5 mg/kg in rats and 0.44 mg/kg in rabbits. These doses are 0.73 and 0.43 times, respectively, the maximum recommended human dose (MRHD) of 10 mg b.i.d. given sublingually on a mg/m^2 basis. Plasma level of asenapine was measured in the rabbit study, and the AUC at the highest dose tested was 2 times that in humans receiving the MRHD.
In a study in which rats were treated from day 6 of gestation through day 21 postpartum with i.v. doses of asenapine of 0.3, 0.9, and 1.5 mg/kg/day (0.15, 0.44, and 0.73 times the MRHD of 10 mg b.i.d. given sublingually on a mg/m^2 basis), increases in post-implantation loss and early pup deaths were seen at all doses, and decreases in subsequent pup survival and weight gain were seen at the two higher doses. A cross-fostering study indicated that the decreases in pup survival were largely due to prenatal drug effects. Increases in post-implantation loss and decreases in pup weight and survival were also seen when pregnant rats were dosed orally with asenapine.
There are no adequate and well-controlled studies in pregnant women. Sycrest should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus".

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Absorption, distribution, metabolism, and excretion (ADME): Asenapine is being developed for the sublingual route of administration. However, it was previously studied as a potential oral drug. Therefore, oral administration was used in initial preclinical studies. The oral route was abandoned because of very low bioavailability in humans (<2%). Bioavailability was also low in animals, i.e. between 20-65% in rats and up to 10% in dogs. The sublingual route of administration is impractical in laboratory animals. Therefore, intravenous and subcutaneous routes were used in animals in preclinical studies. Some studies in dogs were conducted using the sublingual route. Exposures to asenapine achieved in preclinical studies at least at one dose were greater than typical level of human exposure (AUC_{0-12} = 43.4 ng·h/mL; 86.8 ng·h/mL for 10 mg b.i.d dosing; C_{max} = 6.6 ng/mL, based on data from the thorough QTc study). Asenapine was shown to have a relatively high clearance, high volume of distribution and short half-life in laboratory animals. Preclinical studies demonstrated that asenapine is well absorbed when administered by the sublingual route, readily penetrates to tissues, including brain and CSF, crosses the placental barrier and is excreted in milk. Protein binding of asenapine is relatively high in all species, including humans. Asenapine does not appear to be a substrate for P-glycoprotein. Metabolism studies demonstrated multiple pathways of metabolism, with oxidative metabolism via N-demethylation and N-oxidation being predominant in laboratory species. In humans, asenapine is metabolized via oxidation to

N-desmethyl and hydroxy metabolites, and via direct conjugation with glucuronic acid. Major pathways of metabolism in humans appear to be present in animals. Further metabolism of metabolites has been also observed. Over 40 metabolites have been detected in circulation and excreta of laboratory animals and human subjects. 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. The enzymes involved in oxidative and conjugative metabolism of asenapine in humans are CYP1A2 and UGT1A4. Mass balance studies with radiolabeled asenapine were conducted in several laboratory species (mouse, rat, rabbit, and dog) and in humans. Unchanged asenapine was present in human feces at 5-15% of the dose. Total recovery values were in excess of 80%. Asenapine does not inhibit most human cytochrome P450 enzymes, but does inhibit CYP2D6. No pharmacokinetic differences were observed between the asenapine 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.

General toxicity studies: Effects of asenapine were assessed in single and repeated dose general toxicology studies in rats, mice and dogs. 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.

Toxicologically important observations in rats in repeated oral toxicity studies consisted of palpebral ptosis, reduced motor activity, miosis, decreased body weight and decrease in food consumption in males, decreased body weight and increase in food consumption in females, deteriorating overall body condition and death, slight reductions in blood glucose, cholesterol and triglycerides, slight increases in blood urea nitrogen, creatinine values and urinary volume (indicating renal effect but without histopathological findings in kidneys), disturbance in the estrus cycle, and stimulation of the mammary glands. The effects on the CNS and those caused by deregulation of prolactin release by interference with dopaminergic and serotonergic regulation of the hypothalamus-pituitary axis were considered related to the pharmacologic activity of asenapine. Such effects were not considered adverse unless the degree was such that it led to severe deterioration of the health of animals.

Toxicologically important observations in dogs in repeated oral toxicity studies consisted of miosis, reduced activity, motor incoordination, tremors, abnormal behavior, increased heart rate, reduced body weight and food consumption (in some animals at doses $\geq$ 20 mg/kg/day), inhibition of spermatogenesis in males, disturbance of the estrus cycle and increased secretory activity of mammary glands in females, and an increased liver enzyme activity in blood accompanied by various degrees of liver cell damage and

inflammatory cell reactions. Moreover, accumulation of grayish-brownish intrahepatocellular pigment was noted in dogs in the 52-week study. However, this effect was not accompanied by morphological lesions or liver dysfunction in this study. In the 13- and 39-week intravenous toxicity studies no hepatotoxicity was observed.

The reversibility of asenapine-induced effects was investigated in the oral 13- and 52-week studies. Most effects were partially reversible. In adult and juvenile rats hyperactivity was observed after the treatment with asenapine was stopped, followed by a gradual decrease over 8 weeks in adult animals and no decrease in young animals during a 4-week recovery period.

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 22/60 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. 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.

Local tolerance: 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.

Special toxicology: 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:

Impurity (b) (4). Drug substance impurity (b) (4) has been present in the drug substance commercial size clinical/stability batches at (b) (4). However, the Sponsor proposed to set a specification limit for this impurity in asenapine drug substance at (b) (4), thus above the ICH Q3A(R) qualification limit of (b) (4). The content of (b) (4) 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 of (b) (4) 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 (b) (4) 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) (b) (4) 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 (b) (4) 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 (b) (4) in the rabbit to qualify this impurity during phase IV or reduce the specification of (b) (4) to the ICH Q3A(R) qualification limit of (b) (4).

Other studies conducted to qualify (b) (4) 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 (b) (4) is considered non-genotoxic and qualified for general toxicity.

Impurity (b) (4)**.** Studies conducted to qualify the impurity (b) (4) directly included genotoxicity studies (Ames tests and chromosomal aberrations study in cultured human lymphocytes) and an intravenous 2-week toxicity study with (b) (4) in Wistar rats. Reproductive toxicology study (embryofetal toxicity study in rats) recommended by the Agency for qualification of impurities was not conducted with (b) (4). However, (b) (4) is considered to be qualified based on the presence of adequate amounts of this impurity in reproductive toxicology studies conducted with asenapine.

Qualification of degradants in drug product:

The following degradation products have been identified in asenapine tablets: (b) (4) .

(b) (4), 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, (b) (4) is an aliphatic N-oxide (usually aromatic N-oxides are known for structural alerts for mutagenicity). (b) (4) 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 (b) (4) achieved in rats and dogs following long term treatment (oral and intravenous administration) is similar to or exceeds that to 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 (b) (4) will significantly increase with time up to (b) (4) respectively. It is concluded that (b) (4) is qualified with respect to its general toxicity, reproductive toxicity and genotoxicity based on its presence as the metabolite in laboratory animals.

(b) (4)**:** As indicated by the reviewing chemist Dr. Tele**,** (b) (4) is present at levels less than (b) (4) 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)**:** 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 of (b) (4). 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 (b) (4) 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 (b) (4) 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. Pharmacologic activity

Nonclinical pharmacology studies have demonstrated that asenapine is a potent multi-receptor antagonist with a high affinity for several serotonin, dopamine, noradrenaline and histamine receptors. Asenapine has subnanomolar affinity for the serotonin 5-HT_{2C}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_6 and 5-HT_7 receptors and nanomolar affinity for the serotonin 5-HT_5, 5-HT_{1A}, 5-HT_{1B}, α_1, α_{2A}, α_{2C}, and dopamine D_2 receptors. Asenapine was also shown to act a as a functional antagonist at serotonin, dopamine, noradrenaline and histamine receptors. It potently blocked agonist-induced activation of 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{1A}, 5-HT_{1B}, 5-HT_6, 5-HT_7, D_1, D_2, D_3, α_1, α_{2A}, α_{2B}, α_{2C}, H_1, and H_2 receptors. *In vivo* in acute rodent behavioral studies asenapine showed potent antiserotonergic and antidopaminergic properties. It also demonstrated activity in animal models with potential relevance to schizophrenia and bipolar disorder. Thus, prolonged administration of asenapine attenuated cognitive impairment in monkeys and chronic mild stress-induced loss of reward sensitivity in rats. Based on its pharmacological activity, asenapine is expected to show efficacy in the treatment of positive symptoms of schizophrenia and mania associated with bipolar disorder.

C. Nonclinical safety issues relevant to clinical use

Target organs of asenapine toxicity in the species used in the toxicology studies include the central nervous system, cardiovascular system, female reproductive organs and mammary glands, thyroid and adrenal glands. Effects of treatment of humans with asenapine on these tissues, organs or systems are possible based on preclinical studies.

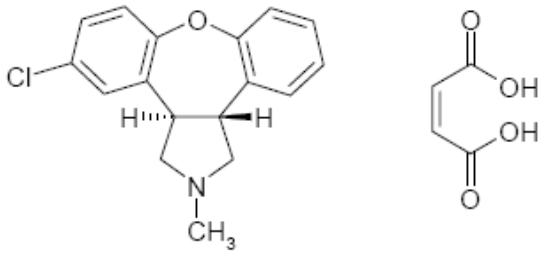
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-117
Review number: 1
Sequence number/date/type of submission: 0000
Information to Sponsor: Yes (x) No ( )
Sponsor and/or agent: Organon USA, Inc.
Manufacturer for drug substance: N.V. Organon, The Netherlands
Reviewer name: Elzbieta Chalecka-Franaszek, Ph.D.
Division name: Division of Psychiatry Products
HFD #: 130
Review completion date: April 14, 2008

Drug:

Trade name: Sycrest
Generic name: Asenapine maleate
Code name: Org 5222
Chemical name: (3aR,12bR)-rel-5-chloro-2,3,3a, 12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole(2Z)-2-butenedioate (1:1)
CAS registry number: 85659-56-2
Molecular formula/molecular weight (g/mol): $C_{17}H_{16}ClNO.C_4H_4O_4$/asenapine maleate: 401.84; active entity: 285.8
Structure:



Asenapine maleate

Relevant INDs/NDAs/DMFs: IND 51,641 for asenapine (Org 5222) sublingual tablets for schizophrenia; IND 70,329 for asenapine (Org 5222) sublingual tablets for bipolar disorder; DMF (b) (4)
Drug class: Asenapine is a potent multi-receptor antagonist with a high affinity for several serotonin, dopamine, noradrenaline, and histamine receptors, indicated for the treatment of schizophrenia or acute manic or mixed episodes associated with bipolar I disorder.
Intended clinical population: adults with schizophrenia or adults with acute manic or mixed episodes associated with bipolar I disorder

Clinical formulation: fast dissolving sublingual tablets
Route of administration: sublingual
Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: All pivotal studies submitted to the NDA 22-117 except toxicology studies reviewed previously by Drs. Lois Freed and Sonia Tabacova under INDs 51,641 and 70,329. Their reviews are available in the Division's file and DARRTS. In addition, the summaries of pivotal studies and conclusions taken directly from Drs. Freed and Tabacova reviews are included in relevant sections of this review.

Studies not reviewed within this submission: Selected dose-ranging and other supportive, non-pivotal studies that do not have impact on the evaluation of the safety of asenapine administration have not been reviewed.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary (based on the Sponsor's summary)

Nonclinical pharmacology studies have demonstrated that asenapine is a potent multi-receptor antagonist with a high affinity for several serotonin, dopamine, noradrenaline and histamine receptors. Asenapine has subnanomolar affinity for the serotonin 5-HT_{2C}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_6 and 5-HT_7 receptors and nanomolar affinity for the serotonin 5-HT_5, 5-HT_{1A}, 5-HT_{1B}, α_1, α_{2A}, α_{2C}, and dopamine D_2 receptors. Asenapine was also shown to act a as a functional antagonist at serotonin, dopamine, noradrenaline and histamine receptors. It potently blocked agonist-induced activation of 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{1A}, 5-HT_{1B}, 5-HT_6, 5-HT_7, D_1, D_2, D_3, α_1, α_{2A}, α_{2B}, α_{2C}, H_1, and H_2 receptors. However, asenapine is devoid of binding activity at muscarinic, β1- and β_2-adrenergic, H_3 histamine, and 5-HT_3 receptors. In monoamine uptake assays, asenapine showed no appreciable activity at dopamine, norepinephrine, and 5-HT transporters, and was inactive in a wide general specificity screen for many other receptors. In studies conducted *in vivo* asenapine demonstrated potent antiserotonergic and antidopaminergic properties. It demonstrated also activity in animal models with potential relevance to schizophrenia and bipolar disorder. Prolonged administration of asenapine attenuated cognitive impairment in monkeys and chronic mild stress induced loss of reward sensitivity in rats. Treatment with asenapine induced catalepsy in rats at dosages higher then those needed to show activity in tests predictive of antipsychotic activity. Therefore, the Sponsor suggested its low potential propensity for the induction of extrapyramidal symptoms. Asenapine is a 50:50 mixture of two enantiomers (Org 10968 and Org 10969). Both of them have similar activity and contribution towards the pharmacological effects of asenapine. Based on its pharmacological activity, asenapine is expected to show efficacy in the treatment of positive symptoms of schizophrenia and mania associated with bipolar disorder.

In safety pharmacology studies, behavioral effects of asenapine were tested in Cebus monkeys previously sensitized to neuroleptics. Single i.m. doses (0.01-0.25 mg/kg) of asenapine tested blindly at weekly intervals dose dependently decreased locomotor activity and reactivity to environmental stimuli at the dose that moderately affected other parameters of motor function (dystonia and bradykinesia). Org 5222 exhibited anxiolytic effects in several animal models, e.g., shock-induced lick suppression, the elevated X-maze (0.05- 1 mg/kg i.p.). Org 5222 exhibited sedative properties (related to α_1 and/or H_1 receptor activity) in the hexobarbital titration, rotarod, and hot plate tests (ED_{50}'s = 0.13-10 mg/kg s.c.). Other effects of asenapine on the CNS were evaluated in the general toxicology studies. Cardiovascular safety studies indicated that sublingual administration of asenapine induced dose-dependent increase in heart rate and hypotension in dogs, and orthostatic hypotension and increase in heart rate in rats. A decrease in arterial blood pressure was probably a result of α_1-adrenoceptor blocking activity. Sublingually administered asenapine has fewer cardiovascular effects than orally administered asenapine. QTc prolongation in dogs following oral and sublingual administration was minimal (at most 15 msec above control levels). Two enantiomers demonstrated the same cardiovascular activity as racemate. The metabolite desmethyl-asenapine was less active than asenapine in its effects on arterial blood pressure and heart rate in dogs. The effects of asenapine and desmethyl-asenapine in the hERG assay demonstrated the IC_{50} values of 0.3 μM and 0.7 μM, respectively. Therefore, asenapine and desmethyl-asenapine may interact with the hERG channel at clinically relevant concentrations. The N-glucuronide metabolite (the major human metabolite) had no hERG activity. Respiratory activity studies in conscious rats following subcutaneous administration of asenapine at 5 mg/kg demonstrated transient central respiratory depression (increased tidial and expired volume, enhanced pause). Endocrinological studies showed that subcutaneous administration of asenapine induced increases in prolactin release in rat plasma. No other effects on the endocrine system were observed in the safety pharmacology studies. Asenapine showed short-lasting effects on the intestinal system in guinea pigs following i.v. dosing and showed an acute ulcerogenic activity in rats following p.o. administration but no effect in gastrointestinal transit time. Asenapine showed a stronger local anesthetic activity compared to lidocaine.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

The pharmacological effects of asenapine were investigated *in vitro* and *in vivo*. The human and rodent CNS receptor binding properties of asenapine were determined in multiple studies. Asenapine binds with high affinity (expressed as pKi values) to a range of human serotonin, dopamine, noradrenaline and histamine receptor subtypes. The human receptor binding profile was asenapine was compared to several other antipsychotic drugs (aripiprazole, ziprasidone, quetiapine, olanzapine, risperidone, clozapine, and haloperidol). From 27 receptors examined in detail, asenapine showed greater than 5 nM affinity for 18 of these receptor subtypes. Asenapine has subnanomolar affinity for several human 5-HT receptor subtypes: 5-HT_{2C}, 5-HT_{2A}, 5-HT_7, 5-HT_{2B}, and 5-HT_6. Subnanomolar affinity was also observed for α_{2B}-adrenergic and dopamine D_3

receptors. Asenapine has nanomolar affinity for a range of other receptors. For the majority, but not all (eg. 5-HT_{1A}, 5-HT_{1B}, and D_2 receptors) of tested receptors asenapine has the highest affinity of all the drugs tested. Asenapine is the only drug with appreciable affinity for histamine H_2 receptors. In contrast to olanzapine, quetiapine and clozapine, asenapine has no appreciable affinity for muscarinic receptors. The summary of human receptor binding affinities of asenapine and comparator drugs is shown in the following Sponsor's table:

Receptor	asenapine	aripiprazole	ziprasidone	quetiapine	olanzapine	risperidone	clozapine	haloperidol
5-HT_{1A}	8.60 ± 0.04	8.57 ± 0.04	9.01 ± 0.03	6.78 ± 0.03	5.82 ± 0.05	6.75 ± 0.02	7.06 ± 0.02	6.29 ± 0.03
5-HT_{1B}	8.40 ± 0.08	8.55 ± 0.11	9.05 ± 0.11	<6.50	6.60 ± 0.04	7.29 ± 0.11	6.57 ± 0.07	<6.00
5-HT_{2A}	10.15 ± 0.09	8.02 ± 0.16	9.51 ± 0.06	6.81 ± 0.02	8.88 ± 0.03	9.69 ± 0.01	8.39 ± 0.03	7.28 ± 0.02
5-HT_{2B}	9.75 ± 0.03	9.59 ± 0.17	9.08 ± 0.01	7.33 ± 0.01	8.41 ± 0.11	7.99 ± 0.13	8.79 ± 0.09	6.48 ± 0.10
5-HT_{2C}	10.46 ± 0.15	7.55 ± 0.14	9.01 ± 0.05	5.98 ± 0.05	8.41 ± 0.12	8.17 ± 0.11	8.56 ± 0.06	5.79 ± 0.01
5-HT_{5A}	8.84 ± 0.21	6.05 ± 0.12	5.95 ± 0.07	5.70 ± 0.19	7.00 ± 0.07	7.23 ± 0.10	7.60 ± 0.13	6.10 ± 0.09
5-HT_6	9.60 ± 0.04	6.64 ± 0.07	6.78 ± 0.13	5.64 ± 0.04	8.49 ± 0.06	5.66 ± 0.02	8.05 ± 0.10	5.44 ± 0.06
5-HT_7	9.94 ± 0.04	7.46 ± 0.18	8.60 ± 0.23	7.25 ± 0.09	7.43 ± 0.08	9.13 ± 0.06	8.19 ± 0.01	7.05 ± 0.04
D_1	8.85 ± 0.04	6.09[a]	8.45 ± 0.08	6.71[a]	7.93 ± 0.13	7.68 ± 0.02	7.64 ± 0.12	8.20 ± 0.14
D_{2A}	8.90 ± 0.08	8.94 ± 0.04	8.09 ± 0.15	6.38 ± 0.13	7.67 ± 0.04	8.21 ± 0.04	6.87 ± 0.08	8.84 ± 0.06
D_{2B}	8.84 ± 0.05	8.91 ± 0.06	7.99 ± 0.09	6.32 ± 0.06	7.58 ± 0.00	8.07 ± 0.06	6.81 ± 0.04	8.76 ± 0.14
D_3	9.38 ± 0.06	8.85 ± 0.02	8.35 ± 0.03	6.41 ± 0.03	7.46 ± 0.07	8.16 ± 0.08	6.66 ± 0.08	8.56 ± 0.08
D_4	8.95 ± 0.07	6.89 ± 0.08	7.33 ± 0.08	5.85 ± 0.07	7.75 ± 0.07	8.21 ± 0.04	7.33 ± 0.07	8.83 ± 0.08
α_{1A}	8.93 ± 0.04	6.49 ± 0.06	7.81 ± 0.09	7.19 ± 0.04	7.65 ± 0.05	8.29 ± 0.03	7.90 ± 0.03	7.60 ± 0.03
α_{2A}	8.94 ± 0.05	7.16 ± 0.16	6.59 ± 0.17	6.25 ± 0.21	6.83 ± 0.08	8.09 ± 0.05	7.54 ± 0.04	6.06 ± 0.05
α_{2B}	9.49 ± 0.02	6.72 ± 0.10	6.62 ± 0.19	7.08 ± 0.06	6.48 ± 0.02	8.02 ± 0.02	7.55 ± 0.09	6.25 ± 0.03
α_{2C}	8.91 ± 0.12	7.93 ± 0.19	7.38 ± 0.19	7.42 ± 0.09	7.39 ± 0.01	8.74 ± 0.03	8.80 ± 0.11	6.88 ± 0.05
H_1	9.00 ± 0.13	7.69 ± 0.16	6.89[a]	7.96 ± 0.07	8.47 ± 0.06	7.09 ± 0.03	8.76 ± 0.06	5.68[a]
H_2	8.21 ± 0.10	5.15[a]	4.55[a]	5.18[a]	5.50[a]	6.32[a]	5.91[a]	5.50[a]
M_1	5.09 ± 0.03	5.41 ± 0.10	<5.00	6.55 ± 0.03	7.92 ± 0.21	4.57 ± 0.03	8.29 ± 0.16	5.25 ± 0.03
M_2	4.50 ± 0.09	4.92 ± 0.17	<5.00	6.22 ± 0.04	7.40 ± 0.14	4.41 ± 0.03	7.15 ± 0.02	5.05 ± 0.05
M_3	4.67 ± 0.03	5.11 ± 0.06	<5.00	6.29 ± 0.04	7.47 ± 0.10	4.60 ± 0.07	7.61 ± 0.08	4.87 ± 0.02
M_4	5.04 ± 0.10	5.23 ± 0.23	<5.00	6.61 ± 0.07	7.65 ± 0.08	4.97 ± 0.02	7.68 ± 0.04	5.25 ± 0.05

Drug activity related to proposed indication:

In studies conducted *in vivo,* asenapine demonstrated potent antiserotonergic and antidopaminergic properties. It demonstrated also activity in animal models with potential relevance to schizophrenia and bipolar disorder. Prolonged administration of asenapine attenuated cognitive impairment in monkeys and chronic mild stress induced loss of reward sensitivity in rats. Treatment with asenapine induced catalepsy in rats at dosages higher then those needed to show activity in tests predictive of antipsychotic activity. Therefore, the Sponsor suggested its low potential propensity for the induction of extrapyramidal symptoms. Asenapine is a 50:50 mixture of two enantiomers (Org 10968 and Org 10969). Both of them have similar activity and contribution towards the pharmacological effects of asenapine. Based on its pharmacological activity, asenapine is expected to show efficacy in the treatment of positive symptoms of schizophrenia and mania associated with bipolar disorder.

2.6.2.3 Secondary pharmacodynamics

No secondary pharmacodynamics studies have been performed with asenapine.

2.6.2.4 Safety pharmacology

Pivotal safety pharmacology studies of asenapine (neurological, cardiovascular, gastrointestinal, and endocrine effects) submitted under the NDA have been previously submitted under the IND 51,641 (Serial No. 000) and reviewed by Dr. Lois Freed (Studies No.: SDG RR 2750, 3325, 4130, 4297, 3695, 2820, and 2749). Please see her review of June 11, 1997 in the Division's file for further details. The summary of pharmacology/safety pharmacology findings taken directly from Dr. Freed's review is provided below:

"The CNS pharmacology data were provided in summary form. Org 5222 exhibited highest *in vitro* binding affinity for the $5HT_{2A}$ and $5HT_{2C}$ receptors (human, pig, rat; K_i = 0.025-0.8 nM). High affinity was also exhibited for the D_2, D_3, and D_4 (human, K_i = 1.6, 0.8, and 1.25 nM, respectively), α_1 and α_2 adrenergic (K_i = 0.63 and 8 nM, respectively), and H_1 (K_i = 8 nM) receptors. Org 5222 had no appreciable affinity for the muscarinic receptor ($K_i \geq 1$ μM**).** Org 5222 exhibited ~64-fold greater potency for the $5HT_{2A}$ than for the D_{2A} receptor.

Dosing rats with Org 5222 (3-100 μg/kg s.c.) for 10-21 days resulted in a dose-dependent decrease in $5HT_2$ receptors (42-35% at 20-200 μg/kg, 80% at 30-100 μg/kg), but had no effect on D_l or D_2 receptors.

In vivo binding studies in rat demonstrated selective binding of ^{3}H-Org 5222 in areas high in $5HT_{2A, 2C}$ receptors, i.e., claustrum, choroid plexus, cortex. At a dose of 0.25 mg/kg i.p., the extent of receptor occupancy was estimated as 84, 7, and 44% at the $5HT_2$, D_1, and D_2 receptors, respectively.

In animal models thought to have predictive validity for antipsychotic potential, Org 5222 was active at ED_{50}'s of 0.02-0.04 mg/kg s.c. (apomorphine-induced climbing), 0.3-0.64 mg/kg i.p., p.o. (conditioned avoidance), and at 0.1 mg/ kg s.c. (prepulse inhibition). Also, Org 5222 antagonized D_l and D_2-mediated circling behavior at doses ≥ 0.05 and 1.5 s.c., respectively. Org 5222 induced catalepsy, considered to predict side-effect liability (i.e., eps) at doses of 0.84-4 mg/kg s.c. [In comparison, ED_{50} values for haloperidol, clozapine, and risperidone for induction of catalepsy were 0.2-2, 107 to >220, and 2.4-20 mg/kg s.c., respectively]. Org 5222 was only minimally effective in inducing perioral movements (species not specified in the summary) with repeat dosing (=2 mg/kg p.o.).

Org 5222 antagonized serotonin-agonist effects indicative of activity at the $5HT_{lA}$, $5HT_{2A}$, and $5HT_{2C}$ receptors. Org 5222 was a full antagonist at the $5HT_2$ receptor, but functioned as a partial agonist at the $5HT_{lA}$ receptor.

Org 5222 exhibited anxiolytic effects in several animal models, e.g., shock-induced lick suppression, the elevated X-maze (0.05- 1 mg/kg i.p.).

In neurochemical studies, Org 5222, given acutely, increased dopamine turnover in mesolimbic and striatal brain regions (≥3-10 pg/kg s.c.), but had minimal effect on 5HT turnover. Following multiple dosing, dopamine turnover was increased in the caudate, but

not in mesolimbic areas, and had no effect on 5HT turnover.

Org 5222 exhibited sedative properties (related to α_1 and/or H_1 receptor activity) in the hexobarbital titration, rotarod, and hot plate tests (ED_{50}'s = 0.13-10 mg/kg s.c.).

In terms of peripheral receptors, Org 5222 exhibited potent antagonism at the α_1 receptor in rat vas deferens, the $5HT_{2B}$ receptor in rat fundus strip, and at the H_1 receptor in guinea pig ileum. No activity was observed at the β-adrenergic or muscarinic receptors. In *in vivo* studies, Org 5222 exhibited ulcerogenic potential in rat (ED_{50} = 32 mg/kg p.o.) and a potent local anesthetic effect (potency ratio of 2.5 relative to lidocaine) as measured in an isolated toad nerve preparation.

Cardiovascular effects of Org 5222 were studied in rat, dog, cat, rabbit, and guinea pig. In anesthetized cats, Org 5222 (0.1-10 mg/kg i.v.) had no effect on heart rate or ECG parameters. Blood pressure, LV dP/dt, and nictitating membrane tone were dose-dependently reduced. Org 5222 did antagonized the effects of various agonists (histamine, NE, isoprenaline), vagal stimulation, and bilateral carotid artery occlusion.

In dog, the cardiovascular effects of Org 5222 were tested in anesthetized and conscious animals. In conscious dogs (Mongrel, Beagle), Org 5222 had the following effects: (1) decreases in blood pressure (0.05-10 mg/kg i.v., 1-50 mg/kg p.o., 0.1- 1 mg/kg s.l.), (2) reduced peripheral resistance (TPR, femoral resistance), CO and Cl (0.1-10 mg/kg i.v.), (3) decreased PR and QRS, and increased heart rate and QTc at 1-50 mg/kg p.o. and 0.1-1 mg/kg s.l., (4) potentiation of blood pressure and heart rate changes in response to head tilt (1-50 mg/kg p.o., 0.1-1 mg/kg s.1.; the heart rate response was only minimally affected by s.1. dosing. (5) increased LV peak d,P,/d,t, V_{max} peak velocity, and velocity 40 (0.1-10 mg/kg i.v.) (6) decreased carotid flow (0.1-10 mg/kg i.v.).

In anesthetized dog, Org 5222 inhibited the blood pressure response to head tilt, carotid occlusion (10 mg/kg i.v.), and NE (1, 10 mg/kg i.v.), but not to ACh or isoprenaline-induced changes. Systolic segmental shortening following head tilt was enhanced at 0.1-0.5 mg/kg i.v. Org 5222, when infused into isolated carotid sinus (10^{-7}- 10^{-1} M), potentiated the baroreceptor response to sinus pressurization at lower concentrations, but inhibited the baroreceptor response at higher concentrations.

Org 30526, a metabolite of Org 5222, reduced blood pressure, LVSSS, and PR, increased QTc, and potentiated heart rate and blood pressure changes following head tilt (5 mg/kg i.v.), but was less potent than Org 5222. When infused into isolated carotid sinus in anesthetized dog, had a slight inhibitory effect on the vasopressor response. Org 30526 exhibited a greater effect (i.e., enhancement) on systolic segmental shortening following head tilt than did Org 5222 (1-5 mg/kg i.v.).

In rat**,** the effects of Org 5222 and Org 30526 on the Bezold-Jarish reflex were compared. The Bezold-Jarish reflex (i.e., reflex bradycardia, diastolic and systolic hypotension) was induced by i.v. bolus injections of veratrine every 10 min. The primary effect of both compounds was an increase in heart rate, which was more prolonged with Org 30526 than Org 5222. At 3.0 mg/kg, Org 5222's effect on heart rate interfered with the analysis. Both compounds also lowered blood pressure (1.0 mg/kg); Org 30526's effect was greater than after either vehicle or Org 5222.

In conscious rabbit, Org 5222 reduced blood pressure (0.1, 1 mg/kg i.v. infusion) and heart

rate (0.01-1 mg/kg i.v. infusion) in the resting state, and inhibited the effects of NE, ACh, and isoprenaline on blood pressure (0.1-1 mg/kg i.v.) and enhanced isoprenaline's effect on hr (0.01-0.1 mg/kg i.v.). In isolated cardiac tissue from male rabbits, Org 5222 and Org 30526 (at doses >1 and ≥0.1 mg/kg) both exhibited negative inotropic effects, inhibiting the force of contraction in ventricular and atrial tissue. Org 5222 also exhibited negative chronotropic effects, reducing the frequency of spontaneous beating in atria (> 1 pM); Org 30526 had no chronotropic effects (at up to 100 pM). [There was, however, a large increase in the SEM at 10 μM, suggesting the possibility of an effect in tissue from individual animal(s).] In aortic ring preparations from New Zealand White rabbits, Org 5222 and Org 30526 both inhibited the spasmogenic effects of KCl in a concentration-dependent manner (1-30 μM), and to a similar extent.

In guinea pig ventricular papillary muscle preparations, Org 5222 had the following effects at 30-100 μM: (1) shortening of the APD_{50} with no effect on APD_{90}, (2) reduced V_{max}, and (3) increased effective refractory period. Org 30526 had the following effects: (1) increase in APD_{90} (10-100 pM), reduced V_{max}, (30-100 μM), and increase in the effective refractory period (concentration-dependent, 1-100 μM, i.e., all concentrations tested). The Sponsor indicated that these effects, indicative of Ca- (shortened APD_{50}), Na- (reduced V_{max}), and K- (prolongation of ERP) channel effects would have no clinical relevance (even though they could result in cardiac asystole) since they were observed at concentrations greater than those predicted to be achieved in humans. It should be noted, however, Org 30526's effect on ERP was noted at all concentrations tested.

GI, local anesthetic effects: the effect of Org 5222 (10 mg/kg p.o., b.i.d. for 4 days) had no effect on either GI motility or fecal production in rats. Org 5222 did exhibit slight ulcerogenic potential in rats at doses of 1 and 10 mg/kg p.o. The incidence and severity of ulcers was similar at the two doses, and markedly less than the response to indomethacin, the positive control.

In ligated ileum (guinea pig), Org 5222 increased the force of ileal contractions at doses of 1 and 5 mg/kg i.v. (bolus). At 5 mg/kg, there was a decrease in, the amplitude of the response.

Org 5222 exhibited local anesthetic properties in an isolated toad nerve preparation. According to the Sponsor, Org 5222's effect was greater than that of the positive control, lidocaine, with a potency ratio of 2.5.

Endocrine effects: when given for 7 days at a dose of 0.02 mg/kg to immature rats, Org 5222 produced decreases in adrenal and, to a lesser extent, in thyroid and seminal vesicles in male rats, and a decrease in thyroid wt in females. Changes in vaginal cornification were not observed.

Org 5222 exhibited no progesterone activity in estrogen-primed immature rabbits or antiprogesterone activity in estrogen-primed rabbits. Org 5222 also exhibited no anti-estrogen activity in ovariectomized, estrogen-primed rats. No mineralocorticoid activity was noted in adrenalectomized young male rats at a dose of 0.025 mg/rat (or =0.4 g/kg p.o.).

The effects of Org 5222 on serum prolactin were tested in male rats. In one study, Org 5222 was shown to increase serum prolactin levels for at least 1 'hr at all doses tested (0.032-0.5 mg/kg p.o.). Haloperidol (0.125, 0.5 mg/kg p.o.) produced similar (at 0.5 mg/kg) effect on serum prolactin in one experiment, but greater increases in serum prolactin (>3-fold) in a second experiment. In a second study, Org 5222 elevated serum prolactin levels at s.c. doses

of 0.1-1.0 mg/kg."

The following safety pharmacology study was submitted under the NDA 22-117:

Study title: In vitro hERG patch clamp assay of asenapine (Org 5222), Org 30526 (desmethyl-asenapine), and Org 216761-0 (asenapine N^+-glucuronide). (Study No. 745-04051) Testing facility: Pfizer, Ann Arbor, MI; Study initiation date: April 5, 2004; GLP: no

Asenapine and desmethyl-asenapine in the hERG assay demonstrated the IC_{50} values of 310 nmol/L and 670 nmol/L, respectively. Therefore, asenapine and desmethyl-asenapine may interact with the hERG channel at clinically relevant concentrations. The N-glucuronide metabolite (the major human metabolite) had no hERG activity. A summary of hERG data is presented in the Sponsor's table below:

	Asenapine		Desmethyl asenapine	
	$nmol.L^{-1}$	**$ng.mL^{-1}$**	**$nmol.L^{-1}$**	**$ng.mL^{-1}$**
hERG IC_{20}	55	15	190	52
hERG IC_{50}	310	83	670	182
C_{max} at 10 mg b.i.d.		6.56		2 [a]
Concentration unbound (C_u)		0.39		0.05 [b]
Ratio hERG IC_{20} / C_u	**38**		**1000**	

[a] Estimated from study 041-012 (section 5.3.3.2)

[b] protein binding 97.6% (See section 2.6.4.4.8, Report No DM2005-005222-007)

2.6.2.5 Pharmacodynamic drug interactions

No pharmacodunamic drug interaction studies were carried out with asenapine.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Safety pharmacology studies conducted with asenapine are summarized in the following Sponsor's table:

Table 13: Overview of in vitro safety pharmacology studies performed with asenapine and its desmethyl-metabolite (Org 30526)

TableModel	Concentration range of asenapine and desmethyl asenapine	Assessments	Ref.
Guinea-pig (ventricular papillary muscles)	1-100 $\mu mol.L^{-1}$	Effect on action potentials	4.2.1.3, Report No. 3695 [29]
Rabbit (aortic rings)	1-30 $\mu mol.L^{-1}$	Effect on KCl-stimulated muscle contractions	[29]
Rabbit (left and right atria)	0.001-100 $\mu mol.L^{-1}$	Effects on the amplitude of contraction (left atrial and ventricular tissues) and the rate of spontaneous contraction (right atria)	4.2.1.3, Report No. 4297 [30]
Dog (Purkinje fibers)	30 – 3000 $nmol.L^{-1}$	Resting potential, Vmax, APD50, APD70, APD90	4.2.1.3, Report No. NL0047838 [31], 4.2.1.3, Report No. NL0050226 [32]
hERG (transfected HEK293 cells)	Asenapine: 100 – 1000 $nmol.L^{-1}$ desmethylasenapine: 300 – 3000 $nmol.L^{-1}$ Org 216761-0: 1000 – 30,000 $nmol.L^{-1}$	Tail current inhibition	4.2.1.3, Report No. 745-04051 [33]
Isolated sciatic toad nerve	0.1 - 0.225 $mmol.l^{-1}$	Amplitude of action potentials	4.2.1.3, Report No. 2820 [34]

Table 14: Overview of in vivo safety pharmacology studies performed with asenapine

TableSpecies	asenapine dose[a] ($mg.kg^{-1}$)	Duration	Route	Parameters/assessments	Ref.
Anesthetized rat	1	s.d.	i.v.	Effect on Bezold-Jarish reflex	[30]
Pithed rat	0.1-3	s.d.	i.v.	Effects on tachycardia and pressor responses (elicited by electrical stimulation and i.v. administration of NA)	4.2.1.3, Report No. 4705 [35]
Conscious rabbit	0.01, 0.1 and 1	s.d.	i.v.	Effects on BP and HR together with changes in these variables induced by NA, Ach and Iso	4.2.1.3, Report No. 2750 [36]
Conscious dog	0.05, 0.1 and 0.5	s.d.	i.v.	Hemodynamic parameters, ECG parameters, responses to upright tilt	[30]
Conscious dog	1, 2.5, 5, 10 and 50	s.d.	p.o.	Hemodynamic parameters, ECG parameters, responses to upright tilt	4.2.1.3, Report No. 4130 [37], 4.2.1.3, Report No. INT00002533 [38]
Conscious dog	0.01, 0.1 and 1	s.d.	SL	Hemodynamic parameters, ECG parameters, responses to upright tilt	[37, 38]
Conscious dog	5 (individual enantiomers)	s.d.	p.o.	Hemodynamic parameters, ECG parameters, responses to upright tilt	4.2.1.3, Report No. NL001234 [39]
Conscious dog	0.1 (individual enantiomers)	s.d.	SL	Hemodynamic parameters, ECG parameters, responses to upright tilt	[39]
Anesthetized dog	0.1, 1 and 10	s.d.	i.v.	Effects on HR, arterial, right arterial and LV pressures, cardiac output, carotid and femoral flows, ECG and computer-derived	[36]

TableSpecies	asenapine dose* ($mg.kg^{-1}$)	Duration	Route	Parameters/assessments	Ref.
				parameters.	
Anesthetized dog	0.1, 1 and 10 mg	s.d.	i.v.	Hemodynamic parameters, ECG parameters; effects on changes in BP/HR induced by NA, Ach and Iso; changes in BP during BCO and after head up tilt	4.2.1.3, Report No. 3325 [40]
Anesthetized dog	Intra-sinus concentration increasing from 0.01 - 10 000 µM		Injected into the carotid sinus	Effect on baroreceptor function	[29]
Anesthetized cat	0.1, 1 and 10	s.d.	i.v.	Effects on BP, HR and nictitating membrane tone together with changes in these variables induced by electrical stimulation of the vagus and sympaticus nerves, carotid occlusion and NA, Ach and Iso Effects on BP, LV pressure, dP/dt, HR and nictitating membrane tone together with changes in these variables induced by electric stimulation of the vagus and sympaticus nerves, NA and His	[36]
Rat	0.5 , 1.5 and 5	s.d.	s.c.	Respiratory function	4.3.2.1, Report No. NL0047654 [41]
Rat	10 b.i.d.	5 days	p.o	Gastrointestinal motility	[34]
Rat	1, 10	s.d.	p.o.	Ulcerogenic activity	[34]
Rat	0.4	7 days	p.o.	Biochemical and endocrine profile	4.2.1.3, Report No. 2749 [42]
Anesthetized Guinea-pig	1, 10	s.d.	i.v.	Spontaneous contractions of the ileum	[34]
Rabbit	2 (total dose)	7 days	p.o.	Endocrine profile	[42]

TableSpecies	asenapine dose* ($mg.kg^{-1}$)	Duration	Route	Parameters/assessments	Ref.
Cebus monkey	0.01 – 0.25	s.d.	i.m.	Behavioral effects	4.2.1.3, Report No. NL0017668 [43]

Key: a: expressed as salt unless otherwise specified (AE, active entity). Conversion factor from salt to AE: 0.71
Ach = acetylcholine; BCO = bilateral carotid occlusion; BP = blood pressure; dP/dt = maximum rate of the change in ventricular pressure; His = histamine; HR = heart rate; i.v. = intravenous; Iso = isoprenaline; LV = left ventricular; NA = noradrenaline; p.o. = per os; s.d. = single dose; Ref. = reference; SL = sublingual

Table 15: Overview of in vivo nonclinical safety cardiovascular studies performed with desmethyl-asenapine

Species	Desmethyl asenapine dose[a] ($mg.kg^{-1}$)	Duration	Route	Parameters/assessments	Ref.
Anesthetized rat	1	s.d.	i.v.	Effect on Bezold-Jarish reflex	[30]
Pithed rat	0.3-10	s.d.	i.v.	Effects on tachycardia and pressor responses (elicited by electrical stimulation and i.v. administration of NA)	[35]
Anesthetized dog	0.1, 1 and 10	s.d.	i.v.	Hemodynamic parameters, ECG parameters; effects on changes in BP/HR induced by NA, Ach and Iso; changes in BP during BCO and after head up tilt	4.2.1.3, Report No. 3697 [44]
Anesthetized dog	Intra-sinus concentration increasing from 0.01 - 10 000 µM		Injected into the carotid sinus	Effect on baroreceptor function	[29]
Conscious dog	0.5, 1 and 5	s.d.	i.v.	Hemodynamic parameters, ECG parameters, responses to upright tilt	[44]

Key: [a]: expressed as salt unless otherwise specified (AE, active entity). Conversion factor from salt to AE: 0.71
Ach = acetylcholine; BCO = bilateral carotid occlusion; BP = blood pressure; HR = heart rate; Iso = isoprenaline; i.v. = intravenous; NA = noradrenaline; s.d. = single dose

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 **Brief summary (**based on the Sponsor's summary)

Asenapine is being developed for the sublingual route of administration. However, it was previously studied as a potential oral drug. Therefore, oral administration was used in initial preclinical studies. The oral route was abandoned because of very low bioavailability in humans (<2%). Bioavailability was also low in animals, i.e. between 20-65% in rats and up to 10% in dogs. The sublingual route of administration is impractical in laboratory animals. Therefore, intravenous and subcutaneous routes were used in animals in preclinical studies. Some studies in dogs were conducted using the sublingual route. Exposures to asenapine achieved in preclinical studies at least one dose were greater than typical level of human exposure (AUC_{0-12} = 43.4 ng.h/mL; 86.8 ng.ml/h for b.i.d dosing; C_{max} = 6.6 ng/mL, based on data from the thorough QTc study). Multiple dose AUC and C_{max} values determined in various species and compared with human exposure are shown in the Sponsor's table on page xxx of this review. Asenapine was shown to have a relatively high clearance, high volume of distribution and short half-life in laboratory animals. Preclinical studies demonstrated that asenapine is well absorbed when administered by the sublingual route, readily penetrates to tissues, including brain and CSF, crosses the placental barrier and is excreted in milk. Protein binding of asenapine is relatively high in all species, including humans. Asenapine does not appear to be a substrate for P-glycoprotein. 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 Sponsor's table on page xxx of this review. Metabolism studies demonstrated multiple pathways of metabolism, with oxidative metabolism via N-demethylation and N-oxidation being predominant in laboratory species. In humans,

asenapine is metabolized via oxidation to N-desmethyl and hydroxy metabolites, and via direct conjugation with glucuronic acid. Major pathways of metabolism in humans appear to be present in animals. Further metabolism of metabolites has been also observed. Over 40 metabolites have been detected in circulation and excreta of laboratory animals and human subjects. The major human metabolites were present in laboratory animals. The enzymes involved in oxidative and conjugative metabolism of asenapine in humans are CYP1A2 and UGT1A4. The summary of metabolites observed in animal studies and in humans is shown in the Sponsor's table on page xxx of this review. The proposed metabolic pathways in all species examined are shown on page xxx of this review. Mass balance studies with radiolabel led asenapine were conducted in several laboratory species (mouse, rat, rabbit, dog) and in humans. Unchanged asenapine was present in human feces at 5-15% of the dose. Total recovery values were in excess of 80%.

2.6.4.2 Methods of Analysis

[See under individual study reviews]

2.6.4.3 Absorption

The absorption of asenapine was investgated in several laboratory species. Overall asenapine is characterized by high absorption. Systemic availability for the sublingual route of administration was assessed in dogs in the study, entitled:

"**Comparative haemodynamics, electrocardiography, orthostatic hypotension, and pharmacokinetics of oral and sublingual Org 5222 in the conscious Beagle dog" (SDG RR No. 4130),** reviewed by Dr. Lois Freed under the IND 51642 (please see her review dated June 11, 1997 for further details). In this study, asenapine was administered as an oral capsule (gelatin) or sublingually as lyosoheres at single doses of 1-50 mg/kg p.o. and 0.01-1 mg/kg s.l. to Beagle dogs. Both cardiovascular and TK parameters were assesed. Blood samples were taken at intervals for up to 6 hours and at 24 hours after administration. Oral administrtion yielded non-linear kinetics. Bioavailability was markedly higher in dogs after sublingual as compared to oral administration. Absorption was highly variable (t_{max} ranging from 0.4 to 1.9 h s.l.). The TK data are shown in the following Sponsor's table:

Table 31: Summary of mean (± standard deviation) pharmacokinetic parameters of Org 5222 after a single oral or sublingual dose of Org 5222

Route	Dose (mg/kg)	AUC(0-5) (ng.h/ml)	AUC(0-24) (ng.h/ml)	Normalized AUC(0-5)	Normalized AUC(0-24)	Cmax (ng/ml)	tmax (h)
PO	1	13±16	28±35	13±16	28±35	7±8	1.6±0.4
	2.5	32±21	85±40	13±8	34±16	17±11	1.25±0.5
	5	61±26	217±98	12±5	43±20	25±8	1.8±1.6
	10	461±534	1753±2027	46±53	175±203	207±236	2.8±1.3
	50	1590±1295	8503±5814	32±26	170±116	760±537	3.7±2.3
SL	0.01	2±1	6±8	196±63	637±767	1±1	0.4±0.5
	0.1	31±13	59±23	307±132	592±225	29±18	1.9±2.5
	1	110±83	197±110	110±83	197±110	86±75	0.8±1.9

In study entitled, "A sublingual toxicokinetics study with asenapine (Org 5222) in female Beagle dog" (No. NL0057840), sublingual administration of asenapine at 1 mg twice daily for 2 weeks resulted in rapid absorption (T_{max} of 0.15 h and 0.17 h after single and multiple dosing, respectively) followed by rapid elimination with $T_{1/2}$ of 0.54 h and 0.42 h after single and multiple dosing, respectively. Mean dose-normalized AUC_{0-2h} and C_{max} values were slightly higher (1.7 and 1.9 times, respectively) after multiple than after single dosing.

2.6.4.4 Distribution

Whole body authoradiography study in rats, bioanalysis of plasma and brain concentrations of asenapine and its metabolites study in rats, and serum protein binding study were reviewed by Dr. Lois Freed under the IND 51,641 (please see Dr. Freed's review dated June 11, 1997 for further details). The following summaries of these studies are taken directly from her review:

1. "**Whole-body autoradiography of male and female rats treated with a single oral dose of [^{3}H]-Org 5222** (SDGRR No. 2933, Organon Inc., study dates: 9/90-2/91, non-GLP.

Distribution of [^{3}H]-Org 5222 (Batch E, IP590/0085, unlabeled compound) into tissues was assessed in Lister Hooded rats (5/sex). Org 5222 was administered as a single p.o. dose of 1.8 mg/kg. Tissue distribution was assessed at 0.5, 1, 5, 24, and 90 hr postdosing in 1 rat/sex/time point using whole-body autoradiography. Tissue levels were quantitated using densitometric image analysis (Struers vision AB) and compared to reference tritiated standards. Data were expressed as radioactivity per "area unit tissue".

In males, highest levels of radioactivity were detected in liver, eye choroid, lacrimal gland, kidney (cortex), and adrenal (in that order); substantial amounts were also noted in spleen, pituitary, bone marrow, pancreas, kidney (pelvis), and salivary gland. Accumulation was also detected in brain (cerebrum, cerebellum), with tissue-to-plasma ratios of ~3.5-4.5 at T_{max}. The T_{max} in tissues was 5 hr for most tissues, with liver and eye choroid being notable exceptions (T_{max} = 0.5 and 24 hr, respectively). Tissue radioactivity in all but two tissues was lower at 1 hr than at either 0.5 or 5 hr. This most likely was due to the fact that there was only 1 rat/time point, rather than a real pharmacokinetic finding. By 96 hr postdosing, radioactivity was still detected in a number of tissues, with the highest levels in eye choroid (42% of peak levels vs 2-12% in the other tissues).

In females, highest levels of radioactivity were detected in liver, lung, kidney (cortex), eye choroid, and lacrimal gland (in that order). Substantial levels were also noted in adrenal, bone marrow, kidney (pelvis), spleen, cerebellum, and brown fat. The T_{max} was 0.5-5 hr, except in salivary gland (T_{max} = 24 hr). Tissue-to-plasma ratios for brain regions were ~3.5-4.5 at T_{max}. Except in two tissues (eye choroid, lacrimal gland), low levels of radioactivity were not observed at 1 hr (compared to 0.5 and 5 hrs), as was observed in males. At 96 hr postdosing, radioactivity was still detectable in **a** number of tissues, with the highest levels in eye choroid (~60% of peak levels vs. 2-14% in the other tissues)".

The results of this study are shown in the following Sponsor's tables:

TABLE 3 Contents of radioactivity in organs/tissues at different time points following single oral administration of [^{3}H]-Org 5222 to female Lister Hooded rats.
Data are presented as radioactivity per area unit tissue.

Tissue	Time post dosing (h)				
	0,5	1	5	24	96
	Bq/area unit				
CEREBRUM	523	259	279	138	-
CEREBELLUM	451	263	242	129	-
HYPOPHYSIS	367	383	358	217	-
HEART	189	281	181	115	11*
LUNG	508	1114	314	150	34
LIVER	1111*	1927*	720*	540	58
KIDNEY, CORTEX	977*	731*	615*	435	43
KIDNEY, PELVIS	359	599*	394	274	27
EYE LIQUID	31	5*	43	15*	6*
EYE CHOROID	696*	465	924*	809*	572
SKIN	73	60	62	70	-
ADRENALS	721*	750*	603*	344	-
SPLEEN	474	582	530	335	21
PANCREAS	439	389	330	189	-
INTESTINES	449	253	197	155	-
BLOOD	119	197	97	69	-
BONE MARROW	601*	288	238	138	-
BROWN FAT TISSUE	451	247	183	104	-
LACRIMAL GLAND	763*	452	960*	237	21
SALIVARY GLAND	194	299	350	610*	-

* Relative optical density outside (above or below) the range covered by the calibration curve.
- Not detectable.

TABLE 3 Contents of radioactivity in organs/tissues at different time points following single oral administration of [^{3}H]-Org 5222 to female Lister Hooded rats.
Data are presented as radioactivity per area unit tissue.

Tissue	Time post dosing (h)				
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	Bq/area unit				
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HYPOPHYSIS	367	383	358	217	-
HEART	189	281	181	115	11*
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LIVER	1111*	1927*	720*	540	58
KIDNEY, CORTEX	977*	731*	615*	435	43
KIDNEY, PELVIS	359	599*	394	274	27
EYE LIQUID	31	5*	43	15*	6*
EYE CHOROID	696*	465	924*	809*	572
SKIN	73	60	62	70	-
ADRENALS	721*	750*	603*	344	-
SPLEEN	474	582	530	335	21
PANCREAS	439	389	330	189	-
INTESTINES	449	253	197	155	-
BLOOD	119	197	97	69	-
BONE MARROW	601*	288	238	138	-
BROWN FAT TISSUE	451	247	183	104	-
LACRIMAL GLAND	763*	452	960*	237	21
SALIVARY GLAND	194	299	350	610*	-

* Relative optical density outside (above or below) the range covered by the calibration curve.
- Not detectable.

2. Bioanalysis of: (1) plasma concentrations and brain concentrations of Org 5222 and its N(2)-demethyl (Org 30526) and N(2)-oxide metabolites in rats treated with a single oral dose of Org 5222; (2) plasma concentrations and brain concentrations of Org 30526 in rats treated with a single oral dose of Org 30526 (SDGRR No. 3218, Organon Inc., study dates: 2/92-4/92, GLP).

The purpose of this study was to compare the brain penetration of Org 30526, the N(2)-demethyl metabolite of Org 5222, with that of Org 5222. Male Wistar rats (3/grp) received either ^{3}H-Org 5222 or ^{3}H-Org 30526 at oral doses of 1.7 and 1.8 mg/kg, respectively. At 0.5, 1.5, and 3 hr postdosing, animals (one grp of animals per time point per compound) were sacrificed and trunk blood and brains were collected. Compounds in plasma and brain extracts were quantitated using HPLC.

The data are summarized in the following table:

COMPOUND	SAMPLE	TIME (hr)	Org 5222	Org 30526	N-0*
^{3}H-Org 5222	plasma	0.5	8.9 ± 7.5	16.7 ± 13.2	17.7 ± 16.2
		1.5	4.4 ± 3.8	22.0 ± 23.6	21.1 ± 18.8
		3	16.6 ± 10.6	50.7 ± 16.4	30.0 ± 5.4
	brain	0.5	49.0 ± 44.3	14.5 ± 11.9	6.5 ± 3.3
		1.5	56.4 ± 33.5	40.2 ± 30.9	11.0 ± 10.6
		3	328.1 ± 260.8	127.4 ± 58.5	22.3 ± 0.6
	brain-to-plasma	0.5	5.3 ± 0.3	0.9 ± 0.1	0.5 ± 0.3
		1.5	15.6 ± 5.0	2.2 ± 1.0	0.5 ± 0.0
		3	19.0 ± 4.2	2.4 ± 0.4	0.7 ± 0.2
^{3}H-Org 30526	plasma	0.5	[redacted]	42.8 ± 8.4	[redacted]
		1.5		133.4 ± 77.5	
		3		185.8 ± 65.6	
	brain	0.5		41.9 ± 7.2	
		1.5		280.2 ± 214.7	
		3		335.9 ± 39.8	
	brain-to-plasma	0.5		1.0 ± 0.0	
		1.5		2.0 ± 0.5	
		3		1.9 ± 0.4	

*N(2)-oxide(s) of Org 5222

The data indicate that both Org 5222 and Org 30526 penetrate the blood-brain-barrier, and accumulate in brain up to 3 hr postdosing. Org 5222 accumulated to a greater extent, with a brain-to-tissue ratio of 19 at 3 hr postdosing. Following administration of Org 5222, both Org 30526 and the N-(2)-oxide(s) metabolites are detected in brain; however, only Org 30526 has a brain-to-plasma ratio greater than 1".

3."In vitro binding of [^{3}H]-Org 5222 to male rat, dog and human plasma proteins and in vivo plasma protein binding of radioactivity after a single oral dose of [^{3}H]-Org 5222 to male rats (SDGRR No. 2972, Organon Inc., study dates: 1/91-5/91)

Plasma binding properties of [^{3}H]-Org 5222 (batch H, IP No. 590/112, for unlabeled compound) were assessed by equilibrium dialysis in plasma from male rats (WISW), male Beagle dogs, and a male human volunteer. The extent of <u>in vitro</u> plasma protein binding was high in all species studied (i.e., 93.6 ± 0.6, 94.6 ±2.1, and 94.5 ±0.4% in rats, dogs, and human, respectively).

<u>In vivo</u> binding of Org 5222 to plasma proteins was determined in 5 male rats given a single p.o. dose of 1.8 mg/kg. Blood samples were collected at 15 and 55 min, and 3 hr postdosing. Binding was fairly high, but tended to decrease over time (91.3 ±1.2, 89.2 ±2.9, and 86.4 ± 1.6% at 17, 55, and 199 min postdosing, respectively). Since it is radioactivity that is being detected, this decrease over time, as suggested by the Sponsor, may be due to the formation of

metabolites with lower affinity than the parent compound for plasma proteins".

2.6.4.5 Metabolism

Studies *in vitro:*

1. Study SDGRR2874 (1991): The *in vitro* metabolism of [^{3}H]-asenapine was examined by incubating with hepatic microsomes prepared from 4 male Wistar rats, 1 male Beagle dog, and 2 human male organ donors. Human microsomes were tested separately and resulted in similar profiles. Asenapine was extensively metabolized by rat and dog microsomes. Human microsomes metabolized asenapine to a lesser extent. After 30 min of incubation, asenapine accounted for 19.5%, 36.1%, 79.6% and 68.9% of extracted radioactivity in the rat, dog, human No. 1 and human No. 2, respectively. N-oxide metabolite (2 diastereoisomeric forms) accounted for 74.8%, 31.7%, 3.6% and 5.8% of radioactivity in the same preparations. N-desmethylasenapine accounted for 5.8%, 32.2%, 8.1% and 12.7% in the rat, dog, human No. 1 and human No. 2, respectively. Two additional minor metabolites of unknown structure have been observed in humans. It can be concluded that N-oxidation was the principal metabolic pathway in the rat, although N-demethylation was also involved. N-oxidation and N-demethylation were the most and equally important in the dog. The N-desmethylasenapine was the major human metabolite in this study.

2. Study INT00003054 (2006): The *in vitro* metabolism of [^{14}C]-asenapine was studied by incubating with male mouse, rat, rabbit, dog and human hepatic microsomes. At least 14 peaks were observed in HPLC profiles. Within 30 min, asenapine was extensively metabolized by mouse, rat and dog microsomes. Rabbit and human microsomes metabolized asenapine less effectively. Asenapine accounted for 63.8-81.7% of recovered radioactivity in human samples. Major (more than 10% of the peaks) metabolites identified tentatively included N-desmethylasenapine detected in mouse, rabbit, dog and human, and N-oxide metabolites (2 diastereoisomeric forms) detected in mouse, rat and dog microsome preparations. All metabolites observed in assays with human microsomes were also detected in at least three other species.

3. Study SDGRR5057 (1997): The *in vitro* metabolism of [^{3}H]-asenapine was investigated in cell medium and cell extracts of male rat and female human hepatocytes after 3 hours of incubation. Asenapine was extensively metabolized in the rat, with 49% and 61% remaining unchanged in cell medium and cell extracts, respectively. In humans, the corresponding values were 2% and 10%. Major routes were N-demethylation and N-oxidation in both species. At least 11 and 12 different metabolites were observed in assays with rat and human preparations, respectively. Rat metabolite profile was similar to that of human except one of the N-oxides and a few minor metabolites.

4. Study NL0060905 (2006): The *in vitro* metabolism of [^{14}C]-asenapine was studied in hepatocytes of male mouse, rat, dog, human or female rabbit hepatocytes. Asenapine was extensively metabolized by dog and human hepatocytes, and well metabolized by mouse, rat and rabbit hepatocytes. N-desmethylasenapine was tentatively identified as a major metabolite for mouse, rabbit, dog and human hepatocytes. N-oxide was the major

metabolite for mouse, rat and dog hepatocytes. H5 (O-sulfate asenapine), H9 (unknown metabolite), and N-desmethyl asenapine were the major human metabolites. Several metabolites observed in incubations with dog and human hepatocytes were not observed in other species (i.e. hydroxysulfate metabolite, asenapine N-glucuronide, hydroxy metabolite of N-desmethylasenapine, N-formyl metabolite of N-desmethylasenapine and hydroxy metabolite of N-formyl metabolite of N-desmethylasenapine). However, in general metabolite profiles are similar among species.

5. Study NL0010293 (1998): The potential involvement of human P450 enzymes in the *in vitro* asenapine metabolism was studied using several recombinant human enzymes and different techniques. CYP1A2 was shown to be the main human P450 enzyme involved in the formation of asenapine N-oxide. CYP1A2 and CYP3A4 were involved in the formation of N-desmethyl asenapine. The second study designed to characterize the *in vitro* metabolism of asenapine using male human liver microsomes and recombinant human isoenzymes (Study NL0060848; 2005) confirmed the role of CYP1A2 and CYP3A4 as the major human cytochrome P450 enzymes involved in the Phase I metabolism followed by CYP2D6.

6. Study DM2006-005222-013 (2006): The kinetics of the formation of the N-glucuronide metabolite of asenapine investigated using human liver microsomes demonstrated the K_m of 92.6 μM and V_{max} of 1.8 nmol/min. Studies conducted with a panel of human recombinant UGT enzymes demonstrated that only UGT1A4 is responsible for the formation of N-glucuronide conjugate of asenapine.

Studies *in vivo*:

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

1. Study title: "**The biotransformation of Org 5222 in Wistar rats** (SDGRR No. 2355, Organon Inc., study dates: 11/85-12/88, non-GLP)

The metabolic profile of Org 5222 was assessed in Wistar rats (220-270 g; non-fasted) using ^{3}H (mono, di), ^{14}C, and $^{36}C1$-labeled Org 5222. ^{3}H-labeled 2(N)-demethyl Org 5222 was also administered. Dosing regimens included the following: (1) 1 mg p.o., ^{3}H-Org 5222 to 3/sex/grp for analysis of urinary and fecal radioactivity (samples collected for 4 days postdosing, in 24-hr increments), (2) 0.5 mg i.v., ^{3}H-Org 5222 to 1 male rat for study of plasma kinetics. (3) 14 mg p.o., Org 5222 to 2 male rats to study urinary and fecal metabolites, (4) 14 mg p.o., 10 mg i.d., 3.6 mg p.o., ^{3}H-Org 5222 in bile-duct cannulated animals (1 male/regimen); bile samples collected for 20 hr postdosing, (5) ^{14}C-Org 5222 and ^{3}H-demethyl Org 5222, 1 mg i.d. in bile-duct cannulated animals (n = ?); samples collected for 20 hr postdosing. Enteral preparations were made up in gelatin/mannitol solutions; the vehicle for parenteral administration was a potassium phosphate buffer. Biological samples were analyzed using LSC, HPLC, MS, NMRS, and IS.

Excretion: the major route of elimination of radioactivity following a single p.o. dose was via the feces in both male and female rats (3/sex). By 4 days postdosing, ~22 and 65-75% of dose

radioactivity had been eliminated in the urine and feces, respectively. Fecal radioactivity was slightly higher in females than males. The majority of radioactivity was eliminated in the first 24 (urine) to 48 (feces) hr postdosing. According to the Sponsor, total recovery of radioactivity was 91%.

Identification of metabolites:

Urine: Org 5222, and 3 metabolites, 2(N)-demethyl Org 5222 and 2(N)-oxide (forms 1 and 2) were detected in urine of, according to the Sponsor, 1 male rat. However, data were presented for 3/sex. According to the data table, Org 5222 accounted for 4-7% of dose radioactivity in females, but 12-17% of dose radioactivity in males. On the other hand, the 2(N)-oxide(s) were more prevalent in female urine (5-12% form 1 and 1-6% form 2 in females; 2-4% form 1 and 0% form 2 in males). The 2(N)-demethyl metabolite accounted for 1-5% of dose radioactivity in males and females.

Feces: Org 5222 and 2 metabolites, 2(N)-demethyl and 2(N)-oxide Org 5222, were detected in fecal samples. Demethyl Org 5222 was the predominant identified drug-related species, accounting for 26% (range: 1-39%) of dose radioactivity. Org 5222 and 2(N)-oxide Org 5222 each accounted for 12% (range: 8-15%) and 8% (range: 6-13%) of dose radioactivity, respectively.

Plasma: the kinetics of Org 5222 and metabolites, 2(N)-demethyl and 2(N)-oxide Org 5222, were followed in plasma in 1 male rat for 120 min after i.v. dosing. Org 5222 and 2(N)-oxide Org 5222 were detected at all measurement times (1.3-121 min), whereas the 2(N)-demethyl derivative was detectable only from 30 min on. T_{max}'s were 121 and 30 min for the demethyl and oxide derivatives, respectively.

Bile: only one metabolite was identified in bile (collected from 1 male rat), a carbamate [N-2] glucuronide of Org 5222. This finding was confirmed in a second male rat. This same metabolite was also detected in bile following administration of 3H-2(N)-demethyl Org 5222 in a third male rat".

2. Study title: An excretion balance and metabolite profiling study after a single subcutaneous dose of asenapine (Org 5222) to male and female CD-1 mice. (Study No. INT00003103)

Male and female CD-1 mice received a single s.c. dose of 0.74 mg/kg/5 MBq/kg [^{14}C]-asenapine. Blood samples were collected at 5, 15 and 30 min, and 1, 2, 4, 7 and 24 h post dosing. Urine and feces were collected in 24 h fractions up to 168 h postdosing. Metabolite profiles were analyzed by HPLC in extracts of all plasma samples, 0-24 h urine samples, 0-24 h feces samples and 24-48 feces samples containing more than 5% of administered radioactivity. The metabolite profiles of pooled plasma samples consisted of at least 6 major peaks. Asenapine (6-10% of total radioactivity) and its N-oxide (Org 31437) were major peaks present in plasma. Asenapine was extensively metabolized as indicated by the number of peaks observed and the small amount of asenapine in urine (0.3-1.1% of the dose) and feces (0.2-5.1% of the dose). N-desmethylasenapine and asenapine N-oxide were present in urine and feces. Not all metabolites could be characterized.

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 ± 3,5	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	9,3 ± 2,7	61,8 ± 5,5	71,1 ± 3,5
3,6	Oral	9,3 ± 2,5	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 ± 3,8	66,5 ± 6,0	80,2 ± 2,9
0,1	Oral	15,3 ± 1,2	81,9 ± 3,3	97,1 ± 3,2
0,6	Oral	10,9 ± 2,0	66,9 ± 1,7	77,8 ± 0,3
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 **Excretion** (see the Metabolism section above)

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

1. Study title: The inhibition of the human cytochrome P450 enzymes CYP1A2 and CYP2D6 by Org 5222 (*in vitro*). (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: The inhibition of the human cytochrome P450 enzymes CYP2C19 and CYP3A4 by Org 5222 (*in vitro*). (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 $1x10^5$ 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:

		AUC ($ng \cdot h \cdot mL^{-1}$)		C_{max} ($ng \cdot mL^{-1}$)	
Study/species/dosing route (multiple dose)	Dose (AE $mg \cdot kg^{-1}$)	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	26.2
	1.06	-	-	88.4	80.4
	3.55	-	-	201	166
52 week rat oral[c,10, 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	165
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	26.6		4.23	
	0.15	43.4		6.56	

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

[c] $AUC_{(0\text{-}7h)}$; [d] AUC_{inf}; [e] $AUC_{(0\text{-}2.5h)}$; [f]: highest concentration of three measured time point after 90 weeks; [g]: $AUC_{(0\text{-}1h)}$ for 0.018 $mg \cdot kg^{-1}$ and $AUC_{(0\text{-}3h)}$ for 0.088 and 0.44 $mg \cdot kg^{-1}$, C_{max} is average plasma concentration and first sampling time point (5 min)[h]: $AUC_{(0\text{-}10h)}$, C_{max} is average plasma concentration and first sampling time point (5 min); [i]: AUC_{inf}; - not determined; [j]: Data from the thourough QTc study (see Clinical Trial Report on A7501001, Table 48, $AUC_{(0\text{-}12)}$).

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:

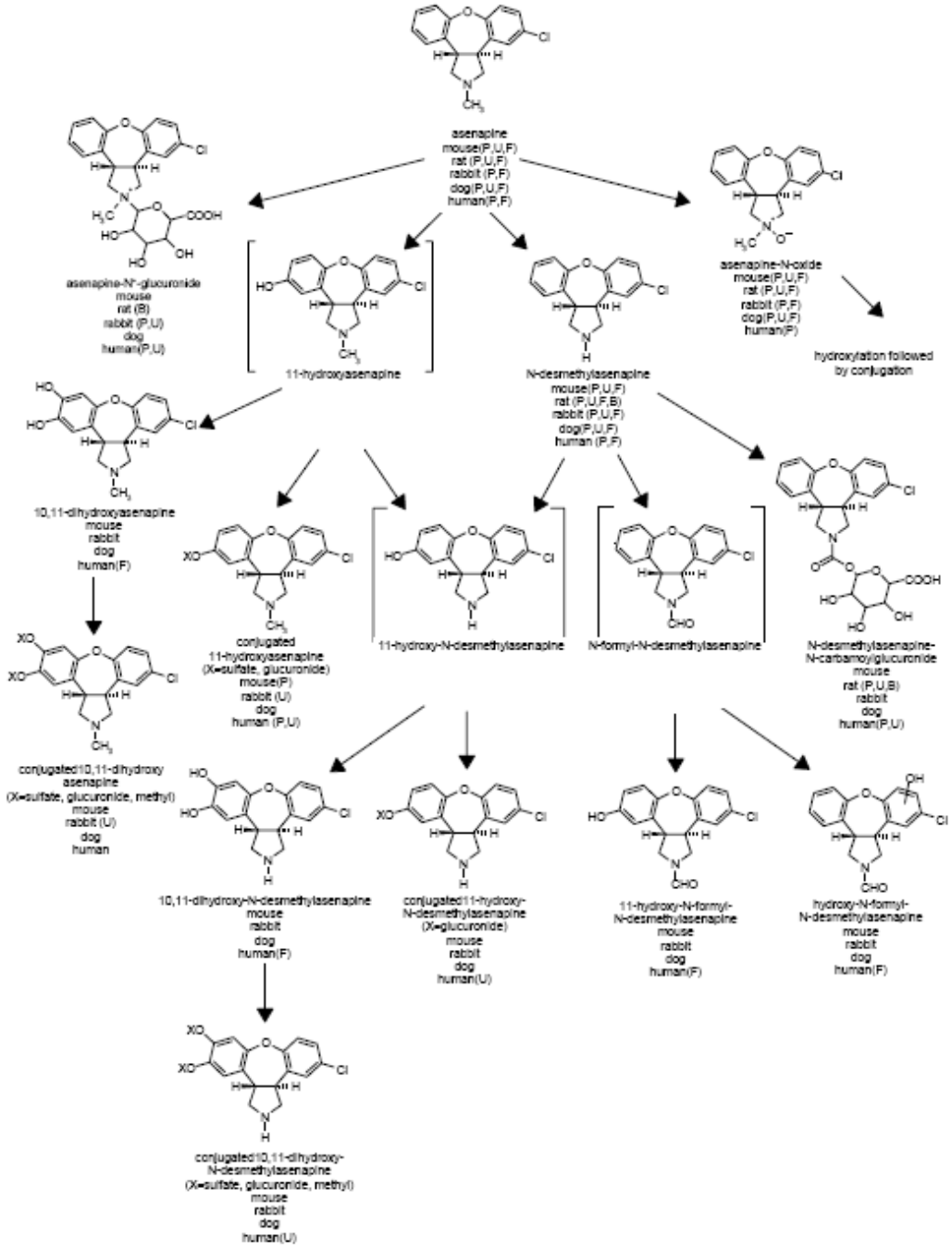
Table 2 Summary of Representative Metabolites of Asenapine in Preclinical Species and Human[d] (2.6.4.5)

	% of Dose in Excreta[a]					% of Total Radioactivity in Circulation[a,e]				
Metabolite/Identity	Mouse	Rat	Rabbit	Dog	Human[g]	Mouse	Rat	Rabbit	Dog	Human[g]
Asenapine	3.9/3.6	15/5.2	3.4/3.3	1.6/1.8	4.8-16	6/10	9.8/15	10/10	10/10	2
N-Desmethylasenapine	16/9.7	20/28	2.6/1.5	11/12	0-1.7	+[b]	ND/5.6	4/4	16/16	+
Asenapine N-Oxide	2.5/2.2	1.8/1.8	0.4/0.5	0.1/1	-	14/8	ND/15	ND/1	7/6	
Asenapine N⁺-Glucuronide	+	+[f]	4.7/5.8	+	10.2-21.3			+[b]		13-19
N-Desmethylasenapine N-Carbamoyl Glucuronide	+	ND/1.6		+	2-3		7.5/6.2			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	+	+		+	+					
x-Hydroxy-N-formyl-N-desmethylasenapine	+	+		+	+					
Hydroxylated and conjugated asenapine N-oxide					+					

[a]male/female; [b]Determined in a separate pharmacokinetic study.
[c]Doses and routes studied were: mouse: 0.74 $mg \cdot kg^{-1}$, sc; rat: 3.0 $mg \cdot kg^{-1}$sc; rabbit: 0.17 $mg \cdot kg^{-1}$, iv; dog: 0.1 $mg \cdot kg^{-1}$, iv; human: 10 mg SL.
[d]ND denotes that the metabolite was not detected by radiometric HPLC; [e]: rough estimates in most cases; [f]: in bile; [g]: 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:

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 Faolity	Report number	Location in CTD
Absorption	mouse	sc	Organon, Schalk, NL	INT00008175	4.2.2.2.
Absorption	rat	iv	Organon, Schalk, NL	INT0039620	4.2.2.2
Absorption	rat	iv	Pfizer, Groton, USA	DM2006-005222-012	4.2.2.2
Absorption	rat	sc	Organon, Schalk, NL	INT00005908	4.2.2.2
Absorption	rabbit	iv	Organon, Schalk, NL	INT00005022	4.2.2.2
Absorption	dog	si	Organon, Schalk, NL	NL0057840 INT00010072	4.2.2.2
Absorption	dog	iv	Organon, Schalk, NL	INT00004015	4.2.2.2
Absorption	monkey	sc	Pfizer, Groton, USA	DM2005-005222-002	4.2.2.2
Absorption	monkey	sc	Pfizer, Groton, USA	DM2005-005222-005	4.2.2.2
Distribution	rat	oral	Organon, Oss, NL	SDG RR 2933	4.2.2.3
Distribution	rat	oral	Organon, Oss, NL	SDG RR 3218	4.2.2.3
Distribution	rat	sc	Pfizer, Groton, USA	DM2004-005222-004	4.2.2.3
Distribution	dog	si	Organon, Schalk, NL	NL0052811	4.2.2.3
Distribution	monkey	sc	Pfizer, Groton, USA	DM2005-005222-011	4.2.2.3
Distribution	rat, dog, human	Plasma in vitro	Organon, Oss, NL	SDG RR 2972	4.2.2.3
Distribution	mouse, rabbit	Plasma in vitro	Organon, Schalk, NL	NL0029221	4.2.2.3.
Distribution	mouse, rat, rabbit, dog, human	Plasma/protein in vitro	Pfizer, Groton, USA	DM2005-005222-007	4.2.2.3
Distribution	rat, rabbit, human	Plasma in vitro	Pfizer, Groton, USA	DM2004-005222-004	4.2.2.3
Distribution	mouse, rat, rabbit, dog, human	Blood (erythrocytes) in vitro	Organon, Schalk, NL	NL0029630	4.2.2.3

Type of study	Test system	Method of administration	Testing Faolity	Report number	Location in CTD
Distribution	rat	iv	HLS, UK	INT00002409	4.2.2.3
Distribution	rabbit	iv	HLS, UK	INT00001997	4.2.2.3
Distribution	MDCK/MDR1 cells	In vitro	Pfizer, Groton, USA	DM2005-005222-008	4.2.2.3
Metabolism (Interspecies comparison)	mouse	sc	Organon, Schalk, NL	INT00003103	4.2.2.5
Metabolism (Interspecies comparison)	rat	Oral, iv, intraduodenally	Organon, Oss, NL	SDGRR2355	4.2.2.4
Metabolism (Interspecies comparison)	rat	sc	Pfizer, Groton, USA	DM2005-005222-014	4.2.2.5
Metabolism (Interspecies comparison)	rabbit	iv	Organon, Schalk, NL	INT00006333*	4.2.2.5
Metabolism (Interspecies comparison)	dog	si	Organon, Schalk, NL	NL0052811*	4.2.2.5
Metabolism (Interspecies comparison)	dog	oral/iv	Organon, Oss, NL	SDG RR 3214	4.2.2.5
Metabolism (Interspecies comparison)	dog	iv	Organon, Schalk, NL	NL0053297*	4.2.2.5
Metabolism (Interspecies comparison)	human	si	Organon, Schalk, NL	INT00003211**	4.2.2.5
Metabolism (Interspecies comparison)	rat, dog, human	Liver microsomes	Organon, Oss, NL	SDG RR 2874	4.2.2.4
Metabolism (Interspecies comparison)	mouse, rat, rabbit, dog, human	Liver microsomes	Organon, Schalk, NL	INT0003054*	4.2.2.4
Metabolism (Interspecies comparison)	rat, human	hepatocytes	Organon, Schalk, NL	SDG RR 5067	4.2.2.4
Metabolism (Interspecies comparison)	mouse, rat, rabbit, dog, human	hepatocytes	Organon, Schalk, NL	NL0060905*	4.2.2.4
Metabolism (Interspecies comparison)	human	Cytochrome P450, liver microsomes	Organon, Schalk, NL	NL00010293*	4.2.2.4
Metabolism (Interspecies comparison)	human	Cytochrome P450, liver microsomes	Organon, Schalk, NL	NL0060848*	4.2.2.4
Metabolism (Interspecies comparison)	human	Recombinant UGT enzymes, liver microsomes	Pfizer, Groton, USA	DM2006-005222-013	4.2.2.4
Metabolism (Interspecies comparison)	various	In vivo and in vitro	Organon, Schalk, NL	INT00008145	4.2.2.4
Excretion	mouse	sc	Organon, Schalk, NL	INT00003103*	4.2.2.5
Excretion	rat	Oral, iv, intraduodenally	Organon, Oss, NL	SDGRR 2355	4.2.2.4
Excretion	rat	sc	Pfizer, Groton, USA	DM2005-005222-014	4.2.2.5

Type of study	Test system	Method of administration	Testing Facility	Report number	Location in CTD
Excretion	rabbit	iv	Organon, Schaijk, NL	INT00006333*	4.2.2.5
Excretion	dog	sl	Organon, Schaijk, NL	NL0052811*	4.2.2.5
Excretion	dog	oral/iv	Organon, Oss, NL	SDG RR 3214	4.2.2.5
Excretion	dog	iv	Organon, Schaijk, NL	NL0053297*	4.2.2.5
Pharmacokinetic drug interactions	human P450	in vitro	Organon, Schaijk, NL	NL0017588*	4.2.2.6
Pharmacokinetic drug interactions	human P450	in vitro	Organon, Schaijk, NL	NL0013163*	4.2.2.6
Pharmacokinetic drug interactions	human P450	in vitro	Organon, Schaijk, NL	NL0048836*	4.2.2.6
Pharmacokinetic drug interactions	human P450	in vitro	Organon, Schaijk, NL	NL0050307*	4.2.2.6
Pharmacokinetic drug interactions	human P450	in vitro	Organon, Schaijk, NL	NL0050059*	4.2.2.6
Pharmacokinetic drug interactions	human liver microsomes	in vitro	Pfizer, Groton, USA	DM2006-005222-009	4.2.2.6
Pharmacokinetic drug interactions	human hepatocytes	in vitro	Pfizer, Ann Arbor, USA	RR 764-04914	4.2.2.6
Pharmacokinetic drug interactions	rat liver microsomes	Ex vivo	Organon, Schaijk, NL	NL0017615*	4.2.2.6
Other pharmacokinetic studies	n.a.	n.a.	n.a.	n.a.	

*: contain GLP compliance statement ; ** contains GLP compliance statement for part of the study
n.a. not applicable

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 two impurities (b) (4) in the asenapine drug substance. Moreover, the *in vitro* and *in vivo* genotoxicity studies were conducted for two structurally related degradants in drug product (b) (4) present in the commercial batches at levels (b) (4) Since the overall metabolic profile in humans and laboratory animal species is similar, individual metabolites or enantiomers have not been tested toxicologically.

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.

Local tolerance: 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.

Special toxicology: 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:

Impurity (b) (4). Drug substance impurity (b) (4) has been present in the drug substance commercial size clinical/stability batches at (b) (4). However, the Sponsor proposed to set a specification limit for this impurity in asenapine drug substance at (b) (4), thus above the ICH Q3A(R) qualification limit of (b) (4). The content of (b) (4) 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 of (b) (4) 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 (b) (4) 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) (b) (4) 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 (b) (4) 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 (b) (4) in the rabbit to qualify this impurity during phase IV or reduce the specification of (b) (4) to the ICH Q3A(R) qualification limit (b) (4)

Other studies conducted to qualify (b) (4) 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 (b) (4) is considered non-genotoxic and qualified for general toxicity.

Impurity (b) (4)**.** Studies conducted to qualify the impurity (b) (4) directly included genotoxicity studies (Ames tests and chromosomal aberrations study in cultured human lymphocytes) and an intravenous 2-week toxicity study with (b) (4) in Wistar rats. Reproductive toxicology study (embryofetal toxicity study in rats) recommended by the Agency for qualification of impurities was not conducted with (b) (4). However, this impurity is considered to be qualified based on reproductive toxicology study conducted with asenapine.

Qualification of degradants in drug product:

The following degradation products have been identified in asenapine tablets: (b) (4)

(b) (4), 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, (b) (4) is an aliphatic N-oxide (usually aromatic N-oxides are known for structural alerts for mutagenicity). (b) (4) 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 (b) (4) 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 (b) (4) will significantly increase with time up to (b) (4) respectively. It is concluded that (b) (4) is qualified with respect to its general toxicity, reproductive toxicity and genotoxicity based on its presence as the metabolite in laboratory animals.

(b) (4)**:** As indicated by the reviewing chemist Dr. Tele**,** (b) (4) is present at levels less than (b) (4) 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)**:** 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 (b) (4) 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 (b) (4) 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 (b) (4) 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

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, l/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 l/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 withlwithout 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)	$AUC_{(0\text{-}420\ min)}$ (µg•min/mL)	$t_{1/2}$ (min)	F (%)
2979	0.3	single	p.o.	M	4	26 ± 5	16 ± 11	2.7 ± 1.7	[redacted]	64
				F		62 ± 81	14 ± 3	2.8 ± 1.4		40
			i.v.	M	3	9 ± 6	47 ± 10	4.2 ± 0.8	212 ± 15	[redacted]
				F		6 ± 3	73 ± 23	6.7 ± 1.9	370 ± 199	
	1.8		p.o.	M	3	40 ± 43	35 ± 15	5.3 ± 0.8	[redacted]	25
				F		23 ± 6	38 ± 24	6.2 ± 2.8		21
	10.8			M	3	127 ± 102	238 ± 46	70.6 ± 5.7		62
				F		245 ± 162	337 ± 223	96.0 ± 67.9		49
3210/2979	0.3#	52-wk	p.o.	M	3	32 ± 13	15 ± 15	2.3 ± 1.6		63
	1.8				4	34 ± 35	26 ± 17	5.6 ± 2.6		26
	10.8				4	46 ± 37	110 ± 119	21.3 ± 11.5		21

#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.

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

STUDY	DOSE (mg/kg)	DURATION	ROUTE	SEX	N	T_{max} (min)	C_{max} (ng/mL)	$AUC_{(0-420\ min)}$ (µg•min/mL)
2979	0.3	single	p.o.	M	4	329 ± 189	18 ± 7	5.9 ± 2.2
				F		237 ± 221	18 ± 5	5.5 ± 1.2
			i.v.	M	3	319 ± 173	8 ± 2	2.8 ± 0.4
				F		310 ± 143	18 ± 8	5.9 ± 2.3
	1.8		p.o.	M	3	86 ± 39	66 ± 13	20.7 ± 3.2
				F		360 ± 106	88 ± 47	19.3 ± 14.7
	10.8		p.o.	M	3	402 ± 33	664 ± 56	207.2 ± 24.2
				F		294 ± 174	561 ± 214	155.4 ± 27.4
3210/2979	0.3	52-wk	p.o.	M	3	291 ± 226	12 ± 3	3.6 ± 0.8
	1.8				4	159 ± 112	101 ± 51	30.7 ± 12.4
	10.8				4	307 ± 65	290 ± 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)	$AUC_{(0-420\ min)}$ (µg•min/mL)
2979	0.3	single	p.o.	M	4	42 ± 8	13 ± 3	2.5 ± 0.7
				F		94 ± 74	11 ± 7	2.4 ± 0.6
			i.v.	M	3	16 ± 12	12 ± 2	2.2 ± 0.6
				F		12 ± 14	15 ± 3	3.6 ± 0.6
	1.8		p.o.	M	3	55 ± 32	82 ± 37	14.4 ± 3.3
				F		180 ± 212	69 ± 53	11.0 ± 6.7
	10.8			M	3	41 ± 9	327 ± 94	76.3 ± 16.5
				F		245 ± 162	305 ± 103	83.3 ± 19.3
3210/2979	0.3	52-wk	p.o.	M	3	341 ± 140	12 ± 5	2.1 ± 0.1
	1.8				4	25 ± 8	66 ± 50	11.5 ± 3.9
	10.8				4	97 ± 107	146 ± 67	28.9 ± 11.8

Toxicokinetic data (Beagle dog), Org 5222

STUDY	DOSE (mg/kg)	DURATION	ROUTE	SEX	N	T_{max} (min)	C_{max} (ng/mL)	$AUC_{(0-\leq 8\ hr)}$* (µg•min/mL)	$t_{1/2}$ (min)	V_{ss} (L)	CL (mL/min)	F (%)
3034	0.5	single	i.v.	M	3			6.7 ± 3.0	327 ± 225	241 ± 58	982 ± 358	
				F				7.9 ± 2.5	168 ± 44	120 ± 35	821 ± 208	
3034	0.1	single	p.o.	M	3	17 ± 2	4 ± 6	0.2 ± 0.3				22.6 ± 29.9
				F		20 ± 9	1 ± 0	0.1 ± 0.0				4.9 ± 2.1
	0.6			M		36 ± 8	2 ± 1	0.3 ± 0.2				3.9 ± 0.9
				F		43 ± 2	3 ± 3	0.4 ± 0.4				3.7 ± 2.8
	3.6			M		54 ± 3	23 ± 13	3.8 ± 2.4				7.5 ± 1.4
				F		37 ± 9	28 ± 8	3.4 ± 1.7				6.2 ± 3.2
# 4417	0.1	13-wk	i.v.	M	3			1.4 ± 0.3##		4.9 ± 1.0$	4.96 ± 1.44$$	
				F				1.1 ± 0.4		6.2 ± 2.3	5.91 ± 1.87	
	0.5			M				5.6 ± 1.9		7.4 ± 3.0	5.99 ± 1.74	
				F				5.7 ± 0.4		6.5 ± 1.1	5.38 ± 0.30	
	2.5			M				34.5 ± 1.9		4.9 ± 1.3	4.47 ± 0.26	
				F				32.2 ± 7.8		4.6 ± 1.6	4.96 ± 1.12	
3209	0.1#	52-wk	p.o.	M	2	67	0.3	n.d.**				
				F		63	0.5	n.d.				
	0.6			M	2-3	57 ± 29	1.6 ± 1.2	0.2 ± 0.0				
				F		42 ± 14	2.1 ± 0.6	0.2				
	3.6			M	3	54 ± 29	43.9 ± 25.2	3.6 ± 1.5				
				F		47 ± 11	34.6 ± 16.7	2.6 ± 0.6				

*AUD calculated to the last sample collection time point; #animals were dosed at these levels b.i.d. (total doses 0.2, 1.2, 7.2 mg/kg); data collected after one daily dose only; **n.d. = not determined; levels <LLOQ
##AUC calculated from 0-24 hr for the 13-wk study
$in L/kg for the 13-wk study, $$in L/h/kg fopr the 13-wk study

Toxicokinetic data (Beagle dog), N(2)-demethyl

STUDY	DOSE (mg/kg)	DURATION	ROUTE	SEX	N	T_{max} (min)	C_{max} (ng/mL)	$AUD_{(0-\leq 8\ hr)}$* (µg•min/mL)	$t_{1/2}$ (min)
3034	0.1	single	p.o.	M	3	102 ± 123	5 ± 2	1.0 ± 0.5	211 ± 0
				F		46 ± 16	4 ± 1	1.0 ± 0.3	241 ± 67
	0.6			M		52 ± 6	31 ± 13	7.0 ± 4.5	206 ± 44
				F		48 ± 4	45 ± 17	8.8 ± 3.4	191 ± 37
	3.6			M		43 ± 11	478 ± 210	70.9 ± 41.1	162 ± 13
				F		33 ± 5	704 ± 181	115.1 ± 39.0	159 ± 6

*AUD calculated to the last sample collection time point

Toxicokinetic data (Beagle dog), N(2)-oxides

STUDY	DOSE (mg/kg)	DURATION	ROUTE	SEX	N	T_{max} (min)	C_{max} (ng/mL)	$AUD_{(0-\leq 8\ hr)}$* (µg•min/mL)
3034	0.1	single	p.o.	M	3	41 ± 23	3 ± 1	0.5 ± 0.6
				F		184 ± 266	1 ± 0	0.2 ± 0.1
	0.6			M		27 ± 8	11 ± 7	1.6 ± 0.9
				F		43 ± 2	12 ± 8	2.1 ± 0.9
	3.6			M		35 ± 5	166 ± 128	14.9 ± 17.4
				F		30 ± 4	253 ± 118	17.4 ± 13.6

*AUD calculated to the last sample collection time point

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	Animal No	Sex	Animal Status	Exp. Day	Exp.day -14 to -7	Exp.day 7	Exp.day 266 to 269
1	1002	F	ST	274	60-	120-	144-
1	1004	F	ST	274	77-	95-	98-
1	1006	F	ST	276	106-	81-	115-
1	1008	F	ST	281	101-	98-	107-
Mean					86	98	116
St. Deviation					21	16	20
Number of Data					4	4	4
2	2002	F	ST	281	85-	79-	144-
2	2004	F	ST	274	140-	187-	142-
2	2006	F	ST	276	74-	156-	171-
2	2008	F	ST	276	99-	122-	116-
Mean					99	136	143
St. Deviation					29	46	23
Number of Data					4	4	4
3	3002	F	ST	281	83-	182-	161-
3	3004	F	ST	281	107-	106-	102-
3	3006	F	ST	274	120-	143-	158-
3	3008	F	ST	276	162-	143-	117-
Mean					118	144	134
St. Deviation					33	31	29
Number of Data					4	4	4
4	4002	F	ST	276	117-	105-	126-
4	4004	F	ST	281	101-	129-	128-
4	4006	F	ST	281	120-	140-	139-
4	4008	F	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 : 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	Animal No	Sex	Animal Status	Exp. Day	Exp.day -14 to -7	Exp.day 7	Exp.day 266 to 269
1	M	1001	ST	274	93-	99-	86-
1	M	1003	ST	274	80-	121-	116-
1	M	1005	ST	276	158-	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. 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	Animal No	Sex	Animal Status	Exp. Day	Exp.day -14 to -7	Exp.day 7	Exp.day 266 to 269
1	1002	F	ST	274	0.21-	0.27-	0.27-
1	1004	F	ST	274	0.24*	0.25*	0.25*
1	1006	F	ST	276	0.28-	0.27-	0.26-
1	1008	F	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	2002	F	ST	281	0.25-	0.25-	0.29-
2	2004	F	ST	274	0.28-	0.30-	0.27-
2	2006	F	ST	276	0.23-	0.26-	0.25-
2	2008	F	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	3002	F	ST	281	0.23-	0.31-	0.30-
3	3004	F	ST	281	0.26-	0.27-	0.26-
3	3006	F	ST	274	0.31-	0.29-	0.27-
3	3008	F	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	4002	F	ST	276	0.26-	0.27-	0.29-
4	4004	F	ST	281	0.26-	0.28-	0.28-
4	4006	F	ST	281	0.28-	0.29-	0.28-
4	4008	F	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 : XS004

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	Animal No	Sex	Animal Status	Exp. Day	Exp.day -14 to -7	Exp.day 7	Exp.day 266 to 269
1	1001	M	ST	274	0.26-	0.25-	0.25-
1	1003	M	ST	274	0.25-	0.28-	0.27-
1	1005	M	ST	276	0.31-	0.29-	0.28-
1	1007	M	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	2001	M	ST	281	0.26-	0.30-	0.29-
2	2003	M	ST	274	0.30-	0.30-	0.29-
2	2005	M	ST	276	0.25-	0.29-	0.28-
2	2007	M	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	3001	M	ST	281	0.22-	0.29-	0.29-
3	3003	M	ST	281	0.26-	0.29-	0.28-
3	3005	M	ST	274	0.30-	0.28-	0.29-
3	3007	M	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	4001	M	ST	281	0.27-	0.26-	0.25-
4	4003	M	ST	281	0.23-	0.27-	0.30-
4	4005	M	ST	276	0.25-	0.29-	0.30-
4	4007	M	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: (1) A 13-week s.c. toxicity and toxicokinetic study with Org 5222 in CD-1 mice (Study No. SDG RR 7026) and (2) 13 week subcutaneous administration range-finding study in the mouse (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; $AUC_{(0\text{-}24)}$ = 17.74 ng•hr/mL). The sponsor's summary table for the second 13-wk study is provided below:

Study group:	(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 sores/lesions (%)	+		+/-		++	
Hematology						
Hemoglobin					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				

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:

Tabulated Summary Report first 13-week pilot toxicity study of Org 5222 in th rat (SDGRR 7025) [continued]

Name of company: NV Organon	TABULATED STUDY REPORT referring to III.B.111	SQ060
Name of finished product		
Name of active ingredient: Org 5222		
REPEATED DOSE TOXICITY 1. Subacute toxicity (up to 3 months)	Report date/Number: /SDSGRR 7025 Study period (years): January-may 1997 Referring to documentation Volume: Page: to:	
Page: 2 of: 3	Addendum No.:	

Important findings	Group 2		Group 3		Group 4	
Sex (m/f)	m	f	m	f	m	f
Periodical determinations						
• mean relative body weights at 13 wks	-8%	9%	-8%	3% (ns)	-13%	5%
• Food consumption	D+	I+	D+	I+	D+	I+
• Reduced activity	+	+	+	+	+	+
• Ptosis		I+			I+	I+
Clinical chemistry						
• GPT		D+		D+		D+
• Bilirubin				D+		D+
• Calcium			D+		D+	
• Potassium					I+	
• Creatinin						
• 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+
Hematology and blood clotting						
• Hemoglobin	I+		I+		I+	I+
• Packed cell volume	I+		I+		I+	
• Mean corpuscular hemoglobin	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+

Explanations: D = decrease I = increase
ns = not significant
+ = mild ++ = moderate +++ = severe

Name of company: NV Organon	TABULATED STUDY REPORT referring to III.B.111	SQ060
Name of finished product		
Name of active ingredient: Org 5222		
REPEATED DOSE TOXICITY 1. Subacute toxicity (up to 3 months)	Report date/Number: /SDSGRR 7025 Study period (years): January-May 1997 Referring to documentation Volume: Page: to:	
Page: 3 of: 3	Addendum No.:	

Important findings	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++	

Explanations: D = decrease I = increase
ns = not significant
+ = mild ++ = moderate +++ = severe

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 group:	(2)		(3)		(4)	
Important findings:	m	f	m	f	m	f
Prostration	+++	+++	+++	+++	+++	+++
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 prolactin	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 *
Hematology						
WBC/Leucocytes	d **		d ***		d ***	
Organ weights						
Adrenals	i ns	i *	i *	i *	i *	i ns
Uterus		d *		d ***		d ***
Histopathology						
Injection sites (4 per animal)						
Hemorrhage (placebo m: 2/40, f: 4/40)	7/40	6/40	6/40	7/40	3/40	9/40
Fasciitis/fibrosis (placebo m: 9/40, f: 2/40)	5/40	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
Conclusion:						
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 group:	(2)		(3)		(4)	

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						
Diestrus (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: 8/10, f: 0/10)	ne	ne	ne	ne	3/10	0/10

Conclusion: administration of Org 5222 was associated with clinical signs of prolonged prostration after dosing, a reduced white blood cell count and increased incidence of hemorrhage and fasciitis/fibrosis (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			

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

Maximum anticipated human dose: 2x2500 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	3-4
	3	334	286	16-19
13-week rat	0.5	63	52	3-4
	1	121	111	6-7
	2	315	234	13-18
2nd 13-week rat	3	279**	183**	21-27[a]
	4	335**	256**	26-36[b]
	5	458**	362**	33-44[b]
2-week mouse	0.12	13	8	0.45-0.7
	0.6	77	44	2-4
	3	291	271	15-16
13-week mouse	0.5	40	23	1
	1	75	54	3-4
	2	145	131	7-8
2nd 13-week mouse	3	426**	366**	> 24 (males) > 21 (females)
	4	507**	435**	> 29 (males) > 25 (females)
	5	*	*	*
	7.5	912**	879**	> 50-51

nd: no data (too limited measurable data)

* values of 5 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 6 onwards

** $AUC_{0-2.5}$ 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 structural 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)
46	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	6	4		
	B	100	5	5		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		1.8
	Totals	200	6	4	p ≤0.01	(1.4)
NQO 2.5	A	25	14	12		
	B	25	17	16		
	Totals	50	31	28	p ≤0.001	

* Additional analysis
§ Statistical significance (Appendix 5a)

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	200	0	0	0	0	0
	B	201	0	0	1	1	0.5
	A+B	401	0	0	1	1	0.25
46	A	103	0	0	3	3	2.9
	B	100	0	0	0	0	0
	A+B	203	0	0	3	3	1.5
55	A	206	1	0	5	6	2.9
	B	178	1	0	1	2	1.1
	A+B	384	2	0	6	8	2.1
64	A	109	0	0	9	9	8.3
	B	107	0	0	7	7	6.5
	A+B	216	0	0	16	16	7.4
NQO 2.5	A	25	0	0	0	0	0
	B	25	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	101	1	0	0	1	1.0
	B	100	0	0	0	0	0
	A+B	201	1	0	0	1	0.5
75	A	100	0	0	0	0	0
	B	102	1	0	1	2	2.0
	A+B	202	1	0	1	2	1.0
100	A	108	1	1	6	8	7.4
	B	103	0	0	3	3	2.9
	A+B	211	1	1	9	11	5.2
125	A	103	2	0	1	3	2.9
	B	113	0	2	11	13	11.5
	A+B	216	2	2	12	16	7.4
CPA 25	A	27	2	0	0	2	7.4
	B	25	0	0	0	0	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 (b) (4) was not tested or present at (b) (4), 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:

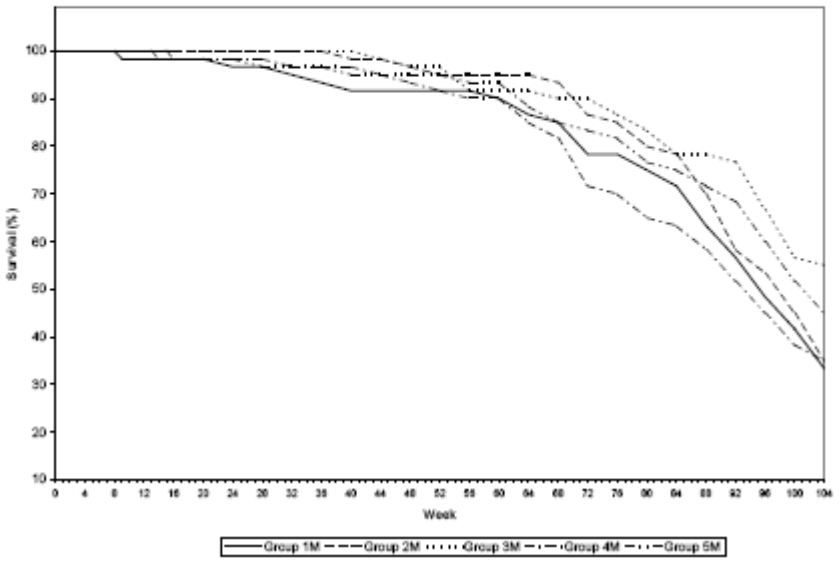
	Group and sex									
	1M	2M	3M	4M*	5M	1F	2F	3F	4F*	5F
Dose level (mg/kg/day):	0	0.3	1.2	3/5	0	0	0.3	1.2	3/5	0
Number animals/group:	60	60	60	60	60	60	60	60	60	60
Number of decedents:	41	40	27	41	36	45	39	33	36	34

* 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:

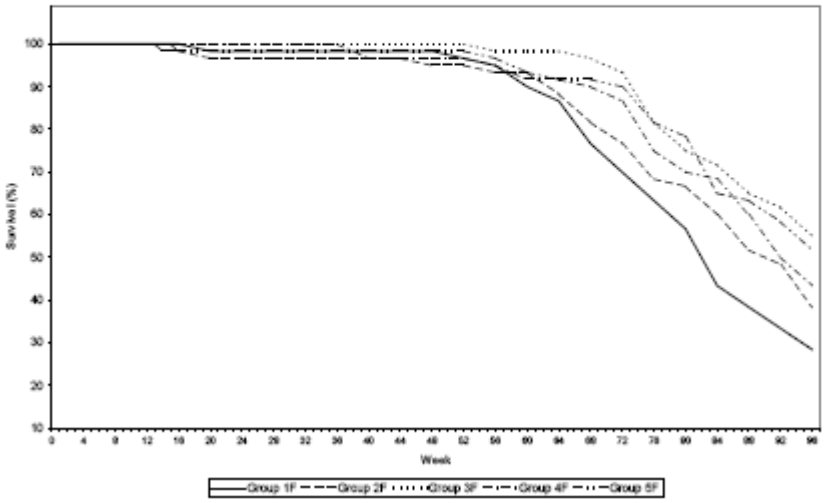
Group mean survival - males

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



Group mean survival - 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



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):

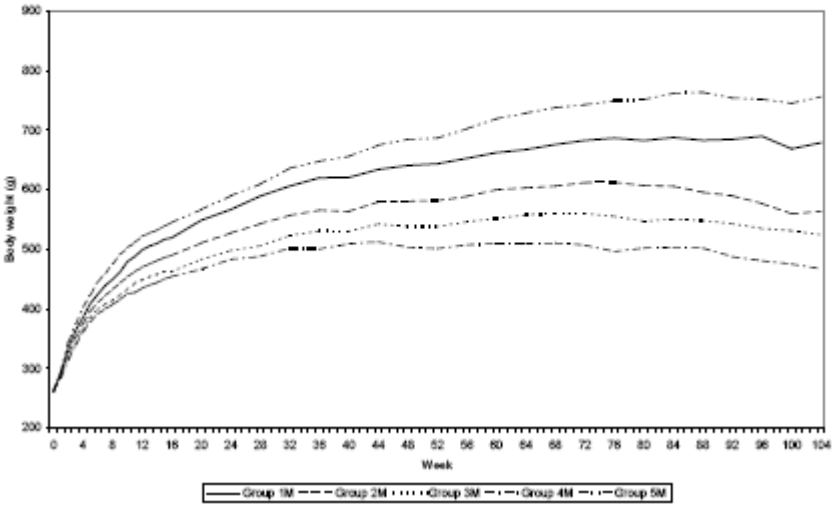
Sex	MALES					FEMALES				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	0.3	1.2	5.0	-	0	0.3	1.2	5.0	-
Number of animals	60	60	60	60	60	60	60	60	60	60
Factors contributing to death/morbidity — Number decedents	**41**	**40**	**27**	**41**	**36**	**45**	**39**	**33**	**36**	**34**
- Clinical observation(s) (killed for humane reasons)	22	21	16	20	13	22	12	17	23	13
- Traumatic lesion(s)				1				1		
- Haematological changes (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	3			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	5	4	12	6		1		1	3

Note: In the case a clear microscopic 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:

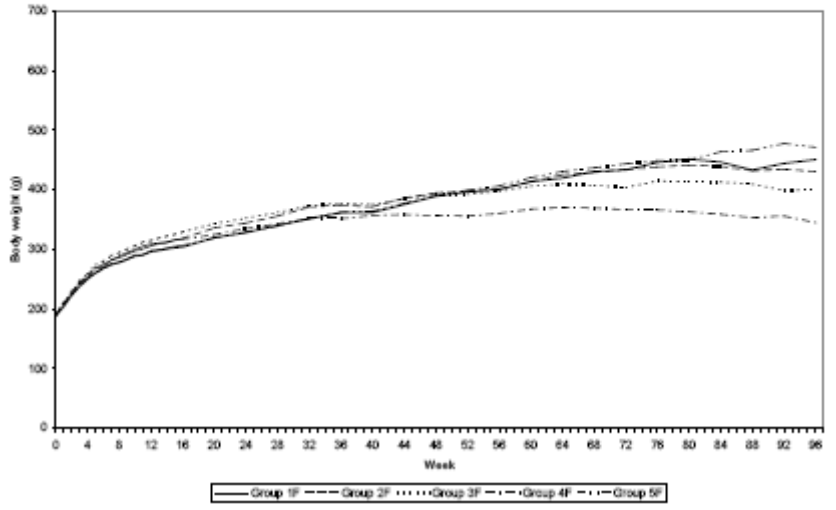
Group mean body weights - males

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



Group mean body weights - females

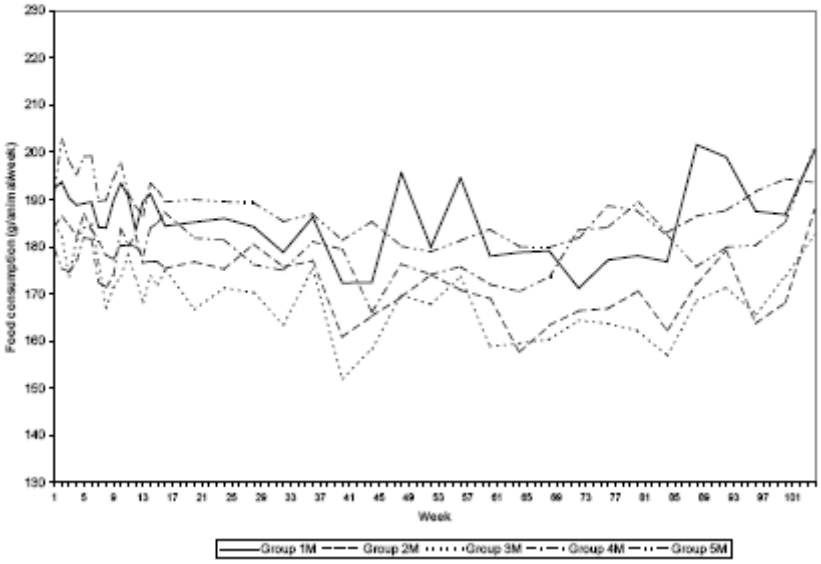
Test article	Vehicle control		Org 5222		Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	0.3	1.2	3.0/5.0	0



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:

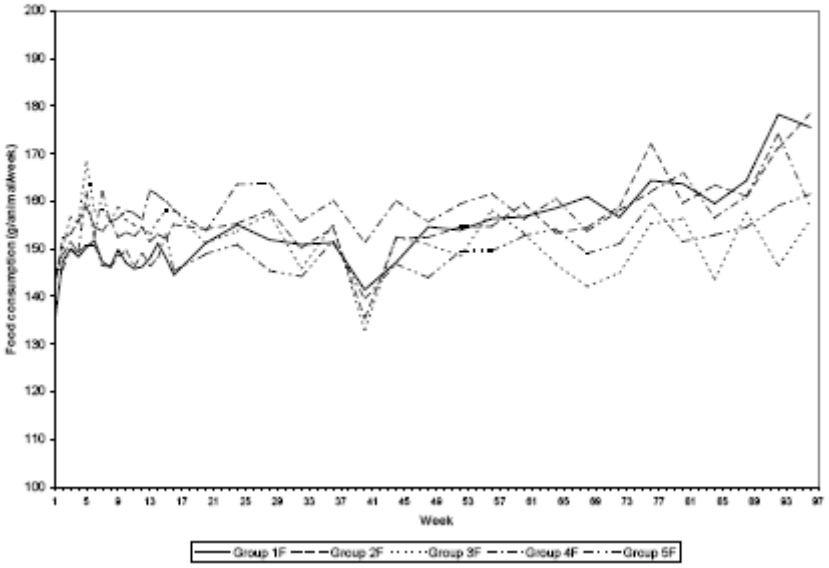
Group mean food consumption - males

Test article	Vehicle control		Org 5222		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 5222		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	0.3	1.2	5.0	-	0	0.3	1.2	5.0	-
FINDING(S)	No. examined	60	50	44	60	60	60	44	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	0.3	1.2	5.0	-	0	0.3	1.2	5.0	-
FINDING(S)	No. examined	60	59	60	60	60	60	60	55	60	60
- Cystic / haemorrhagic degeneration	Grade: 1				2	1	3	4	2		5
	2	2	9	7	14	1	17	15	13	14	14
	3	7	5	14	12	2	17	16	20	30	22
	4	4	2	4	7	5	15	11	13	3	7
	Total	13	16	25	35	9	52	46	48	47	48

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	5.0	-	0	0.3	1.2	5.0	-
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	3	3	15	5
	D	6	8	14	19	4	1	2	6	14	3
	Total	9	16	25	23	10	4	5	9	29	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	2	9	6
Brain	1			1				1		2
Eye(s)				1						
Injection site(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	30	20	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	
Auditory sebaceous gland(s)	1		1						1	
Brain	1	1					1	1	1	1
Haematopoietic system	8	3	3	2		1	3		1	1
Injection site(s)	19	2	14	4	13	3	6	2	6	
Jejunum				1						
Liver		1							1	
Mammary gland(s)		45		26		40		38		20
Mandibular salivary gland(s)									1	
Oral cavity & related structures				1		1				1
Pancreas		1		1	1		1	1	2	
Pineal gland									1	
Pituitary gland				1		1				1
Prostate gland	1									
Rectum									1	
Skin			1						2	
Soft tissues	2		4	1	1		2		2	
Testes	1									
Thymus				1						
Thyroid gland	2	1	3	2	1	1	1	1	2	
Uterus		1								
Vagina						1				
TOTAL NO OF MALIGNANT TUMOURS	**37**	**57**	**26**	**42**	**18**	**49**	**15**	**44**	**23**	**24**
TOTAL NO OF BENIGN AND MALIGNANT TUMOURS	**93**	**165**	**84**	**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 ($ng \cdot mL^{-1}$) [n=3]							
	Low dose*: 0.2 $mg \cdot kg^{-1}$ 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
	Mid dose*: 0.8 $mg \cdot kg^{-1}$ once daily							
	Single dose		Week 26		Week 85		Week 97	
	M	F	M	F	M	F	M	F
0.25	65.1	45.1	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*: 3.5 $mg \cdot kg^{-1}$ once daily							
	Single dose		Week 26		Week 85		Week 97	
	M	F	M	F	M	F	M	F
0.25	135	110	160	158	182	138	298	282
0.5	129	105	215	215	204	136	317	354
1	86.9	86.9	148	123	136	120	235	216

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: (b) (4)

Date of study initiation: August 15, 2000
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 (b) (4) was not tested or present at (b) (4), 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

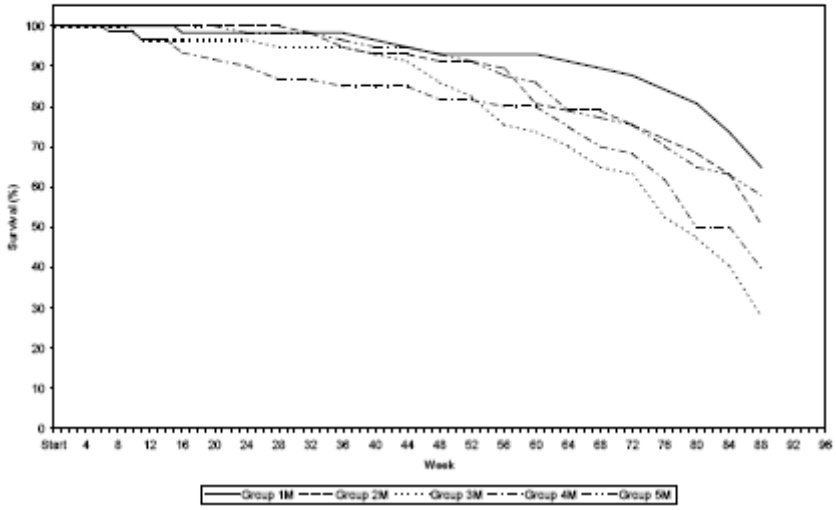
Mortality: A total of 149 males and 160 females died or were killed during the study. The incidence of morbidity/mortality in general increased with the increasing dose in animals of both sexes (except for HD males). These findings are reflected in statistically significant test for linear trend. The 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:

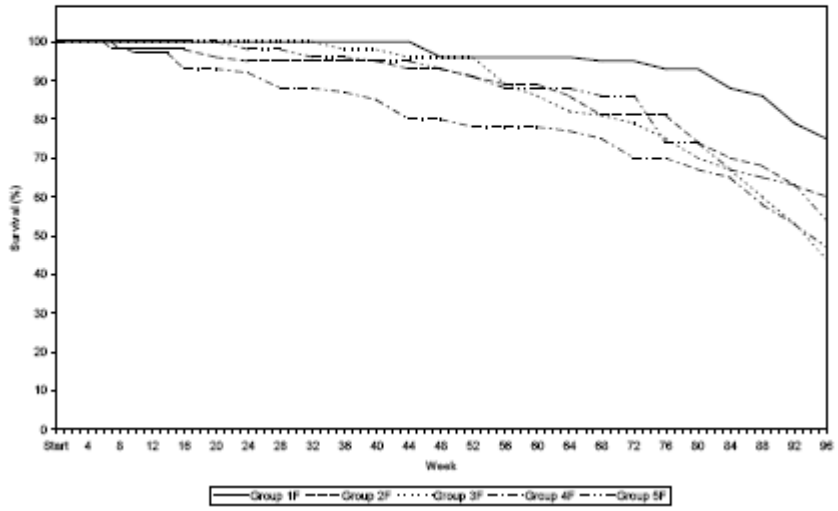
Group survival - males

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



Group survival - 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



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:

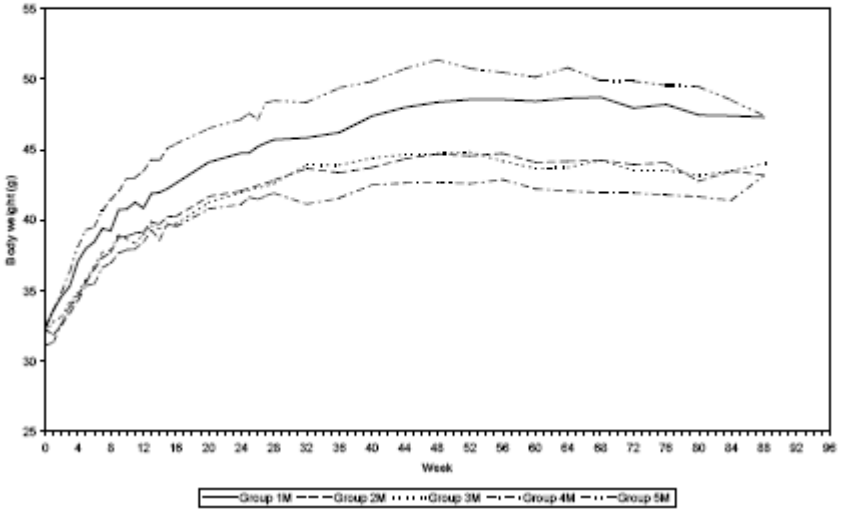
Group incidence of selected factors contributing to morbidity and mortality

		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	2	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:

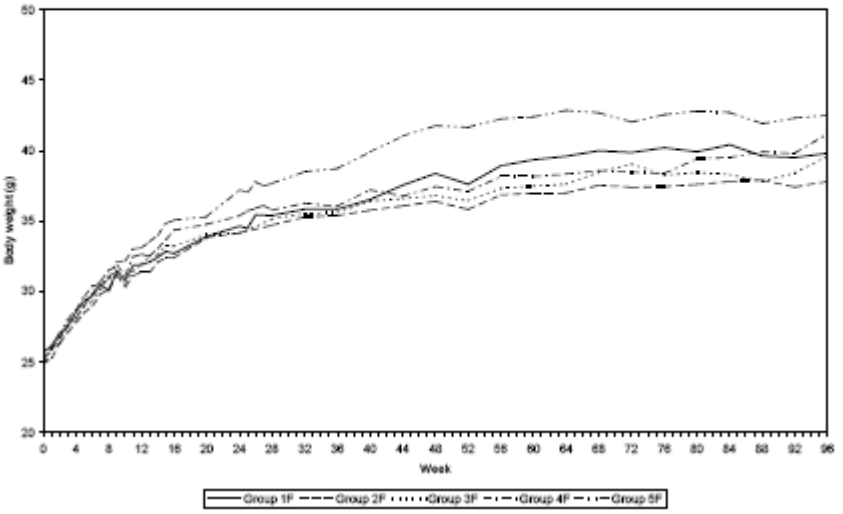
Group mean body weights - males

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



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

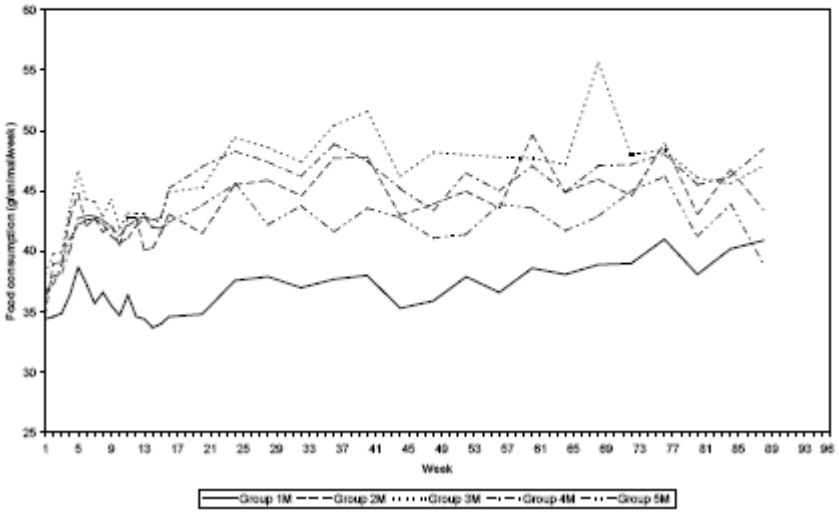


Food consumption: The overall food consumption was statistically significantly increased in mice administered asenapine when compared to the vehicle control, but was similar to that of the untreated control. Males given the LD, MD, and HD were eating 16%, 22%, or 22% more food, respectively, when compared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

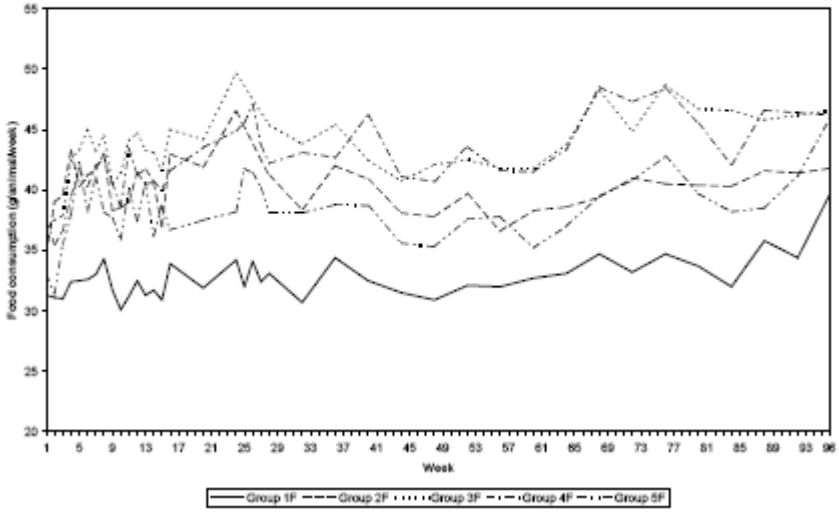
Group mean food consumption - males

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



Group mean food consumption - 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



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

		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Tissue and finding	Level (mg/kg/day)	0	0.5	1.5	5.0/4.0	0	0	0.5	1.5	7.5/5.0	0
Neck (IJ1)	No. examined:	57	39	42	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	12	25	0	13	9	7	14	0
	3	0	2	2	1	1	3	1	1	1	0
Right hip (IJ2)	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 (IJ3)	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

		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Tissue and finding	Level (mg/kg/day)	0	0.5	1.5	5.0/4.0	0	0	0.5	1.5	7.5/5.0	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	5.0/4.0	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
Haemolymporeticular system	No. examined:	57	28	42	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]					
	0.35 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	66.4	50.6		
1	61.2	42.8	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	164	263	156 [a]	166 [b]		

key: F=female, M=male
* 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

content (implantation sites) and weight, position of fetuses in the uterus, number of corpora lutea, pre- and postimplantation loss. Examination of fetuses included survival, sex, body weight, gross external abnormalities, either visceral and brain examination, or examinations of skeletal abnormalities. Part B: The parameters assessed for the F1 generation during lactation up to weaning included daily observations of pups, nesting and nursing behavior of dams, individual pup weight, developmental parameters (pinna unfolding, incisor eruption, onset of coat development, eye opening, testes descent, vagina opening) and behavioral tests (righting reflex, photophobotaxis, cliff avoidance, palmar grasp ability, negative geotaxis, exploratory locomotor pattern, direct pupillary reflex, hearing ability, and water maze test). The parameters assessed for the F1 generation following culling included daily observation of clinical symptoms and body weight. Following delivery of the F2 pups, nursing behavior of dams and the signs and symptoms of the F2 pups were observed, and the sex was determined. All F1 and F2 animals were killed on day 21 post partum and examined macroscopically. The mating and pregnancy rates and the sex ratio of the F2 pups were calculated.

Observations and results

Mortality: There were no treatment-related mortalities. Five animals (2 HD males, 2 HD females, 1 LD male) died due to gavage error or other trauma.

Clinical signs: Animals were observed twice daily. F0 females allowed to deliver naturally were observed for assessment of lactation performance once daily during lactation. Piloerection, lethargy and abnormal posture were observed in MD and HD animals throughout the dosing period. Increased activity/nervousness was observed prior to the first daily dose for some males in these groups. Less marked piloerection and lethargy were observed in LD animals. The incidence and severity of these findings were dose-related.

Body weight: Body weights were recorded daily during the dosing period. In males, body weight was reduced at the MD and HD (compared to controls) throughout the dosing period. The final mean body weight at these doses was 9% and 11% lower than in the control group. Body weight gain was reduced in males during the 60-day pre-mating period by 35% and 70% at the MD and HD, respectively. Body weight gain during the mating and postmating period was similar among groups. In females, body weight gain was similar among groups during premating period. During the mating period, body weight was reduced relative to control at the MD and HD (11%-12% on day 21). Body weight continued to be reduced at the MD and HD (12% and 17%, respectively, relative to control throughout lactation period. Body weight gain was reduced by 37% and 30% in MD and HD females, respectively, during the gestation, and by 30%, 46% and 52% in LD, MD, and HD females, respectively, during lactation.

Food consumption: Food consumption was recorded weekly during the pre-mating period, in males until necropsy, and in females on days 6, 11, 16, and 21 of gestation. Food consumption was decreased in HD males during the first week of dosing, but increased during the pre-mating (<20%) and post-mating (≤32%) periods, except for the first week of dosing. Food consumption was increased during dosing in LD and MD females (12%-16%) and in HD females (21%). A decrease in food consumption during gestation was observed in MD females (8%-12%). During lactation, food consumption decreased in MD females (23%-25%) and HD females (32%-35%).

Toxicokinetics: not conducted

Fertility parameters, terminal and necroscopic evaluations (C-section data):
Treatment delayed mating in all treatment groups: 71%, 23%, 14%, and 30% of animals mated during the first 2 days in the control, LD, MD, and HD groups, respectively. The mean precoital times were 3.7, 7.4, 8.5, and 8.1 days in the control, LD, MD, and HD groups, respectively. 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 was an increase in pre-implantation loss expressed as a percentage of corpora lutea in all dose groups (6.1%, 11.2%, 20.4% and 17.1% in the control, LD, MD and HD groups, respectively). Total resorption and total litter loss was noted only in HD females; there were 2 HD animals with total resorption and 4 HD animals which lost their litters by day 7 postpartum. For Part B female rats, a decrease in number of implantations and a slight increase in the incidence of post-implantation loss (6.9%, 7.0%, 14.3% and 13.3% in the control, LD, MD, and HD groups, respectively) resulted in a marked reduction in live litter size in MD and HD groups. There was a slight decrease in pup survival on days 1-4 post partum (0%, 3.8%, 7.9% and 22.6% in the control, LD, MD, and HD groups, respectively). No treatment-related macroscopic changes were observed at terminal necropsy. These data are shown in the following Sponsor's tables:

FERTILITY
FO GENERATION

FEMALES SCHEDULED FOR CAESAREAN SECTION AND BREEDING

	GROUP 1 2 X 0.0 MG/KG	GROUP 2 2 X 0.5 MG/KG	GROUP 3 2 X 2.5 MG/KG	GROUP 4 2 X 15.0 MG/KG
Percentage mating	24/24 100.0	24/24 100.0	24/24 100.0	22/23 95.7
Fertility index (%)	23/24 95.8	22/24 91.7	22/24 91.7	20/23 87.0
Conception rate (%)	23/24 95.8	22/24 91.7	22/24 91.7	20/22 90.9
Gestation index (%) (Part A)	12/12 100.0	11/11 100.0	12/12 100.0	10/10 100.0
Gestation index (%) (Part B)	11/11 100.0	10/11 90.9	10/10 100.0	8/10 80.0

Percentage mating = (Females mated / Females paired) * 100
Fertility index = (Females achieving a pregnancy / Females paired) * 100
Conception rate = (Females achieving a pregnancy / Females mated) * 100
Gestation index = (Number of females with living pups / Number of females pregnant) * 100

REPRODUCTION DATA SUMMARY
PART A - CAESAREAN SECTION

	GROUP 1 2X0.0 MG/KG	GROUP 2 2X0.5 MG/KG	GROUP 3 2X2.5 MG/KG	GROUP 4 2X15.0 MG/KG
NUMBER OF DAMS	12	11	12	10
CORPORA LUTEA	148	125	142	123
MEAN (+)	12.3	11.4	11.8	12.3
ST.DEV.	1.0	1.6	2.0	2.3
PRE-IMPLANTATION LOSS	9	14	29	21
% OF CORP. LUTEA (#)	6.1	11.2	20.4 ##	17.1 ##
MEAN (+)	0.8	1.3	2.4	2.1
ST.DEV.	1.8	1.5	3.9	1.8
NUMBER OF DAMS AFFECTED	3	7	7	7
IMPLANTATION SITES	139	111	113	102
% OF CORP. LUTEA (#)	93.9	88.8	79.6 ##	82.9 ##
MEAN (+)	11.6	10.1	9.4	10.2
ST.DEV.	1.9	2.5	3.0	2.7
POST-IMPLANTATION LOSS	13	9	11	5
% OF IMPL. SITES (#)	9.4	8.1	9.7	4.9
MEAN (+)	1.1	0.8	0.9	0.5
ST.DEV.	1.8	1.3	0.7	0.8
NUMBER OF DAMS AFFECTED	5	5	9	3
IMPLANTATION SITE SCARS	0	0	0	0
EMBRYONIC DEATHS : TOTAL	13	9	11	5
EMBRYONIC RESORPTIONS	13	9	11	3
% OF IMPL. SITES (#)	9.4	8.1	9.7	2.9 #
MEAN (+)	1.1	0.8	0.9	0.3
ST.DEV.	1.8	1.3	0.7	0.7
NUMBER OF DAMS AFFECTED	5	5	9	2
FETAL RESORPTIONS	0	0	0	2
% OF IMPL. SITES (#)				2.0
MEAN (+)				0.2
ST.DEV.				0.6
NUMBER OF DAMS AFFECTED				1
FETUSES				
TOTAL FETUSES	126	102	102	97
% OF IMPL. SITES (#)	90.6	91.9	90.3	95.1
MEAN (+)	10.5	9.3	8.5	9.7
ST.DEV.	2.5	3.0	3.0	2.7
LIVE FETUSES	126	102	102	97
DEAD FETUSES	0	0	0	0
ABNORMAL FETUSES	0	2	0	0
% OF FETUSES (#)		2.0		
MEAN (+)		0.2		
ST.DEV.		0.4		
NUMBER OF DAMS AFFECTED		2		
ABNORMAL LIVE FETUSES AT EXTERNAL EXAMINATION	0	2	0	0
ABNORMAL DEAD FETUSES AT EXTERNAL EXAMINATION	0	0	0	0

*/** : Dunnett-Test based on pooled variance significant at level 5% (*) or 1% (**)
#/## : Fisher's Exact Test significant at level 5% (#) or 1% (##)
+ : Steel Test significant at level 5%

BREEDING DATA PER GROUP
FO GENERATION

	GROUP 1 2 X 0.0 MG/KG	GROUP 2 2 X 0.5 MG/KG	GROUP 3 2 X 2.5 MG/KG	GROUP 4 2 X 15.0 MG/KG
LITTERS				
TOTAL	11	10	10	6
EXCLUDED	0	0	1	2
EVALUATED	11	10	9	4
DURATION OF GESTATION				
MEAN (+)	21.5	21.6	21.9	21.5
ST.DEV	0.52	0.52	0.38	0.58
N	11	8	7	4
IMPLANTATIONS				
TOTAL	131	114	76	37
MEAN (+)	11.9	11.4	8.4 +	9.3
ST.DEV	1.45	2.22	3.09	2.50
N	11	10	9	4
POST IMPLANTATION LOSS				
% OF IMPLANTATIONS	6.9	7.0	14.5	13.5
LITTERS AFFECTED (#)	8	5	5	3
TOTAL	9	8	11	5
MEAN (+)	0.8	0.8	1.2	1.3
ST.DEV	0.60	1.23	1.30	1.26
N	11	10	9	4
DEAD PUPS AT FIRST LITTER CHECK				
TOTAL	0	0	2	1
MEAN (+)	0.0	0.0	0.2	0.3
ST.DEV	0.00	0.00	0.67	0.50
N	11	10	9	4
LIVING PUPS AT FIRST LITTER CHECK				
% OF MALES / FEMALES (#)	48 / 52	40 / 60	57 / 43	58 / 42
TOTAL	122	106	63	31
MEAN (+)	11.1	10.6	7.0 +	7.8
ST.DEV	1.51	2.17	3.16	3.40
N	11	10	9	4
POSTNATAL LOSS DAYS 0 - 4 P.P.				
% OF LIVING PUPS	0.0	3.8	7.9	22.6
LITTERS AFFECTED (#)	0	4 #	3	3 ##
TOTAL	0	4	5	7
MEAN (+)	0.0	0.4	0.6	1.8
ST.DEV	0.00	0.52	1.01	2.22
N	11	10	9	4
LIVING PUPS DAY 4 P.P.				
TOTAL	122	102	58	24
MEAN (+)	11.1	10.2	6.4 +	6.0
ST.DEV	1.51	2.10	3.36	2.16
N	11	10	9	4
POSTNATAL LOSS DAYS 5 - 21 P.P.				
% OF LIVING PUPS AT DAY 4 P.P.	1.6	0.0	3.4	4.2
LITTERS AFFECTED (#)	2	0	2	1
TOTAL	2	0	2	1
MEAN (+)	0.2	0.0	0.2	0.3
ST.DEV	0.40	0.00	0.44	0.50
N	11	10	9	4
LIVING PUPS DAY 21 P.P.				
% OF MALES / FEMALES (#)	48 / 53	41 / 59	57 / 43	57 / 43
TOTAL	120	102	56	23
MEAN (+)	10.9	10.2	6.2 +	5.8
ST.DEV	1.30	2.10	3.11	2.22
N	11	10	9	4

+ : Steel-test significant at 5% level
# / ## : Fisher's Exact Test significant at 5% (#) or 1% (##) level

Offspring (malformations, variations, etc.): Visceral examination demonstrated one MD fetus with a heart defect. Only 2 LD abnormal fetuses were reported upon external examination. There were no other teratogenic effects. General retardation of development was observed at all dose levels as demonstrated by an increased incidence of abnormal findings and variants detected during the skeletal examinations. Abnormal findings included wavy ribs, four or more anomalous sternebrae, small fetuses with generally delayed ossification or fetuses with incompletely ossified thoracic vertebral centra. The incidence of fetuses with abnormal skeletal findings was as follows: 2/66 fetuses in 2/12 litters in the control group; 7/54 fetuses in 5/11 litters in the LD group; 4/55 fetuses in 3/12 litters in the MD group; 8/51 fetuses in 6/11 litters in the HD group.
F_1 physical development: Mean body weight of the F1 offspring was reduced in day 1 post partum by 7% in the HD group. Body weight gain was also decreased for these animals during the lactation period. There were developmental delays of one day or more in coat development, eye opening, and testes descent at the HD. No other treatment-related findings were observed in pups during the lactation period. These data is shown in the following Sponsor's table:

DEVELOPMENTAL INDICES PER GROUP (DAYS P.P.)
F1 PUPS

		GROUP 1 2 X 0.0 MG/KG	GROUP 2 2 X 0.5 MG/KG	GROUP 3 2 X 2.5 MG/KG	GROUP 4 2 X 15.0 MG/KG
Pinna unfolding	Mean	3.3	3.2	3.0	3.7
	St.dev.	0.67	0.61	0.57	1.31
	N	11	10	10	4
Incisor eruption	Mean	8.0	8.1	8.1	8.2
	St.dev.	0.00	0.18	0.33	0.33
	N	11	10	9	4
Onset of coat development	Mean	9.3	9.4	9.4	10.9
	St.dev.	0.46	0.50	0.47	1.01
	N	11	10	9	4
Opening of eye	Mean	14.7	15.0	14.4	16.8
	St.dev.	0.73	0.65	0.69	1.02
	N	11	10	9	4
Descensus of testes	Mean	22.6	22.8	22.6	23.6
	St.dev.	0.40	0.90	0.54	0.48
	N	10	10	9	4
Opening of vagina	Mean	33.3	33.9	34.3	35.9
	St.dev.	0.84	1.57	2.11	1.02
	N	11	10	9	3

F_1 behavioral evaluation: There were significantly lower values for some of the behavioral tests conducted with F1 pups (palmar grasp ability and water maze) in the HD group. These data is shown in the following Sponsor's table:

BEHAVIOURAL TESTS PER GROUP
F1 PUPS

		GROUP 1 2 X 0.0 MG/KG	GROUP 2 2 X 0.5 MG/KG	GROUP 3 2 X 2.5 MG/KG	GROUP 4 2 X 15.0 MG/KG
Righting reflex	Mean (#)	100 %	100 %	100 %	100 %
	N	11	10	9	4
Photo-phobotaxis	Mean (#)	72 %	70 %	76 %	60 %
	N	11	10	9	4
Pupillary reflex	Mean (#)	100 %	100 %	100 %	96 %
	N	11	10	9	4
Hearing ability	Mean (#)	100 %	100 %	100 %	100 %
	N	11	10	9	4
Palmar grasp ability	Mean (#)	99 %	100 %	100 %	74 % ##
	N	11	10	9	4
Activity test	Mean (+)	1.9	1.8	2.0	1.6
	N	11	10	9	4
Cliff avoidance	Mean (#)	98 %	99 %	99 %	100 %
	N	11	10	9	4
Negative geotaxis	Mean (#)	60 %	73 %	71 % #	54 %
	N	11	10	9	4
Water maze learning	Mean (#)	100 %	100 %	94 %	100 %
	N	11	10	9	4
Water maze memory	Mean (#)	82 %	92 % #	93 % #	87 %
	N	11	10	9	4
Water maze relearning	Mean (#)	90 %	90 %	79 % #	67 % ##
	N	11	10	9	4

F_1 reproduction: There no treatment-related changes in F1 reproduction.
F_2 findings: The implantation rate was slightly lower in the HD group. There no other treatment-related changes in F2 generation.
(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).

Embryofetal development - studies in rats

PILOT STUDIES

1. Study title: A pilot embryotoxicity study with Org 5222 in rats. (Study No. SDG RR 2316). Test facility: Organon Inc; study initiation date: February 7, 1988; GLP: no

Key study findings: In a pilot study designed to assess effects of asenapine at 30 mg/kg/day (21.3 mg/kg/day AE) on pregnancy and embryofetal development, oral administration to pregnant female rats during the period of organogenesis induced slight transient reduction in mean body weight during pregnancy. The abnormal litter ratio and the malformation rate were increased, although the differences were not statistically significant. The degree of ossification of various skeletal elements was also decreased in fetuses of asenapine treated females.

Methods: Asenapine (Batch G) was administered orally to pregnant Sprague-Dawley female rats (15/group) at 30 mg/kg/day (21.3 mg/kg AE) once daily from day 6 to day 17 of pregnancy. Dams were examined for clinical signs and body weight. On day 21 of

pregnancy all females were terminated and necropsy was conducted to determine any signs of gross visceral changes in the parent animals and pregnancy performance (pregnancy rate, number of implantation sites, number and distribution of live fetuses and dead embryos, number of corpora lutea, ovarian weight). The fetuses were examined for sex ratio, fetal/placental weight, abnormal litter ratio, malformation rate and ossification variance (50% of fetuses of each litter was examined for brain and visceral malformations and the remaining 50% for skeletal malformation).

Results: Two unscheduled deaths of asenapine treated females were attributed to dosing error. Mean body weight was reduced in females by 11%-13% on days 11 to 21 of pregnancy. The abnormal litter ratio was 13.3% and 40.0% in the control and asenapine-treated group, respectively. Malformations were detected in 2/213 control fetuses in 2 litters and 4/107 treated fetuses in 4 litters (hydronephrotic kidney in 2 control and 2 asenapine-treated fetuses, and bilateral anophthalmia in 2 additional asenapine-treated fetuses). The degree of ossification of various skeletal elements (e.g. sternebrae 5, 5th proximal phalange) was slightly less in fetuses of asenapine treated group. The abnormal litter ratio and the malformations rate in the asenapine treated group were not statistically significantly higher than that of the control group. These data are shown in the following Sponsor's table:

Table 4 Major-defect-foetuses per litter

F0 - females

ORG 5222
Gr. 1: 0 mg/kg
Gr. 2: 30 mg/kg

	Group no. 1	2
No of females with live foetuses	15	9
Number of females with major-defect-foetuses	2	4
Abnormal litter ratio (%)	13,3	40,0
Significance		//
Number of foetuses	213	107
Number of foetuses displaying major defects	2	4
Malformation rate (%)	1,0	6,1
Significance		//

Note: This study has been reviewed also by Dr. L. Freed; see her review of the IND 51,641 of January 9, 1998 for additional details.

PIVOTAL STUDIES

2. Study title: Embryotoxicity study (including teratogenicity) with Org 5222 in the rat.

Key study findings: 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 and increased post-implantation loss. The mean fetal body weight was also slightly reduced (by 8%) in this group. 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 NOAEL for maternal toxicity was considered to be below the LD. The NOAEL for the reproduction and F1 parameters was the MD. However, it is unclear whether the external and visceral malformations were properly examined. Only one malformed fetus was reported upon external or visceral examinations in the LD group. It appears extremely unlikely that no spontaneous external or visceral findings were detected in any fetus in all other groups. Therefore, evaluation of the external and visceral teratogenic effects in this study is considered inadequate.

[Note: This study was also reviewed by Dr. Lois Freed under the IND 51,641. She concluded that "the lack of specific findings suggests reduced sensitivity to detect soft tissue abnormalities, variants, etc. Unless data can be provided that adequately document the sensitivity of the methods used to assess fetal effects, the studies may need to be repeated". Please see Dr. Freed's review dated January 9, 1998 for further details. Her conclusions were conveyed to the Sponsor in a letter dated February 11, 1998. The Sponsor did not address these concerns directly in the NDA 22-117. However, an intravenous embryofetal developmental study in rats, designed to characterize teratogenic effects of asenapine, was submitted under this NDA].

Study no.: SDG RR 2961
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: March 27, 1990
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine maleate (Org 5222), Batch: AG, purity: 99.7%
Methods

Doses: 0, 0.5, 2.5 and 15.0 mg/kg b.i.d. with an interval of ~ 5 hours (dosages expressed as the maleate)
Species/strain: rat/Wistar/HAN
Number/sex/group: 36 mated female rats/group
Route, formulation, volume: oral (gavage), solution (or suspension for the high dose only) in gelatin/mannitol/water (final concentration 0.5%/5%), volume: 2 ml/kg body weight
Satellite groups used for toxicokinetics: none
Study design: This study was designed to assess the effects of asenapine on embryonic, fetal and postnatal development in pregnant rats, and to provide information about the reproductive processes of the F1 parent animals and the neonatal viability and growth of the F2 pups. Asenapine was administered orally by gavage twice daily from day 7 through day 17 of pregnancy. The dosages were

selected based on a pilot rat embryotoxicity study. Two thirds (24/group) of the mated F0 female rats (Part A) were sacrificed on day 21 of gestation, the fetuses were removed by C-section, weighed, examined externally and allocated to either visceral or skeletal examination. The remaining third (12/group) of the F0 mated females (Part B) was allowed to give birth and rear their litters up to day 21 after birth. Developmental and behavioral parameters were assessed for the F1 offspring. Selected F1 animals were reared to maturity, paired and their reproductive performance was assessed. These animals were not dosed.
Parameters and endpoints evaluated: Maternal parameters: mortality, clinical signs, food consumption, and body weights. C-section and postmortem parameters: gross pathology changes of the dams, number and weight of individual live fetuses, number of embryonic and fetal deaths, number of implantation sites in the uterus, and number of corpora lutea. Litter parameters: Fetuses from Part A females were examined for external abnormalities, sex, weight, visceral and skeletal malformations and ossification variance. Pups from Part B females were examined for sex, number of missing or dead, abnormal findings, individual and mean weights. On day 4 postpartum, litters were culled to 4/sex and later tested in physical development tests, including pinna unfolding, incisor eruption, onset of coat development, eye opening, testes descent, vaginal opening, and behavioral tests, including righting reflex (day 14), photophobotaxis (day 21), cliff avoidance (day 21), palmar grasp ability (day 16), negative geotaxis (day 21), exploratory locomotor pattern (day 21), direct pupillary reflex (day 21), hearing ability (day 21), water maze test (days 35-43) (days refer to days pospartum). F1 reproduction parameters of selected animals mated at 12 weeks of age and macroscopic abnormalities of F2 at necropsy were assessed.

Results
Mortality (dams): There was no mortality in the females of the F0 generation.
Clinical signs (dams): Animals were observed twice daily during the dosing period. Dose-related clinical signs of ruffled fur, somnolence, hunched posture and ventral recumbency were observed during the dosing phase of the study in female rats of all dose groups. Bite wounds and injuries to paws, and swelling and bleeding of paws were noted at 15.0 mg/kg b.i.d.
Body weight (dams): Body weights were recorded daily during the pregnancy and on days 1, 4, 7, 14, and 21 postpartum. Marked decrease in body weight between days 7 and 8 of pregnancy (by 10%) and slightly reduced body weight gain during the rest of the dosing period (gain of 27% compared to 39% in the control group) were observed in dams treated with asenapine at the HD. Minimal decrease in body weight between days 7 and 8 of pregnancy and slightly reduced body weight gain during the first half of the dosing period were observed in dams treated with asenapine at the LD and MD. In addition, dams administered the MD and HD had reduced corrected (for uterus weight) body weight gain. In dams allowed to deliver, body weight on day 1 was slightly lower but thereafter was similar among all groups. Body weight gain during lactation was moderately higher in the MD and HD groups compared to controls.
Food consumption (dams): Food consumption was recorded on days 0-7, 7-10, 10-14, 14-18, and 18-21 of pregnancy, and in females allowed to deliver, on days 1-7 and 7-14

postpartum. Treatment-related decrease in food consumption from the control values was observed in dams administered the HD during the treatment and post-treatment periods, in particular between days 7 and 10 of pregnancy by 62%, and in dams administered the MD during the post-treatment period by 14%.

Toxicokinetics: not conducted

Terminal and necroscopic evaluations: Increased pre-implantation loss was noted in Part A HD dams (16.1% of the corpora lutea) compared to controls (8.3% of the corpora lutea). However, the Sponsor considered the increase in pre-implantation loss not to be drug-related since dosing started after implantation in female rats. In addition, 5/24 HD females in Part A had total resorption (no resorptions were observed in controls), and an increased post-implantation loss was observed at the HD (15.8%) compared to the control group (5.7%). There were no effects on these parameters in other asenapine-treated groups. In females allowed to deliver (Part B), there were no drug-related effects on any litter parameters. There were no treatment related macroscopic findings in F0, F1 or F2 generations. The summary of data reproduction performance data is shown in the following Sponsor's table:

REPRODUCTION DATA SUMMARY
PART A - CAESAREAN SECTION

	GROUP 1 2X0.0 MG/KG	GROUP 2 2X0.5 MG/KG	GROUP 3 2X2.5 MG/KG	GROUP 4 2X15.0 MG/K
NUMBER OF DAMS	22	22	23	19
CORPORA LUTEA	289	280	286	249
MEAN (+)	13.1	12.7	12.4	13.1
ST.DEV.	1.3	1.6	1.6	1.5
PRE-IMPLANTATION LOSS	24	22	29	40
% OF CORP. LUTEA (#)	8.3	7.9	10.1	16.1 ##
MEAN (+)	1.1	1.0	1.3	2.1
ST.DEV.	1.8	1.7	1.9	2.6
NUMBER OF DAMS AFFECTED	11	12	13	14
IMPLANTATION SITES	265	258	257	209
% OF CORP. LUTEA (#)	91.7	92.1	89.9	83.9 ##
MEAN (+)	12.0	11.7	11.2	11.0
ST.DEV.	2.2	2.4	1.9	2.9
POST-IMPLANTATION LOSS	15	14	13	33
% OF IMPL. SITES (#)	5.7	5.4	5.1	15.8 ##
MEAN (+)	0.7	0.6	0.6	1.7
ST.DEV.	0.8	1.1	0.5	2.5
NUMBER OF DAMS AFFECTED	11	7	13	12
IMPLANTATION SITE SCARS	0	0	0	0
EMBRYONIC DEATHS : TOTAL	15	14	13	33
EMBRYONIC RESORPTIONS	15	13	13	32
% OF IMPL. SITES (#)	5.7	5.0	5.1	15.3 ##
MEAN (+)	0.7	0.6	0.6	1.7
ST.DEV.	0.8	1.1	0.5	2.5
NUMBER OF DAMS AFFECTED	11	7	13	12
FETAL RESORPTIONS	0	1	0	1
% OF IMPL. SITES (#)		0.4		0.5
MEAN (+)		0.0		0.1
ST.DEV.		0.2		0.2
NUMBER OF DAMS AFFECTED		1		1
FETUSES				
TOTAL FETUSES	250	244	244	176
% OF IMPL. SITES (#)	94.3	94.6	94.9	84.2 ##
MEAN (+)	11.4	11.1	10.6	9.3
ST.DEV.	2.1	2.7	2.1	3.8
LIVE FETUSES	250	244	244	176
DEAD FETUSES	0	0	0	0
ABNORMAL FETUSES	0	1	0	0
% OF FETUSES (#)		0.4		
MEAN (+)		0.0		
ST.DEV.		0.2		
NUMBER OF DAMS AFFECTED		1		
ABNORMAL LIVE FETUSES AT EXTERNAL EXAMINATION	0	1	0	0
ABNORMAL DEAD FETUSES AT EXTERNAL EXAMINATION	0	0	0	0

*/** : Dunnett-Test based on pooled variance significant at level 5% (*) or 1% (**)
#/## : Fisher's Exact Test significant at level 5% (#) or 1% (##)
+ : Steel Test significant at level 5%

REPRODUCTION DATA SUMMARY
PART A - CAESAREAN SECTION

	GROUP 1 2X0.0 MG/KG	GROUP 2 2X0.5 MG/KG	GROUP 3 2X2.5 MG/KG	GROUP 4 2X15.0 MG/K
NUMBER OF DAMS	22	22	23	19
SEX OF FETUSES				
TOTAL MALES	125	122	124	81
% OF FETUSES (#)	50.0	50.0	50.8	46.0
MEAN	5.7	5.5	5.4	4.3
ST.DEV.	2.0	1.9	1.8	1.8
TOTAL FEMALES	125	122	120	95
% OF FETUSES (#)	50.0	50.0	49.2	54.0
MEAN	5.7	5.5	5.2	5.0
ST.DEV.	1.9	2.8	2.3	2.9
LIVE MALES	125	122	124	81
LIVE FEMALES	125	122	120	95
WEIGHTS OF LIVE FETUSES (LITTER BASIS)				
TOTAL FETUSES				
N (LITTERS)	22	22	23	19
MEAN (*)	4.8	4.8	4.8	4.5 **
ST.DEV.	0.2	0.2	0.2	0.4
MALES				
N (LITTERS)	22	22	23	18
MEAN (*)	4.9	4.9	4.9	4.5 **
ST.DEV.	0.2	0.3	0.2	0.4
FEMALES				
N (LITTERS)	22	21	22	17
MEAN (*)	4.7	4.7	4.7	4.4 **
ST.DEV.	0.2	0.2	0.2	0.4
WEIGHTS OF LIVE FETUSES (INDIVIDUAL BASIS)				
TOTAL FETUSES				
N (FETUSES)	250	244	244	176
MEAN	4.8	4.8	4.8	4.4
ST.DEV.	0.3	0.4	0.3	0.5
MALES				
N (FETUSES)	125	122	124	81
MEAN	4.9	4.9	4.9	4.6
ST.DEV.	0.3	0.4	0.3	0.4
FEMALES				
N (FETUSES)	125	122	120	95
MEAN	4.7	4.6	4.6	4.3
ST.DEV.	0.3	0.3	0.3	0.4

Offspring: Mean fetal body weight was slightly reduced (by 8%) in group administered the HD (the mean value was 4.4 g compared to 4.8 g in all other groups). External findings were detected only in 1 fetus administered the LD. On visceral examinations, "no abnormal findings" was reported for all groups, including control. Drug-related effects on skeletal parameters were noted, including both increases and decreases in ossification. The incidence of incomplete ossification and non-ossified skeletal elements (sternebra, vertebra, and limbs) was slightly increased in the asenapine-treated groups. These findings were generally not statistically significant, except non-ossified metatarsalia 1 (hind limb) at the HD and decreases in non-ossified digit 5 distal phalanx (forelimb) at all doses. Selected skeletal data are shown in the following Sponsor's table:

SKELETAL EXAMINATION SUMMARY

	GROUP 1 2X0.0 MG/KG		GROUP 2 2X0.5 MG/KG		GROUP 3 2X2.5 MG/KG		GROUP 4 2X15.0 MG/KG	
NUMBER OF FETUSES EXAMINED	132		128		126		90	
LEFT FORELIMB								
NON-OSSIFIED								
DIGIT 4 DISTAL PHALANX, LEFT	3	2%	2	2%	2	2%	0	
METACARPALIA 5, LEFT	0		1	1%	0		0	
DIGIT 5 PROXIMAL PHALANX, LEFT	64	48%	47	37% *	40	32% **	52	58%
DIGIT 5 DISTAL PHALANX, LEFT	70	53%	46	36% **	34	27% **	18	20% **
RIGHT FORELIMB								
NON-OSSIFIED								
DIGIT 1 DISTAL PHALANX, RIGHT	1	1%	3	2%	0		0	
METACARPALIA 2, RIGHT	0		1	1%	0		0	
DIGIT 2 PROXIMAL PHALANX, RIGHT	35	27%	21	16% *	15	12% **	31	34%
DIGIT 2 DISTAL PHALANX, RIGHT	12	9%	5	4%	7	6%	4	4%
METACARPALIA 3, RIGHT	0		1	1%	0		0	
DIGIT 3 PROXIMAL PHALANX, RIGHT	1	1%	2	2%	0		0	
DIGIT 3 DISTAL PHALANX, RIGHT	0		1	1%	0		0	
METACARPALIA 4, RIGHT	0		1	1%	0		0	
DIGIT 4 PROXIMAL PHALANX, RIGHT	4	3%	4	3%	1	1%	0	
DIGIT 4 DISTAL PHALANX, RIGHT	2	2%	3	2%	1	1%	0	
METACARPALIA 5, RIGHT	0		1	1%	0		0	
DIGIT 5 PROXIMAL PHALANX, RIGHT	64	48%	45	35% *	37	29% **	51	57%
DIGIT 5 DISTAL PHALANX, RIGHT	70	53%	46	36% **	29	23% **	17	19% **
LEFT HIND LIMB								
NON-OSSIFIED								
TALUS LEFT	105	80%	100	78%	87	69% *	83	94% **
METATARSALIA 1, LEFT	14	11%	13	10%	16	13%	26	29% **
TOE 1 DISTAL PHALANX, LEFT	1	1%	1	1%	0		0	
METATARSALIA 2, LEFT	0		1	1%	0		0	
TOE 2 PROXIMAL PHALANX, LEFT	79	60%	83	65%	75	60%	70	78% **
TOE 2 DISTAL PHALANX, LEFT	0		1	1%	0		0	
METATARSALIA 3, LEFT	0		1	1%	0		0	
TOE 3 PROXIMAL PHALANX, LEFT	51	39%	50	39%	39	31%	54	60% **
TOE 3 DISTAL PHALANX, LEFT	0		1	1%	0		0	
METATARSALIA 4, LEFT	0		1	1%	0		0	
TOE 4 PROXIMAL PHALANX, LEFT	48	36%	39	30%	37	29%	52	58% **
TOE 4 DISTAL PHALANX, LEFT	0		1	1%	0		0	
METATARSALIA 5, LEFT	0		1	1%	0		0	
TOE 5 PROXIMAL PHALANX, LEFT	113	86%	105	82%	105	83%	84	93%
TOE 5 DISTAL PHALANX, LEFT	0		2	2%	0		0	
RIGHT HIND LIMB								
NON-OSSIFIED								
TALUS RIGHT	112	85%	101	79%	85	67% **	85	94% *
METATARSALIA 1, RIGHT	16	12%	13	10%	16	13%	27	30% **
TOE 1 DISTAL PHALANX, RIGHT	1	1%	1	1%	0		0	
METATARSALIA 2, RIGHT	0		1	1%	0		0	
TOE 2 PROXIMAL PHALANX, RIGHT	81	61%	82	64%	75	60%	69	77% *
TOE 2 DISTAL PHALANX, RIGHT	0		1	1%	0		0	
METATARSALIA 3, RIGHT	0		1	1%	0		0	
TOE 3 PROXIMAL PHALANX, RIGHT	55	42%	53	41%	42	33%	56	62% **
TOE 3 DISTAL PHALANX, RIGHT	0		1	1%	0		0	
METATARSALIA 4, RIGHT	0		1	1%	0		0	
TOE 4 PROXIMAL PHALANX, RIGHT	50	38%	45	35%	38	30%	54	60% **
TOE 4 DISTAL PHALANX, RIGHT	0		1	1%	0		0	
METATARSALIA 5, RIGHT	0		1	1%	0		0	
TOE 5 PROXIMAL PHALANX, RIGHT	111	84%	106	83%	108	86%	84	93% *
TOE 5 DISTAL PHALANX, RIGHT	0		2	2%	0		0	
LEFT FORELIMB								
NON-OSSIFIED								
DIGIT 1 DISTAL PHALANX, LEFT	1	5%	3	14%	0		0	
METACARPALIA 2, LEFT	0		1	5%	0		0	
DIGIT 2 PROXIMAL PHALANX, LEFT	16	73%	14	64%	12	52%	10	53%
DIGIT 2 DISTAL PHALANX, LEFT	8	36%	3	14%	6	26%	3	16%
METACARPALIA 3, LEFT	0		1	5%	0		0	
DIGIT 3 PROXIMAL PHALANX, LEFT	2	9%	3	14%	1	4%	0	
DIGIT 3 DISTAL PHALANX, LEFT	0		1	5%	0		0	
METACARPALIA 4, LEFT	0		1	5%	0		0	
DIGIT 4 PROXIMAL PHALANX, LEFT	7	32%	4	18%	2	9%	0	**
LEFT FORELIMB								
NON-OSSIFIED								
DIGIT 4 DISTAL PHALANX, LEFT	2	9%	2	9%	2	9%	0	
METACARPALIA 5, LEFT	0		1	5%	0		0	
DIGIT 5 PROXIMAL PHALANX, LEFT	19	86%	16	73%	16	70%	16	84%
DIGIT 5 DISTAL PHALANX, LEFT	21	95%	17	77%	16	70% *	9	47% **
RIGHT FORELIMB								
NON-OSSIFIED								
DIGIT 1 DISTAL PHALANX, RIGHT	1	5%	3	14%	0		0	
METACARPALIA 2, RIGHT	0		1	5%	0		0	
DIGIT 2 PROXIMAL PHALANX, RIGHT	17	77%	13	59%	10	43% *	11	58%
DIGIT 2 DISTAL PHALANX, RIGHT	8	36%	4	18%	5	22%	3	16%
METACARPALIA 3, RIGHT	0		1	5%	0		0	
DIGIT 3 PROXIMAL PHALANX, RIGHT	1	5%	2	9%	0		0	
DIGIT 3 DISTAL PHALANX, RIGHT	0		1	5%	0		0	
METACARPALIA 4, RIGHT	0		1	5%	0		0	
DIGIT 4 PROXIMAL PHALANX, RIGHT	3	14%	4	18%	1	4%	0	
DIGIT 4 DISTAL PHALANX, RIGHT	2	9%	2	9%	1	4%	0	
METACARPALIA 5, RIGHT	0		1	5%	0		0	
DIGIT 5 PROXIMAL PHALANX, RIGHT	19	86%	15	68%	16	70%	16	84%
DIGIT 5 DISTAL PHALANX, RIGHT	21	95%	16	73% *	15	65% *	9	47% **

Wavy ribs were observed in the control, LD and MD, but not in the HD fetuses. There were no increases in skeletal malformations in dosed groups.
F_1 physical development, behavioral evaluation and reproduction: The only drug-related effect on behavioral development was a dose-related decrease in negative geotaxis assessed once during the postpartum period (97%, 88%, 79% and 75% in the control, LD, MD and HD groups, respectively). There were no other treatment-related changes in F1 parameters.
F_2 findings: There were no treatment-related changes in parameters examined for the F2 generation.

3. Study title: An intravenous embryo-fetal development study with asenapine in Sprague-Dawley rats.

Key study findings: This study was designed to examine the effects of asenapine on pregnant female rats as well as embryonic and fetal development when administered intravenously from implantation to closure of the hard palate (days 6-17 of pregnancy). 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 in 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.

There were no test article-related external or visceral findings in fetuses at any dose level. 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 from the HD dam no. 85. Macroscopic observations indicated a mass in the chest wall region of this dam, which was considered an incidental occurrence. Therefore, findings in the litter from the dam no. 85 can be excluded from the assessment of teratogenic effects. In conclusion, findings at the HD are not considered drug-related. The NOAEL for maternal toxicity and for fetal and skeletal abnormalities is the HD of 1.5 mg/kg/day AE (2.11 mg/kg/day expressed as the maleate). This dose is equal the MRHD of 10 mg b.i.d. on mg/m^2 basis.

Study no.: INT00002826
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: April 7, 2005
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine maleate, batch AG, purity: 99.7%

Methods

Doses: 0, 0.3, 0.9, 1.5 mg/kg body weight/day expressed as active entity (0, 0.42, 1.27, 2.11 expressed as maleate)
Species/strain: rat/Sprague Dawley
Number/sex/group: 22 mated female rats/group
Route, formulation, volume, and infusion rate: intravenous application into the tail vein, solution in isotonic pyrogen-free citrate buffer, 2.5 ml/kg body weight, injection speed of 1.2 ml/minute
Satellite groups used for toxicokinetics: none
Study design: Asenapine was administered intravenously once daily from day 6 to day 17 of pregnancy. Dose levels were selected based on previously conducted pre- and postnatal developmental study with asenapine (study NL0052638). Female rats treated with asenapine and vehicle control were sacrificed on day 21 of pregnancy and the fetuses were removed by C-section. Examination of dams and fetuses was conducted.
Parameters and endpoints evaluated: Maternal parameters: mortality rate, clinical signs, body weights, food consumption. C-section and postmortem examinations: gross macroscopic examination of all internal organs with emphasis on the uterus, uterine weight and content, position of fetuses in the uterus, the number of corpora lutea, post-implantation loss, embryonic/fetal resorptions or death, number of fetuses per dam. Fetal parameters: body weights and sex ratios, external, visceral and skeletal examinations of the fetuses (50% fetuses for each) for abnormalities (malformations) conducted for the control and the HD groups (in case of abnormal finding, examinations were extended to all groups).

Results

Mortality (dams): There was no test article-related mortality.
Clinical signs (dams): From day 6 to day 17 of pregnancy at all dose levels "within short time after application animals show short-lasting ataxia and then persist in a motionless condition. Muscle tone was in increased. Animals remain conscious, but high-grade reduced in motoric activity".
Body weight (dams): Body weight gains were minimally lower after initiation of the treatment (days 6-12) when compared with that of the control group. These changes were graded as slight by the Sponsor. 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). These findings are summarized in the following Sponsor's table:

DIFFERENCES IN MEAN BODY WEIGHT GAIN OF DAMS (G) POST COITUM

Group	Days post coitum							
	0 - 6		6 - 12		12 - 18		6 - 18 *	
(mg/kg)	g	(%)	g	(%)	g	(%)	g	(%)
1 (0)	28	(+10.6)	16	(+5.5)	47	(+15.3)	63	(+21.6)
2 (0.3)	26	(+9.7)	11	(+3.7)	49	(+16.0)	60	(+20.3)
3 (0.9)	27	(+10.5)	7	(+2.5)	46	(+15.9)	53	(+18.7)
4 (1.5)	24	(+9.2)	11	(+3.9)	44	(+14.9)	55	(+19.4)

* The calculations of body weight gain during the treatment period started on day 6 post coitum (immediately prior to the first administration) and ended on day 18 post coitum (24 hours after the last administration)

Food consumption (dams): There were no treatment-related effects on food consumption.
Toxicokinetics: TK assessment was not conducted. However, in the toxicology written summary, the Sponsor provided the AUC value of 212 ng·h/mL and the C_{max} value of 147 ng/mL at the level of 0.9 mg/kg/day. It is unclear how this data is obtained.
Terminal and necroscopic evaluations: Post-implantation loss, embryonic/fetal resorptions or death and number of fetuses per dam or the sex ratio of fetuses were not affected by asenapine administration.
Offspring (malformations, variations, etc.): Mean fetal weights at the HD was minimally lower (by 4%) compared to that of the control group (males/females combined 5.0 g versus 5.2 g, respectively). There were no external or visceral findings related to the test article. Skeletal abnormal findings (malformations and variations) were noted in 4 fetuses in 3 litters (14% of all litters) of the control group, 3 fetuses in 3 litters (14% of all litters) at the LD, 4 fetuses in 3 litters (14% of all litters) at the MD, and 10 fetuses in 5 litters (24% of all litters) at the HD. The percentage of all fetuses affected was 2.6% (4/152), 1.9% (3/154), 2.7% (4/148), and 6.6% (10/151). Abnormal findings at the HD included zygomatic arch fusion, rudimentary cervical rib, misshapen scapula, fused thoracic vertebral arch, dumbbell-shapen or bipartite lumbar or thoracic vertebral body, misshapen cervical vertebral arch, and fused rib. These findings were of low incidence and restricted to one to three litters. The majority of findings occurred in several fetuses of one single litter delivered by dam no. 85). Dam no. 85 was more sensitive than other animals since its body weight development was lower than that of all other animals in this group on days 6-10 of pregnancy. Macroscopic observations indicated a mass in the chest wall region (d=20 mm) of dam no. 85, which was considered an incidental occurrence by the Sponsor. Excluding the litter delivered by the dam no. 85, malformations were observed in 6 fetuses in 4 litters (20% of all litters; 3.9% of all fetuses) at the HD. Overall, based on the low incidence, skeletal abnormalities at the HD are not considered clearly drug-related. The summary of findings in the HD group is shown below:

ABNORMAL FINDINGS FROM SKELETAL EXAMINATION OF FETUSES

Group 4 (1.5 mg/kg)

Number of fetuses examined: 151

Type of abnormal finding	Litter No.	Fetus No.
Thoracic vertebral body 12 bipartite ossification Thoracic vertebral body 13 dumbbell-shaped Lumber vertebral bodies 1 and 2 bipartite ossification Rudimentary cervical rib, left Scapula misshapen, left	68	79
Rudimentary cervical rib, bilateral Ribs 6 and 7 fused, distal, right Scapula misshapen, left		85
Ribs 10 and 11 wavy, slight, right	70	120
Rudimentary cervical rib, left	77	528
Rudimentary cervical rib, left	85	1007
Thoracic vertebral body 12 dumbbell-shaped Thoracic vertebral body 13 bipartite ossification Lumbar vertebral body 1 dumbbell-shaped Pelvic girdle left caudal displacement (27 pre-pelvic vertebral arches) Zygomatic arch fusion bilateral (process of jugal to zygomatic process of squamosal) Ribs 6 + 7 and 8 + 9 fused, distal, right		1009
Thoracic vertebral body 11 dumbbell-shaped Thoracic vertebral body 12 bipartite ossification Lumbar vertebral body 1 dumbbell-shaped Zygomatic arch fusion bilateral (process of jugal to zygomatic process of squamosal) Suture bone between frontals		1015
Zygomatic arch fusion right (process of jugal to zygomatic process of squamosal) Ribs 5 and 6 fused, distal, left Ribs 5 and 6 fused, medial, right Thoracic vertebral arch 4 and 5 fused, right Thoracic vertebral body 10 and 13 bipartite ossification Pelvic girdle right caudal displacement (27 pre-pelvic vertebral arches) Cervical vertebral arch 2 misshapen, left		1017
Ribs 10 and 11 wavy, slight, bilateral	87	1023
Zygomatic arch fusion left (process of maxilla to jugal)		1033

For percentage incidences of abnormalities and variations see skeletal examinations summary, pp. 57-60.

The summary of the incidence of findings in all groups is shown in the following Sponsor's table:

ABNORMAL FINDINGS FROM FETAL SKELETAL EXAMINATION - SUMMARY

	Group 1 0 mgkg		Group 2 0.3 mg/kg		Group 3 0.9 mg/kg		Group 4 1.5 mg/kg	
Number of litters examined	21		21		22		21	
Number of fetuses examined	152		154		148		151	
Incidences of fetuses with	N	%	N	%	N	%	N	%
Suture bone between cranial bones	0	0	1	1	0	0	1	1
Zygomatic arch fusions	0	0	0	0	2	1	4	3
Lower mandible with unossified areas	1	1	0	0	0	0	0	0
Rudimentary cervical rib	0	0	1	1	0	0	4	3
Scapula misshapen	0	0	0	0	0	0	2	1
Thoracic vertebral arch fused	0	0	0	0	0	0	1	1
Thoracic vertebral body dumbbell-shaped	0	0	1	1	2	1	3	2
Thoracic vertebral body bipartite ossification	1	1	1	1	1	1	4	3
Cervical vertebral arch misshapen	0	0	0	0	0	0	1	1
Ribs wavy	4	3	0	0	0	0	2	1
Ribs fused	0	0	0	0	0	0	3	2
Lumbar vertebral body bipartite ossification	0	0	0	0	0	0	1	1
Lumbar vertebral body dumbbell-shaped	0	0	0	0	0	0	2	1
Pelvic girdle caudal displacement (27 pre-pelvic vertebral arches)	0	0	0	0	0	0	2	1
Litters with any finding	3	14	3	14	3	14	5	24
Fetuses with any finding	4	3	3	2	4	3	10	7

* = For one fetuses in group 2 the Alcian blue stain could not be performed, due to incomplete skinning of the fetus.

[Note: Historical controls for Wistar Han rats were provided. However, this study was conducted in Sprague-Dawley rats.]

Embryofetal development – studies in rabbits

PILOT STUDIES

1. Study title: A pilot embryotoxicity study with Org 5222 in Dutch rabbits (SDG RR 2328). Test facility: Organon Oss, Schaijk, The Netherlands; Date of study initiation: February 8, 1982; GLP: no

Key study findings: Oral administration of asenapine to pregnant Dutch rabbits during the period of organogenesis (from day 6 to day 18 of pregnancy) at 30 mg/kg/day did not induce any maternal and/or embryotoxic or teratogenic effects that could be attributed to treatment. However, the trunks were not examined for visceral malformations. Nevertheless, the dose of 30 mg/kg/day was chosen as the HD for the definitive study in rabbits.

Methods: Asenapine (Batch D, purity: not provided) was administered orally to 12 pregnant female Dutch rabbits from day 6 to day 18 of pregnancy at 30 mg/kg/day as a suspension in vehicle [vehicle: 0.5% (w/v) gelatin containing 5% (w/v) mannitol; volume: 2 ml/kg body weight]. A group of 12 vehicle treated females served as a control group. Mortalities, clinical signs, body weight and food consumption were recorded for parent animals. The females were necropsied on day 29 of pregnancy and dissected to determine gross changes in the parent animals, pregnancy performance (i.e., pregnancy rate, number of implantation sites, number and distribution of live/dead fetuses and embryos, number of resorptions, corpora lutea, post-implantation loss) and ovarian weights. Litter data included postimplantation loss, fetal/placental weigh, sex of fetuses, abnormal litter index, malformation rate, ossification variance. Fetal examinations included external malformations, sectioning for brain and eye defects, and trunks examined only for skeletal malformations (alizarin red stain) (Individual animal data for malformations were not submitted).

Results: There were no unscheduled deaths**,** drug related clinical signs, significant changes in body weight or food consumption. One control and two drug treated females were not pregnant, resulting in a slight decrease in pregnancy rate (91.7% and 83.3%, respectively). There were no other drug-related effects on any other parameters, except malformed brain in one fetus in group administered asenapine. (Note: This study has been also reviewed by Dr. Lois Freed; please see her review of the IND 51,641 of January 9, 1998 for additional details).

PIVOTAL STUDIES

2. Study title: An oral embryotoxicity study with Org 5222 in rabbits administered twice daily (including teratogenicity).

Key study findings: An oral embryotoxicity/teratogenicity study 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. Two HD females died about 5 minutes after the second daily administration: No. 62 (day 10 of gestation, day 5 of dosing) and No. 52 (day 15 of gestation, day 10 of dosing). In female No. 62 dyspnea and ventral recumbency were observed prior to deaths. These symptoms started about 20 minutes after the first daily administration in the morning. No clinical signs were observed in female No. 52. At necropsy, reddened and incompletely collapsed lungs were noted in female No. 52. Both deaths are considered to be drug related. Clinical sign of dyspnea and ventral recumbency were observed also in HD female No. 54 on days 18 and 19 of pregnancy. 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 by 17% (male and female fetuses combined). Therefore, the NOAEL for the maternal and fetal toxicities was the MD. 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.

[Note: This study was also reviewed by Dr. Lois Freed under the IND 51,641. She concluded that "the lack of specific findings suggests reduced sensitivity to detect soft tissue abnormalities, variants, etc. Unless data can be provided that adequately document the sensitivity of the methods used to assess fetal effects, the studies may need to be repeated". Please see Dr. Freed's review dated January 9, 1998 for further details. Her conclusions were conveyed to the Sponsor in a letter dated February 11, 1998. The Sponsor did not address these concerns directly in the NDA 22-117 submission. However, an i.v. embryofetal developmental study in the rabbits, designed to characterize teratogenic effects of asenapine, was submitted under this NDA].

Study no.: SDG RR 2914
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: March 27, 1990
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine maleate, lot G, purity 100.6%
Methods

Doses: 0 (vehicle), 0.5, 2.5, and 15.0 mg/kg b.i.d at an interval of 5 hours
Species/strain: rabbit/Chinchilla (initial age: 18-36 weeks, initial body weight: 2944-4207 g)
Number/sex/group: 16 mated female rabbits/group
Route, formulation, volume: oral (gavage), solution (or suspension for the HD only) in gelatin/mannitol/water (final concentrations 0.5%/5%), volume: 2 ml/kg body weight (solutions/suspensions contained 92-97% of intended concentrations)
Satellite groups used for toxicokinetics: none
Study design: Asenapine was administered orally to pregnant female rabbits twice daily from day 6 to day 18 of pregnancy. Dose levels were selected based on previously conducted pre- and postnatal developmental study with asenapine (reference information or number not provided). Control animals were similarly dosed with the vehicle alone. All groups were sacrificed on day 28 of pregnancy and the fetuses were removed by C-section. Examination of dams and fetuses was conducted.
Parameters and endpoints evaluated: Maternal parameters: mortality, clinical signs, body weights and food consumption. C-section and postmortem parameters: Examinations included gross pathology of all internal organs with emphasis on the uterus, uterine weight and content, position of fetuses in the uterus and number of the corpora lutea. Moreover, pre- and post-implantation loss, embryonic/fetal resorptions or death, and number of fetuses per dam were examined. Fetal parameters: Fetal body weights and sex ratios were determined.

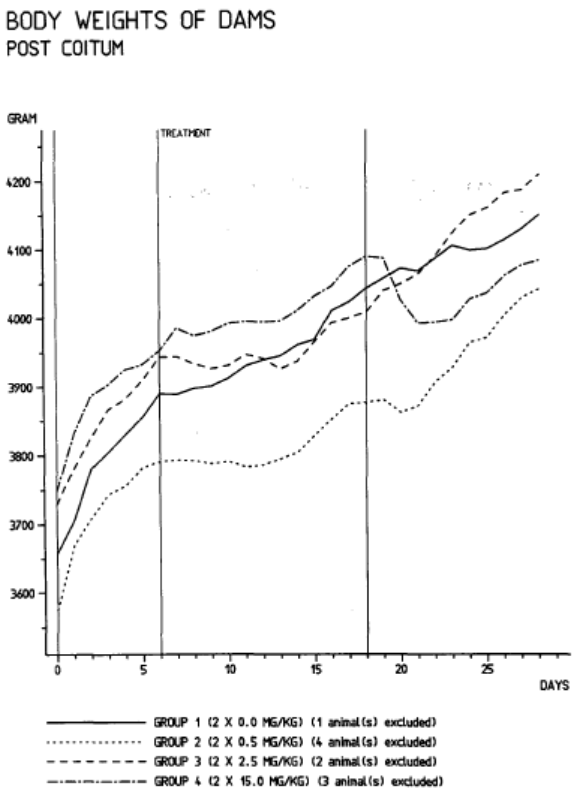
The fetuses were dissected and examined for external and visceral malformations. The heads were fixed, serially sectioned and examined for the degree of ossification and soft tissue abnormalities. The trunks were examined for skeletal abnormalities.

Results

Mortality (dams): Two HD females (No. 62 and No. 52) died about 5 minutes after the second daily administration: No. 62 (day 10 of gestation, day 5 of dosing) and No. 52 (day 15 of gestation, day 10 of dosing). In female No. 62 dyspnea and ventral recumbency were observed prior to death. These symptoms started about 20 minutes after the first daily administration in the morning. No clinical signs were observed in female No. 52. At necropsy, reddened and incompletely collapsed lungs were noted in female No. 52. The Sponsor considered both deaths to be drug related.

Clinical signs (dams): Animals were observed twice daily. Dyspnea and ventral recumbency were observed in HD female No. 54 on days 18 and 19 of pregnancy. These signs were similar to the observations in HD female No. 62 that died as described above. There were no other treatment-related clinical signs.

Body weight (dams): Body weights were recorded daily throughout gestation period. Body weight gain was similar in all groups from day 6 to day 19 of pregnancy (+ 4.3% and + 3.4% in the control and HD, respectively). Body weight gain was reduced from day 19 to day 28 and by 50% from day 6 to day 28 in HD females. Mean body weight loss of 95 g (approximately 2.5%) was noted between days 19 and 21 of pregnancy at the HD. During the same time period, body weight increased by 12 g in the control group. Slight decreases in body weight were observed also in all other groups during the treatment phase of the study, as shown in the following Sponsor's figure:



Food consumption (dams): Food consumption was recorded on Days 6, 11, 15, 19, 24, and 28 of gestation. Food consumption was slightly increased from day 6 to 11 of pregnancy (by 10% from day 0 compared with 5% increase in the control group) and markedly reduced (by approximately 50% of control) between days 19 and 24 of pregnancy in HD females.
Toxicokinetics: not conducted
Terminal and necroscopic evaluations: The final number of pregnant females per group was less than recommended 16/group as a result of non-pregnant females mortality at the HD and loss of entire litters in 2 LD females. There were no abnormal findings at necropsy of parental animals. There were no effects on the following parameters: number of corpora lutea, pre- or postimplantation loss, resorptions (early/late), number of live/dead fetuses, and sex ratio.
Offspring (malformations, variations, etc.): There were a total of 130, 137, 140, and 152 live fetuses examined in control, LD, MD, and HD females, respectively. Mean fetal body weight was reduced at the HD by 17% (male and female fetuses combined). The higher incidence of runts (small fetuses <19.0 grams) was observed at the HD when compared to other groups (5/130 fetuses in 2/15 liters, 4/137 fetuses in 1/12 litters, 1/140 fetuses in 1/14 litters, and 11/152 fetuses in 3/13 liters in the control, LD, MD and HD groups, respectively). "Runt" was the only abnormal finding reported upon external and visceral findings detected in any fetus. Head/brain examinations did not show abnormal findings. There was a minimal increase in fetuses with malformations and variations at the HD (2, 2, 2, and 4 in control, LD, MD, and HD females, respectively; in terms of litters, 2, 1, 2, and 3 were affected, respectively). Skeletal examinations revealed findings of sternebrae abnormally ossified and fused ribs in 2 fetuses from 2 litters in each the

control, LD, and MD mg/kg/day groups. In the HD group, the incidence of abnormal findings was slightly higher since abnormally ossified and fused sternebrae, fused or missing ribs, hemicentric or missing thoracic vertebral bodies and fused or missing thoracic vertebral arches were observed in 4 fetuses from 3 litters. Skeletal findings are summarized in the following Sponsor's table:

SKELETAL EXAMINATION OF FETUSES (ABNORMAL FINDINGS)

Group (mg/kg)	No. of fetuses examined	Type of abnormal finding	Individual data of fetus(es)*	Fetus(es) of litter number
1 (2 X 0.0)	130	Sternebrae nos. 4 and 5, abnormally ossified and fused	11/38.1/M	2
		Ribs nos. 8 and 9 medial fused	363/32.6/F	12
2 (2 X 0.5)	137	Sternebrae nos. 2-4, abnormally ossified and fused	52/27.8/M	18
		Scoliosis; thoracic vertebral body no. 12, missing; right side, 12 thoracic vertebral arches; left side, 11 thoracic vertebral arches	384/18.7/F	27
3 (2 x 2.5)	140	Sternebrae nos. 3 and 4, abnormally ossified and fused	292/32.1/F	40
		Sternebrae nos. 3 and 4, abnormally ossified and fused	298/22.2/F	40
4 (2 X 15.0)	152	Rib no. 5 (left side), distal bifurcated; ribs nos. 6 and 7 (left side), proximal fused; ribs nos. 6 and 7 (right side), proximal and medial fused; rib no. 9 (right side) missing; ribs nos. 11 and 12 (right side), proximal fused; between thoracic vertebrae nos. 6 and 12, several fused arches and 3 vertebral bodies hemicentric and partially fused	202/20.8/F	54
		Ribs nos. 9-10 and 10-11 (right side), proximal fused; thoracic vertebral body no. 10, hemicentric (right side missing); thoracic vertebral arch no. 10 (right side) missing	213/27.3/F	54
		Thoracic vertebral body and arch no. 5 (left side) missing; one rib (left side) missing	427/22.6/F	60
		Ribs nos. 11 and 12 (right side), proximal fused; thoracic vertebral body no. 12, hemicentric (right side missing), thoracic vertebral arch no. 12 (right side), missing.	516/32.7/F	63

* = Fetus number / body weight in grams / sex (M = male; F = female)

There were dose-related (all doses) increases in non-ossification or incomplete ossification of the number of skeletal elements when expressed as affected fetuses. When expressed as the number of affected litters, drug-related increases were noted primarily at the HD. These developmental delay effects may be related to the decreased maternal body weight and food consumption at the HD. There were certain skeletal elements in which the incidence of non- or delayed ossification was reduced in dosed groups when data are expressed as number of affected fetuses, e.g., non-ossified rib 13 (left, right), decreases in incomplete ossification of digit 5 medial phalanx (right forelimb), toe 4 medial phalanx (left and right) were associated with increases in the incidence of non-ossification of these same sites. There was also an increase in shortened rib and flying rib at the HD.

3. Study title: A parenteral embryotoxicity study, including toxicokinetics, with Org 5222 in the rabbit and Amendment NL0000571 (containing FDA requested detailed individual findings of the fetal examinations performed).

Key study findings: In the second pivotal embryofetal development study, New Zealand White rabbits were administered asenapine maleate intravenously at 0, 0.025, 0.125, and 0.625 mg/kg/day (0.018, 0.088, and 0.444 mg/kg/day AE) from day 6 to day 18 of gestation. There were two drug-related unscheduled deaths. An abdominal subcutaneous hematoma was detected at autopsy in the MD female that died on day 24. Polypnea, ptosis, and many red foci on the surface of all lobes of the lungs were noted at necropsy in the HD female that died on day 9. Clinical signs of polypnea (all animals), occasional motor incoordination (18/26 animals), occasional ptosis (all animals), and occasional hyperactivity (8/26 animals) were observed at HD usually from 5 to 30 minutes after dosing and lasted up to 2 hours after dosing. 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).

[Note: This study was also reviewed by Dr. Lois Freed under the IND 51,641. She concluded in her review of January 9, 1998 that "Due to technical problems, data from a number fetuses could not be used (11 C, 5 LD, 6 MD, 13 HD fetuses). Unfortunately, individual line listings were provided only for those fetuses that could not be used. Due to the lack of adequate fetal examination, the data from this study cannot be considered to have adequately assessed the teratogenic potential of Org 5222". Therefore, the individual line listings for all fetuses included in the final analysis, with each fetus identified by number and litter, were requested from the Sponsor at that time. The requested data were submitted to the NDA 22-117. Although 6.2% and 7.9% of the total number of fetuses were not available for skeletal examinations in this study, the overall number of fetuses examined is sufficient for an adequate study].

Study no.: SDG RR 4428
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: December 6, 1995
GLP compliance: yes
QA reports: yes (x) no ( )

Drug, lot #, and % purity: asenapine maleate (Org 5222), lot K, purity: 99.8%

Methods

Doses: 0 (vehicle), 0.025, 0.125, and 0.625 mg/kg/day (expressed as the maleate); (0.018, 0.088, and 0.444 mg/kg/day AE)
Species/strain: rabbit/New Zealand White
Number/sex/group: control and the HD: 26 pregnant females/group (due to technical problems during skeletal staining of fetuses, additional 10 animals in the control and the high dose group were included); LD and MD: 16 pregnant females/group
Route, formulation, volume, and infusion rate: intravenous injection into an ear vein, solution in cytric acid, volume: 1 mL/kg body weight,
Satellite groups used for toxicokinetics: Blood samples were collected from five animals per dosage group on day 10, 11, 12 or 15 of pregnancy just before dosing and at 5, 15, 30, 60, 120, and 180 min after dosing.
Study design: This study was designed to determine whether daily intravenous administration of asenapine to pregnant rabbits during the period of organogenesis resulted in any maternal toxic and embryotoxic/teratogenic effects and to determine the toxicokinetic profile after multiple dosing. Pregnant female rabbits were treated from day 6 to day 18 of pregnancy. All groups were sacrificed on day 29 of pregnancy and the fetuses were removed by C-section. Examination of dams and fetuses was conducted.
Parameters and endpoints evaluated: Maternal parameters: mortality, clinical signs, body weights, food consumption. C-section and postmortem parameters: pregnancy rate, number of abortions, gross macroscopic changes of the dams, number of implantation sites and corpora lutea, number and distribution of live or dead fetuses, ovary weight, fetus/placenta weight, abnormal litter ratio, malformation rate. Fetal parameters: All fetuses were examined for external malformations and sex. The heads of all fetuses were fixed in Bouin solution and those of fetuses from the control and HD groups were examined for brain and eye defects. The trunks were dissected and internal organs were examined for visceral malformations. Although not clearly stated, it would appear that all fetuses were examined for visceral findings. The skeletons of fetuses of the control and the HD groups were examined for malformations and ossification variance.

Results

Mortality (dams): There were 7 unscheduled deaths. According to the Sponsor, 5 animals were sacrificed after being accidentally paralyzed; these animals were replaced. One MD female died on day 24 of gestation. Subcutaneous hematoma on the abdominal wall was noted in this animal at necropsy. One HD female died on day 9 of gestation. Polypnea and ptosis were observed between 5 min and 2 h after dosing in this animal. At necropsy, many red foci on the surface of all lobes of the lungs were noted. The reason of death was not further explained by the Sponsor.
Clinical signs (dams): Animals were observed daily for clinical signs. Polypnea (all animals), occasional motor incoordination (18/26 animals), occasional ptosis (all

animals), and occasional hyperactivity (8/26 animals) were observed at HD usually from 5 to 30 minutes after dosing and lasted up to 2 hours after dosing.
Body weight (dams): Body weight was recorded on days 3, 6, 9, 12, 15, 19, 21, 24, 27, and 29 of gestation. Slightly reduced body weight gain, most likely of no toxicological significance, was observed from day 9 to 24 of pregnancy in all treatment groups.
Food consumption (dams): Food consumption was recorded daily and summed over days 0-6, 7-12, 13-19, 20-24, and 25-29. Slight increases or decreases in food consumption, were observed during gestation in MD and HD females. A decrease in food consumption (~30%) was observed between days 19 and 24 of gestation in the MD and HD groups.

Toxicokinetics: Blood samples were collected from 5 animals/group (except control) on days 10, 11, or 12 of gestation at 0, 5, 15, 30, 60, 120, and 180 min postdosing. The results are shown in the following Sponsor's table:

Dose (mg/kg/day)	t½ (min)	AUC (0-24) (ng.h/ml)	Normalized AUC (0-24) (ng.h/ml)/(mg/kg)	CL (ml/min/kg)	V.central (l/kg)
0.025	46.60	4.88*	192.56*	115.05	2.73
0.125	52.35	41.53	327.51	51.07	1.93
0.625	58.90	179.02	285.43	59.02	2.25

n = 5 rabbits per dosing group.
* As the AUC could not be calculated up to 24 h for the 0.025 mg/kg/day group, it was calculated up to and including the last measurable concentration (AUC 0-t).

Terminal and necroscopic evaluations (C-section data): There were no clear drug-related effects on pregnancy rate (100%, 100%, 87.5%, and 96.2% in the control, LD, MD, and HD groups, respectively), and the number of corpora lutea, number to implantation sites, number of live/dead fetuses, or ovarian weight. The number of early resorptions was slightly higher in HD females (affected fetuses: 4, 7, 5, and 10 in the control, LD, MD, and HD groups, respectively; affected litters: 0.2, 0.4, 0.4. 0.4). Postimplantation loss was higher in dosed groups (3.7, 7.1, 7.0, and 7.7 in the control, LD, MD, and HD groups, respectively); however, these differences were not statistically significant. Fetal weight and placental weight were minimally lower in all dosed groups (2-6% and 5-9%, respectively); however, these differences were not dose-dependent for the fetal weight and not statistically significant for both parameters; therefore they are not considered drug-related.
Offspring (malformations, variations, etc.): 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. Malformations noted only in the HD fetuses consisted of the following: exencephaly (1), misformed pons cerebelli (1), and umbilical hernia (1). Hydronephrotic kidney was detected in 1 control, 1 LD, and 2 HD fetuses. Major skeletal malformation (flexure of the forelimb) was observed only in one fetus in the MD group. Minor skeletal and visceral anomalies were also observed. However, the LD and MD groups were not examined. The total number of minor variants was 631 and 564 in the control and HD groups, respectively (the mean number of minor variants per fetus was 3.6 and 3.5, respectively). Due to technical problems (disintegration of some fetuses during staining) some skeletons of some fetuses could not be examined (11

fetuses from 7 litters in the control and 13 fetuses from 7 litters in HD group). Therefore, 6.2% and 7.9% of the total number of fetuses were not available for skeletal examinations.

The incidence of major skeletal and visceral malformations is shown in the following Sponsor's table:

Major skeletal and visceral defects

FO females

Experiment no.: XP010

	Org 5222	
Gr. 1:	Placebo	0 mg/kg
Gr. 2:	Org 5222 (low dose)	0.025 mg/kg
Gr. 3:	Org 5222 (mid dose)	0.125 mg/kg
Gr. 4:	Org 5222 (high dose)	0.625 mg/kg

	Group no. 1	2	3	4
No of females with live foetuses	25	16	14	25
No of foetuses examined	177	111	97	164
No of foetuses with major defects	1	2	1	4
Malformation rate (%)	0.5	1.4	1.0	3.9
A. SKELETON				
FLSFL Forelimbs flexure of the -	0	0	1	0
B. VISCERA				
*F EX Foetus Exencephaly	0	0	0	1
PO MI Pons cerebelli Misformed	0	0	0	1
*F UH Foetus Umbilical hernia	0	0	0	1
KL HN Kidney left Hydronephrotic	0	1	0	0
KS HN Kidney left and right Hydronephrotic	1	1	0	2
Number of major defects				
- total	1	2	1	5
- mean per major-defect-foetus	1.0	1.0	1.0	1.3
Statistics (permutation test) on the malformation rate				
- probability		0.280	0.440	0.150
- significance		//	//	//

The incidence of minor skeletal and visceral variants is shown in the following Sponsor's table:

Minor skeletal and visceral variants

F0 females

Experiment no.: XP018

Org 5222		
Gr. 1: Placebo	0	mg/kg
Gr. 2: Org 5222 (low dose)	0.025	mg/kg
Gr. 3: Org 5222 (mid dose)	0.125	mg/kg
Gr. 4: Org 5222 (high dose)	0.625	mg/kg

		Group no. 1	2	3	4
No of females with live foetuses		25	16	14	25
No of foetuses examined		177	111	97	164
No of foetuses with major defects		1	2	1	4
No of foetuses with minor variants		176	2	1	160
A. SKELETON					
I24F	Rib No 1 and No 2 right Fused	0	0	0	1
R25PR	Rib No 13 left Present	14	0	0	17
R26PR	Rib No 13 right Present	5	0	0	10
RT3PR	Rib left and right No 13 Present	118	0	0	76
PHROI	Prox.epiphysis of hum-r ossification incomplete	72	0	0	53
PHROA	Prox.epiphysis of hum-r ossification absent -	76	0	0	90
COLOI	Coracoid left ossification incomplete	39	0	0	33
COLOA	Coracoid left ossification absent -	103	0	0	114
FMOOI	Mid phalanx No 5 right-fl. ossification incomplete	33	0	0	34
FMOOA	Mid phalanx No 5 right-fl. ossification absent -	15	0	0	10
PTLOI	Prox epiphysis of tibia-l ossification incomplete	33	0	0	24
PTLOA	Prox epiphysis of tibia-l ossification absent -	91	0	0	91
B. VISCERA					
BR CT	Brain Cyst	28	0	0	12
LI CT	Liver Cyst	0	0	1	0
G B	Gallbladder bifurcation of the -	2	0	0	0
G R	Gallbladder reduced -	1	1	0	0
SP R	Spleen reduced -	1	0	0	0
*USDI	Ureter left and right dilatation of the -	0	1	0	0
Number of minor variants					
- total		631	2	1	565
- mean per minor-variant-foetus		3.6	1.0	1.0	3.5

[Note: the Sponsor submitted historical control data for litter and fetal examinations (external, visceral, skeletal) for rat and rabbit]

Prenatal and postnatal development

PILOT STUDIES

1. Study title: A pilot segment III study (including cross-fostering) with Org 5222

Key study findings: Administration of asenapine p.o. at 15 mg/kg b.i.d. to pregnant female rats from day 17 of pregnancy to day 14 of lactation had no effect on survival or

body weight of parental animals. However, asenapine caused severe clinical signs of lethargy in the parent animals leading to adverse effects on nursing behavior. No signs of fetal mortality were observed in asenapine-treated animals terminated on day 21 of pregnancy. Body weight of pups delivered by asenapine-treated animals was transiently lower than that of the controls. Neonatal mortality was high (up to 85.7%) in all asenapine-treated groups at 24 hours after delivery. The neonatal mortality in the group of non cross-fostered animals was higher than the neonatal mortality in the group of cross-fostered animals.

These data indicated that the increased neonatal mortality was most likely caused not only by changes in nursing/lactation process due to lethargy of parental animals or effect on lactation but also by the effects of asenapine on offspring development during pregnancy. The results of this study in comparison with the data indicating neonatal mortality in the Segment I rat study (No. SDG RR 3115) with treatment extended to the lactation period and no increase in neonatal mortality in the Segment II rat studies (No. SDG RR 2961) with treatment up to day 17 of pregnancy demonstrated that the neonatal mortality is caused by disturbances induced during the last part of pregnancy. In addition, this study demonstrated that the selected HD (15 mg/kg b.i.d.) exceeded the MTD for segment III oral study in rats.

Study no.: SDG RR 4299
Volume #, and page #: electronic submission
Conducting laboratory and location: Department of Drug Safety, N.V. Organon, Schaijk, The Netherlands
Date of study initiation: February 12, 1992
GLP compliance: yes
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: Asenapine (Org 5222), lot H, purity: 99.8%
Methods

Doses: 0 (vehicle), 15 mg/kg b.i.d. with an interval of approximately 5 hours (dosages expressed as the maleate)
Species/strain: rat/Sprague-Dawley
Number/sex/group: see table below

Group No.	Size	Dose	Treatment	Handling of litters planned and actual
1	16 F	0 mg/kg	vehicle	No fostering
2		0 mg/kg	vehicle	Pups to be fostered by Group 5. Cancelled. Animals included in Group 1.
3	10 F	0 mg/kg	vehicle	Foster parents for pups from group 6.
4	11F	2x15 mg/kg	Org 5222	No fostering
5		2x15 mg/kg	Org 5222	Foster parents for pups from Group 2. Cancelled. Animals included in Group 4.
6	10F	2x15 mg/kg	Org 5222	Animals from Group 4, that underwent C-section on pregnancy day 21; their pups were fostered by Group 3

Route, formulation, volume: oral (gavage), solution in gelatin (0.5% m/v) and mannitol (5% m/v) in water, 2 mL/kg body weight
Satellite groups used for toxicokinetics: none

Study design: This study was designed to determine whether daily oral administration of asenapine to pregnant female rats from day 17 of pregnancy up to and including day 14 of lactation induces any adverse effects with respect to delivery, pregnancy outcome and viability of the pups. The same HD level was used in the Segment I and II oral studies conducted previously with asenapine. As soon as possible after delivery, the litters were exchanged following slight ether anesthesia of dams. However, the parent animals treated with asenapine were unable to serve as foster parents (due to poor condition) and the original grouping plan had to be modified as indicated in the table above. A number of asenapine-treated females from Group 4 underwent C-section and their pups were transferred to vehicle treated animals for fostering. All other animals were sacrificed on day 14 of lactation.

Parameters and endpoints evaluated: Maternal parameters included clinical signs, mortalities, body weight (measured on pregnancy days 0, 7, 14, 17 and on lactation days 0, 7 and 14) and pregnancy performance. F1 offspring parameters included litter size, litter weight and gross visceral changes.

Results: Maternal toxicity: Treatment with asenapine had no significant effect on survival or body weight of parental animals. However, asenapine caused severe clinical signs of lethargy in the parent animals. Nursing behavior was adversely affected. The incidence of cannibalism in Group 4 was high. F1 offspring toxicity: No signs of fetal mortality were observed in animals terminated on day 21 of pregnancy following treatment with asenapine. Mean weight of pups delivered by asenapine-treated animals was significantly lower (up to 30% less) than that of the controls during the first 7 days of lactation. On lactation day 14 the weights were comparable. Neonatal mortality in the group of asenapine-treated and allowed to deliver non fostered animals (Group 4) was 85.7% at 24 hours post partum. Mortality was high in all eleven litters in this group. Neonatal mortality in the group of asenapine-treated and allowed to deliver animals (Group 3) following fostering was 29.3% at 24 hours post partum. This lower pup mortality involved 6/10 litters. During the subsequent 6 days pup mortality in this group reached another 42.4%. These data are shown in the following Sponsor's table.

GROUP MEAN DATA - NEONATAL MORTALITY AND PUP WEIGHT

Group 1 Vehicle
Group 3 Pups from Org 5222-treated females from Group 6 fostered by vehicle-treated parents from Group 3
Group 4 Org 5222

Group No.		1	3	4
Neonatal mortality within 24 Hrs.(%)		2.3	29.3	85.7
No. of litters		16	10	11
Mean pup weight (g)	LD-0	6.6	4.6	4.6@
S.D.		0.6	1.1	0.6
Significance			*	*
Total litter loss		0	1	9
Neonatal mortality after 24 Hrs.(%)		7.3	42.4	50.0
No. of litters		16	9	2
Mean pup weight (g)	LD-7	14.8	11.4	14.0
S.D.		1.5	2.2	
Significance			*	
Mean pup weight (g)	LD-14	29.1	25.8	29.3
S.D.		3.5	2.5	
Significance			//	

@ Mean based on 5 litters
The majority of the pups was killed by the dams within 4 hrs

2. Study title: An intravenous pilot segment III study with Org 5222 in Sprague-Dawley rats (Study No. NL0012545). Testing facility: N.V. Organon, Oss, The Netherlands; study initiation date: October 16, 1998; GLP: no

Key study findings: Administration of asenapine i.v. to pregnant rats at 0.3 and 3 mg/kg/day from day 6 of pregnancy to the day of delivery (day 21) in a pilot segment III study resulted in clinical signs of reduced activity in both groups administered asenapine within 5 minutes up to several hours after treatment. The animals of the 3 mg/kg/day group were stuck with their paws to the grid of the cage immediately after treatment, which lasted for about 2-3 hours. Administration of asenapine resulted also in a decreased mean body weight gain (up to 12% less than in the control group) in both asenapine groups from day 9 of pregnancy to the day of delivery, a slight decrease in the absolute body weight (by up to 7%) in animals administered 3 mg/kg/day after the start of dosing (likely to be a direct consequence of the observed clinical effects of reduced activity), and increased mean length of gestation at the 3 mg/kg/day group (23.3 days as compared to 22.4 days for the control group). During lactation, when dosing stopped, the decrease in body weight gain in parental animals was not observed anymore. In the group administered 3 mg/kg/day one female delivered dead fetuses only, one female had no live fetuses left on day 1 of lactation and one female had no live fetuses left on day 4 of lactation. These three females were sacrificed. In the same group, the mean number of fetuses per litter at delivery was decreased (8.9 as compared to 12.4 and 11.8 in the control and LD groups, respectively), the mean number of live young per litter at delivery was decreased (6.9 as compared to 12.3 and 11.5 in the control and LD groups, respectively), and the mean number of dead young per litter at delivery was increased (2.0 as compared to 0.1 and 0.3 in the control and LD groups, respectively). Neonatal mortality was also increased at HD within 24 h after delivery (42.7% as compared to 0.8% and 3.3% in the control and LD groups, respectively) and from 24 hours after

delivery until the end of lactation period (35.2% as compared to 2.2% and 0.9% in the control and LD groups, respectively). The Sponsor concluded that the dosage of 3 mg/kg/day can be regarded as too high in the subsequent pivotal study because clinical signs observed at this dose are not desirable in the period of nursing. This conclusion appears to be reasonable based on the data obtained in this study.

Methods: This study was designed to evaluate the dose levels to be used in the subsequent pivotal segment III study. Asenapine (batch H; purity 99.8%) was administered i.v. to pregnant Sprague-Dawley rats (7-8/group) at 0 (vehicle), 0.3 and 3 mg/kg/day (dosages expressed as the maleate) from day 6 of pregnancy to the day of delivery. Females were allowed to deliver spontaneously and rear their young for two weeks. In life parameters evaluated included mortality, clinical signs, maternal body weight, and litter number, sex and weight. After autopsy, all females were examined for gross anatomical abnormalities and litters were observed for external abnormalities. Uterine horns of apparently non-pregnant rats were examined for possible implantation sites.

3. Study title: A second intravenous pilot pre- and postnatal development study with Org 5222 in Sprague-Dawley rats. (Study No. NL0048584); Testing facility: (b) (4) ; study initiation date: February 11, 2003; GLP: no

Key study findings: Administration of asenapine i.v. to pregnant rats at 0.5, 1, and 2 mg active entity/kg/day from day 6 of pregnancy to the day of delivery (day 21) in a second pilot segment III study resulted in clinical signs of decreased motor activity and increased muscle tone observed in all groups shortly after asenapine administration and decreasing later during the day. The mean maternal body weight gains were lower from day 6 to day 21 of pregnancy compared to the controls by 6%, 6% and 13% at the LD, MD and HD, respectively. Food consumption was slightly lower in HD females. At day 14 of lactation, the mean body weights of all groups were comparable. Increased post-implantation loss was observed at the HD (16.4% compared to 6% in the control). At parturition all delivered pups were alive. However, post-natal pup loss was noted from day 0 to day 4 at the MD (12% pups in 6 litters) and the HD (29% in 6 litters) compared to control (2% in one litter). Two out of 8 HD females lost their litters during the lactation period. At 24 hours post partum, the mean body weight of pups was decreased in all asenapine-treated groups by 6%, 6% and 13% at the LD, MD and HD, respectively, compared to control. However, during the lactation period body weight gain in pups was similar to that of control and the differences were reduced to the minimal level. At the first check after parturition, 6 pups in each MD and HD groups and 1 pup in the control group were found dead with or without milk in their stomach, partly cannibalized or missing, and with bluish discolorations of the skin indicating hypothermia. Based on these findings, the HD was considered too high for the subsequent pivotal study NL0052638.

Methods: This study was designed to evaluate the dose levels to be used in the subsequent segment III study since there was no sufficient information on the steepness of the dose response from the first pilot study (No. NL0012545). Asenapine (batch S, purity: 100.5%) was administered i.v. to pregnant Sprague-Dawley rats (8/group) at 0

(vehicle), 0.5, 1, and 2 mg/kg/day AE from day 6 of pregnancy to the day of delivery. Females were allowed to deliver spontaneously and rear their young for 2 weeks. In life parameters evaluated included mortality, clinical signs, maternal body weight, the behavior of the dams during parturition, and litter weight. After autopsy, all females were examined for gross anatomical abnormalities and litters were observed for external abnormalities. F1 parameters observed included sex, number of missing (cannibalized) or dead pups and number of live pups.

PIVOTAL STUDIES

4. Study title: An intravenous pre- and postnatal development study with Org 5222 (asenapine) in Sprague-Dawley rats.

Key study findings: Administration of asenapine i.v. at 0.3, 0.9 and 1.5 (AE) 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. These effects may have resulted from impaired nursing ability of parental animals. There were no other signs which were indicative of embryo- and fetotoxicity.

Study no.: NL0052638
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: June 17, 2003
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Asenapine (Org 5222), lot S, purity: 100.5%

Methods

Doses: 0 (vehicle control), 0.3, 0.9, 1.5 mg/kg body weight/day expressed as the AE (0, 0.42, 1.27, 2.11 expressed as maleate)
Species/strain: rat/Sprague-Dawley
Number/sex/group: 21-22 pregnant female rats/group

Route, formulation, volume, and infusion rate: intravenous, solution for injection in citrate buffer; volume: 4 mL/kg body weight; injection speed: 1.2 mL/minute
Satellite groups used for toxicokinetics: none
Study design: This study was designed to detect possible adverse effects of asenapine on the pregnant/lactating female and on the development of the conceptuses and the offspring until sexual maturity. Female rats were treated once daily from implantation (day 6 of gestation) through weaning (day 20 post partum). The i.v route was used to mimic sublingual clinical use in humans. Dose levels were selected based on previously conducted pilot studies No. NL0012545 and NL0048584 (see above). Based on the data from these studies, the dose of 1.5 mg/kg day was "expected to have effects on reproduction parameters". Dams were terminated and necropsy was conducted on day 21 post partum. Developmental and behavioral parameters of F1 generation (randomly selected 4 males and 4 females per litter) were assessed on days 4 and 21 post partum. Water maze test was conducted on day 35 post partum. Selected F1 animals (1 male/1 female per litter) were paired on day 70 post partum. C-section on these animals was performed on day 14 of pregnancy.
Parameters and endpoints evaluated: Maternal parameters: mortality, macroscopic observations of dead animals, food consumption, body weights, perinatal and postnatal observations. F1 offspring parameters: sex of pups, number of missing or dead, abnormal findings in the pups, individual and mean pup weights, presence of milk in the stomach, physical development tests (pinna unfolding, incisor eruption, onset of coat development, eye opening, testes descent, cleavage of balanopreputial gland, vaginal opening) and behavioral tests (righting reflex, photophobotaxis, cliff avoidance, palmar grasp ability, negative geotaxis, exploratory locomotor pattern, direct pupillary reflex, hearing ability, water maze test (three different tests: learning test, memory test, relearning test), reproduction data and macroscopic abnormalities of F2 at necropsy.

Results
F_0 in-life: There were no test article-related deaths. After having lost all pups in their litters, 1 MD female and 4 HD females were sacrificed for humane reasons. All parental animals treated with asenapine exhibited strongly reduced motor activity and increased muscle tone starting immediately after the individual administration and returning to normal during the course of the day. Food consumption was similar to that of control group during pregnancy but was reduced during the lactation period (days 1-14) by 9%, 15% and 15% in LD, MD and HD females, respectively. According to the Sponsor, the mean body weight values were minimally reduced in all groups administered asenapine on the first day of treatment. However, the reviewer concluded that there were no toxicologically significant changes in body weight. Body weight gains were slightly (but not dose-dependently) lower than those of control from day 6 to the end of pregnancy (by 14.9%, 7.9% and 13.9% in LD, MD and HD females, respectively). Body weights were lower in all asenapine treated groups during the lactation period. At weaning (day 21 post partum), the mean body weights were 6%, 7% and 4% lower and the mean body weight

gains were 17%, 29% and 17% lower than those of the control group in LD, MD and HD females, respectively.

F_0 necropsy: The number of pregnant rats that gave birth was 20, 21, 21 and 22 in control, LD, MD and HD females, respectively. The duration of pregnancy was extended by one day in some animals administered asenapine. The number of animals affected was 1, 4, 7 and 3 in control, LD, MD and HD females, respectively. Post implantation loss (i.e. number of implantation sites relative to the number of pups counted at the first litter check) was significantly increased in all groups administered asenapine (9.9, 15.5, and 10.9% at LD, MD and HD, respectively, compared to 2.1% in the control group). However, a more detailed analysis demonstrated evidence of undetected postnatal loss between parturition and performance of the first litter check. These findings indicate that post implantation loss values reflect to a great part postnatal pup loss. Postnatal loss was significantly increased from day 0 to day 4 post partum in the MD group (24 cases; 9% of pups in 10 litters) and in the HD group (72 cases; 25% of pups in 16 litters). Total litter loss occurred in 1 MD female and 4 HD females. These data are shown in the following sponsor's table:

BREEDING DATA PER GROUP
FO GENERATION

	GROUP 1 0 MG/KG	GROUP 2 0.3 MG/KG	GROUP 3 0.9 MG/KG	GROUP 4 1.5 MG/KG
LITTERS				
TOTAL	20	21	21	22
DURATION OF GESTATION				
MEAN (+)	21.1	21.2	21.3	21.1
ST.DEV	0.22	0.40	0.48	0.35
N	20	21	21	22
IMPLANTATIONS				
TOTAL	292	293	310	321
MEAN (+)	14.6	14.0	14.8	14.6
ST.DEV	1.85	1.60	1.97	2.66
N	20	21	21	22
POST IMPLANTATION LOSS				
% OF IMPLANTATIONS	2.1	9.9	15.5	10.9
LITTERS AFFECTED (#)	6	14 #	15 ##	14 #
TOTAL (#)	6	29 ##	48 ##	35 ##
MEAN (+)	0.3	1.4 +	2.3 +	1.6 +
ST.DEV	0.47	1.32	2.53	2.22
N	20	21	21	22
DEAD PUPS AT FIRST LITTER CHECK				
LITTERS AFFECTED (#)	0	0	1	1
TOTAL	0	0	5	1
MEAN (+)	0.0	0.0	0.4	0.0
ST.DEV	0.00	0.00	1.32	0.21
N	20	21	21	22
LIVING PUPS AT FIRST LITTER CHECK				
% OF MALES / FEMALES (#)	53 / 47	58 / 42	52 / 48	52 / 48
TOTAL	286	264	262	286
MEAN (+)	14.3	12.6 +	12.5	13.0
ST.DEV	1.78	1.86	3.59	2.79
N	20	21	21	22
POSTNATAL LOSS DAYS 0 - 4 P.P.				
% OF LIVING PUPS	3.8	4.2	9.2	25.2
LITTERS AFFECTED (#)	2	6	10 ##	16 ##
TOTAL (#)	11	11	24 ##	72 ##
MEAN (+)	0.6	0.5	1.1	3.3 +
ST.DEV	2.24	1.03	2.10	3.56
N	20	21	21	22
LIVING PUPS DAY 4 P.P.				
TOTAL	158	167	151	143
MEAN (+)	7.9	8.0	7.2	6.5
ST.DEV	0.45	0.22	2.04	2.96
N	20	21	21	22
BREEDING LOSS DAYS 5 - 21 P.P.				
% OF LIVING PUPS AT DAY 4 P.P.	0.6	1.2	1.3	1.4
LITTERS AFFECTED (#)	1	2	2	1
TOTAL (#)	1	2	2	2
MEAN (+)	0.1	0.1	0.1	0.1
ST.DEV	0.22	0.30	0.30	0.43
N	20	21	21	22
LIVING PUPS DAY 21 P.P.				
% OF MALES / FEMALES (#)	49 / 51	53 / 47	50 / 50	48 / 52
TOTAL	157	165	149	141
MEAN (+)	7.9	7.9	7.1	6.4
ST.DEV	0.49	0.48	2.02	3.13
N	20	21	21	22

+ : Steel-test significant at 5% level
# / ## : Fisher's Exact Test significant at 5% (#) or 1% (##) level

F_1 physical development: The number of deaths of the pups increased with the increasing dose at the beginning of lactation period. Dates of postnatal losses of F1 pups during the lactation and rearing periods are shown in the following Sponsor's table:

Group	Ascertained on day post partum													
(mg/kg)	1	2	3	4	5	6	7	10	12	13	14	15	16	26
1 (0)		7M 3F	1F			1M								1F
2 (0.3)	1M 4F	4M 1F	1F				2M							
3 (0.9)	5M 3F	8M 6F	1F	1F			1M			1F				
4 (1.5)	7M 9F	20M 26F	4M 2F	2M 2F	1M	1F								

M = Male pup / F = Female pup

In addition, 1 MD pup and 2 LD pups were found cannibalized. All pups were lost between day 1 and 6 post partum in 1/ 21 MD litters and 4/22 HD litters. The mean pup weighs were not affected initially by the treatment. Decreased body weight gain was observed later at the MD and HD, reaching statistical significance from day 7 of lactation onwards. At weaning (day 21 post partum) the mean body weights were decreased by 10% and 11%, in these groups, respectively. During the rearing period (days 21-35), the mean body weight gains in treated groups were similar to those of control, although the mean body weights remained below control values.
F_1 behavioral evaluation: HD pups showed minimally lower photo-phobotaxis (62% of positive outcomes versus 77% in the control group).
All other developmental and behavioral parameters were not affected by asenapine administration.
F_1 reproduction: All animals in all groups mated and all females become pregnant.
F_2 findings: At necropsy on day 14 of pregnancy of F1 animals, all parameters, including the numbers of corpora lutea, pre-implantation loss, implantation sites, post-implantation loss, embryonic resorptions and of embryos were similar in all groups.

5. Study title: A second modified pre-and postnatal developmental study with asenapine in Sprague-Dawley rats.

Key study findings: In the second i.v. prenatal and postnatal developmental study with asenapine at 1.5 mg/kg/day in rat, cross-fostering was included to determine whether the neonatal mortality observed in the first prenatal and postnatal study No. NL0052638 was a result of the effects on the mother or a toxicological effect on offspring. All parental animals treated with asenapine exhibited strongly reduced motor activity and increased muscle tone. The mean body weight gain was 6% lower during gestation in dosed females. Post implantation loss was slightly increased in animals administered asenapine. At first litter check after parturition, 23 dead pups were noted in the group 2 administered asenapine compared to one dead pup in the control group 1. Postnatal pup loss was increased up to 19%-26% up to day 4 of lactation in all groups breeding litters from asenapine treated dams (V/HD, HD/HD, and HD/Control). The number of cannibalized (missing) pups was highest in the group of vehicle-treated nursing animals (V/HD). During lactation days 1 to 10, suckling of individual pups had not occurred at all (or was low) in the HD/HD and HD Control groups. 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.

Study no.: INT00000051
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: January 18, 2005
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine, batch AG; purity: 99.7%

Methods

Doses: 0 (vehicle), 1.5 mg active entity/kg/day
Species/strain: rat/Sprague-Dawley
Number/sex/group: 30/group
Route, formulation, volume, and infusion rate: intravenous, solution for injection, volume: 4 ml/kg body weight, injection speed: 1.2 ml/minute
Satellite groups used for toxicokinetics: none
Study design: This study was designed to assess effects of asenapine on the pregnant and lactating female and on the development of the conceptuses and the offspring until day 10 of lactation. Female rats were treated intravenously with vehicle (group 1) or asenapine (group 2) from implantation (day 6 of pregnancy) through to day 10 of lactation. Cross-fostering (10 litters/group) was performed after littering (at first litter check) as indicated in the table below.

Cross foster groups	**Exchange of litters (dam/litter)**	**Dam from**	**Litter from**
Vehicle/vehicle	V/V (exchange of litter from vehicle treated dams)	Group 1	Group 1
Vehicle/high dose	V/HD (vehicle treated dam with litter from test-item-treated dam)	Group 1	Group 2
High dose/vehicle	HD/V (test item-treated dam with litter from vehicle-treated dam)	Group 2	Group 1
High dose/high dose	HD/HD (exchange of litters from vehicle-treated dam)	Group 2	Group 2
Control groups	**No exchange of litters**		
Vehicle control	V Control		
High dose control	HD Control		

At day 11 of lactation, the necropsy of dams and pups was conducted.

Parameters and endpoints evaluated: mortality rate, clinical signs, body weight, food consumption (dams, F0 generation), peri-postnatal observations (sex of pups, number of missing, live or dead pups, abnormal findings in the pups, pup weight, presence of milk in the stomach), and macroscopic abnormalities at necropsy

Results

F_0 in-life: There were no test article-related deaths. All parental animals treated with asenapine exhibited strongly reduced motor activity and increased muscle tone starting immediately after the individual administration and returning to normal during the course of the day. The mean food consumption was similar in all groups during gestation. However, food consumption was slightly decreased in asenapine treated animals during lactation. The mean body weight gain was minimally lower (by 6%) during gestation in asenapine treated females. In the HD Control and in the HD/HD groups body weight gains were statistically significantly decreased from day 1 to 11 of lactation (+8.5% and +13%, respectively) when compared to the vehicle treated groups (from +16.5% to +20.0%). Post implantation loss (i.e. number of implantation sites minus number of pups counted at the first litter check) was slightly increased in animals administered asenapine (group 2; 17%; 79 out of 24 litters) compared to the control group 1 (9%; 45 out of 22 litters).
F_0 necropsy: There were no test article related findings.
F_1 physical development: At first litter check after parturition, 23 dead pups were noted in the group 2 administered asenapine compared to one dead pup in the control group 1. An increased incidence of missing pups (considered to be cannibalized by the dam or nursing female) was noted in group V/HD (23 pups from 7 litters), HD/HD (10 pups from 5 litters) and in HD Control (11 pups from 4 litters). There was no increase in other groups. 3 pups in 3 litters of asenapine treated females had no milk in the stomach and two pups were bluish discolored in the head or snout area. The mean body weight of pups was significantly (by 8%) decreased at first litter check in the asenapine group (males by 10%; females by 8%). Postnatal pup loss was increased up to 19%-26% in the cross-foster group V/HD group, HD/HD group and HD Control group up to day 4 of lactation as shown in the following table:

	V/V	V/HD	HD/V	HD/HD	VControl	HDControl
Day1-4 post partum						
Pup loss (%)	2.8	19.4	3.3	25.8	0.7	20.2
No. of litters affected	3	7	2	9	1	6
Day 5-10 post partum						
Pup loss (%)	0	0	2.8	0.9	2.1	6.0
No. of litters affected	0	0	3	1	3	1

During lactation days 1 to 10, suckling of individual pups had not occurred at all (or was low) in the HD/HD and HD Control groups as shown below:

No milk in stomach	V/V	V/HD	HD/V	HD/HD	VControl	HDControl
No. of pups affected	0	2	0	28	1	34
No. of litters affected	0	1	0	5	1	8

F_1 behavioral evaluation: not conducted
F_1 reproduction: not conducted
F_2 findings: not conducted

Studies in juvenile animals

1. Study title: Org 5222 toxicity studies in the juvenile rat by subcutaneous administration.

Key study findings: Administration of asenapine to juvenile Sprague-Dawley rats for 56 days from the day 14 of age to day 69 of age resulted in significant reduction in body weight gain in animals of both sexes at all dose levels from the start of dosing until weaning. Body weight gain remained reduced in males to the end of treatment. Neurobehavioral assessment indicated increased activity in animals at all dose levels following the completion of treatment, with the evidence of recovery in males. There was no recovery after the end of treatment in female activity pattern as late as on day 30 following the completion of treatment (last retesting). Therefore, the NOAEL for the juvenile toxicity of asenapine was not determined in this study. There were no treatment-related effects on the startle response, learning/memory, reproductive performance (except minimally reduced conception rate and fertility index in MD and HD males and females), organ weights, and microscopic evaluations of the brain.

Study no.: INT00033485
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: August 31, 2004
GLP compliance: yes (except for the bioanalytical results and related toxicokinetic evaluation of this study, and analysis of the dosing solution).
QA reports: yes (x) no ( )
Drug, lot #, and % purity: asenapine (Org 5222), batch: AG, purity: 99.7%
Formulation/vehicle: solution/solution of sodium citrate

Methods:
Doses: 0 (vehicle control), 0.4, 1.2, and 3.2 mg/kg/day (dosages refer to the active entity) (selected based on a range-finding study INT00018058); vehicle: solution of sodium citrate · $2H_2O$ (9.414 mg/mL) + Na_2HPO_4 (14.48 mg/mL) + NaCl (0.5 mg/mL) + NaOH to pH 5.

Study design: This study was designed to assess the effects on asenapine on juvenile animals. 22 male and 22 female littermate Sprague-Dawley rats/group were administered asenapine by subcutaneous route daily at a volume of 5 mL/kg from day 14 of age for 56 days. A group of sibling rats (22/sex) administered the vehicle served as a control group. An additional set of animals (18/sex/group) served as a satellite group for the assessment of toxicokinetic parameters on the first day of treatment. Three dosing sites were used: the central scapular region and the flanks by the left and right hind limbs.

Parameters evaluated: The effects of asenapine on mortality, general condition, body weight, food consumption, and age at sexual maturation were assessed during the treatment and recovery periods. After one week of recovery, rats were subjected to neurobehavioral examinations. Motor activity, locomotor coordination, auditory startle response habituation, and learning/memory in the Morris water maze were examined.

Motor activity was reassessed at 2 and 4 weeks after the end of treatment. The animals were paired at 14 weeks of age for the assessment of their reproductive performance (sexual maturation, estrus cycle, fertility and precoital interval), with the females killed and examined on day 14 after mating. At necropsy, a full macroscopic examination of the tissues was performed. Brain, pituitary, and reproductive organs were weighed. Brains of selected animals from the control and HD groups (N=10) were examined microscopically. Reproductive assessment included the number of corpora lutea, implantation sites, resorption sites, and live and dead fetuses. Plasma concentrations of asenapine and its toxicokinetic profile were assessed on days 14 and 69 of age (after the first dose and 56 consecutive daily doses). However, according to the report, the non GLP-compliant data could only be used as a qualitative demonstration of exposure.

Results:

Mortality: One HD male was killed for humane reasons on day 15 of age after showing clinical signs of under reactivity, irregular respiration, and reduced body temperature. This death may be drug-related. This animal was replaced.
Clinical signs: Animals were observed at least twice daily. More detailed physical examinations were performed several times throughout the study. Vocalization and struggling, most likely related to the slightly acidic vehicle, were observed when animals were handled for dosing. Minimal, dose-related reduction in activity and ptosis were noted at all dose levels until weaning on day 21 of age (mainly on days 1 and 2 of dosing). Towards the end of the treatment period, these effects were more obvious and were observed within 15 minutes of dosing and lasted for over 4 hours.
Body weights: Recorded weekly for F0 females. For F1 offspring, body weights were determined daily until day 28 of age and then twice weekly. Significant reduction in body weight gain was observed in animals of both sexes at all dose levels from the start of dosing until weaning. An immediate recovery was observed in females at the HD one week after weaning and in males once treatment was stopped. Body weight gain as percentage of control for selected intervals is shown in the following Sponsor's table:

	Males				Females			
Period	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Dosage (mg/kg/day)	0	0.4	1.2	3.2	0	0.4	1.2	3.2
Day 14-21 Pre-weaning	100	82	77	68	100	77	76	66
Day 21 – 70 Continued treatment	100	90	88	82	100	106	109	106
Day 70 – 98 Recovery	100	112	121	118	100	98	90	105

Food consumption: Monitored twice weekly by each cage from day 21 of age until pairing at approximately 99-106 days of age. A slight decrease in food consumption was observed in males in all treated groups from day 35 of age until the end of treatment. In females, food consumption was minimally higher from day 42 of age and remained higher in the MD and HD groups until the end of the study.
Neurobehavioral assessment: Motor activity was significantly increased in all treated groups (up to 2.2-fold and 1.8-fold in males and females at the HD, respectively) when

tested within a week of the end of treatment. Increased activity was also observed in males a week later and again at 30 days after the end of treatment. However, a recovery was noted in males. No recovery was noted in females following the completion of treatment as late as on day 30 (last testing). These data are shown in the following Sponsor's tables:

Text table 2: Cumulative activity scores as percentage of Control

Activity beam height	High beam: rearing activity			Low beam: ground locomotion		
Recovery interval (days)	5/6	12	30	5/6	12	30
Age at test (days)	74/75	81	99	74/75	81	99
Group /sex						
1M: actual score	619	427	372	1325	1078	994
2M: % Control	165	137	129	152	133	125
3M: % Control	202	166	157	179	146	134
4M: % Control	223	206	183	204	184	153
1F: actual score	917	638	592	1405	1225	1326
2F: % Control	135	160	144	132	153	125
3F: % Control	172	182	177	173	172	168
4F: % Control	180	218	215	187	205	177

The increased activity resulted in better performance of both sexes in the rota rod test of muscle coordination. The mean maximum time achieved by male and female rats in the rota rod test in the HD group was 25% longer than that of control. There were no treatment-related effects on the startle response or learning/memory in Morris water maze test.

Reproductive performance. There were no toxicologically significant changes in the reproductive performance of the males or females administered asenapine except minimally reduced (by 14%) conception rate and fertility index in MD and HD males and females.

Organ weights: There were no treatment-related effects on organ weights other than those most likely related to changes in body weight.

Microscopic evaluation of the brain: There were no toxicologically significant differences in brains from the control and HD group animals at the end of the recovery period.

Toxicokinetics: A separate study No. INT00020003 was conducted to provide TK data to support the juvenile toxicity study described above. Plasma concentrations of asenapine and its main metabolite Org 30526 and their TK profile were assessed on days 14 after a single dose or day 69 of age after 56 consecutive daily doses of 0.4, 1.2, and 3.2 mg/kg/day (dosages refer to the active entity) to male and female juvenile Sprague-Dawley rats (6/sex/group). These results are shown below in the Sponsor's table:

Mean toxicokinetic data for asenapine and ORG 30526 are presented in the tables below:

Kinetic parameters asenapine	Sex	0.4 mg·kg^{-1}		1.2 mg·kg^{-1}		3.2 mg·kg^{-1}	
		SD Day 14 of age	MD Day 69 of age	SD Day 14 of age	MD Day 69 of age	SD Day 14 of age	MD Day 69 of age
$AUC_{(0-7)}$ (ng·h·mL^{-1})	M	154	74.7	364	236	1195	579
	F	143	66.1	400	194	1113	586
$NAUC_{(0-7)}$ (ng·h·mL^{-1})/(mg·kg^{-1})	M	386	187	303	197	374	181
	F	358	165	334	161	348	183
$AUC_{(0-24)}$ (ng·h·mL^{-1})	M	-	-	-	-	2227	864
	F	-	-	-	-	-	-
C_{max} (ng·mL^{-1})	M	41.2	30.5	111	101	275	231
	F	44.6	30.5	108	75.2	287	212
NC_{max} (ng·mL^{-1})/(mg·kg^{-1})	M	103	76.2	92.2	83.8	85.8	72.1
	F	112	76.3	90.3	62.7	89.8	66.2
t_{max} (h)	M	0.5[1)]	0.5[1)]	0.5[1)]	0.5[1)]	0.5[1)]	0.5[1)]
	F	0.5[1)]	0.5[1)]	0.5[1)]	0.5[1)]	0.5[1)]	0.52[1)]
$t_{1/2}$ (h)	M	-	2.4	3.0	2.2	3.6	5.2
	F	3.4	2.1	2.4	2.3	4.3	-

SD = single dose, MD = multiple dose, M = male, F = female
[1)] : first sampling point of measurement, hence t_{max} value might be overestimated and C_{max} and AUC values might be underestimated; - : could not be determined

Kinetic parameters Org 30526	Sex	0.4 mg·kg^{-1}		1.2 mg·kg^{-1}		3.2 mg·kg^{-1}	
		SD Day 14 of age	MD Day 69 of age	SD Day 14 of age	MD Day 69 of age	SD Day 14 of age	MD Day 69 of age
$AUC_{(0-24)}$ (ng·h·mL^{-1})	M	270	306	814	980	2586	2776
	F	206	335	694	1117	1917	3022
$NAUC_{(0-24)}$ (ng·h·mL^{-1})/(mg·kg^{-1})	M	676	765	678	817	808	868
	F	516	839	578	931	599	944
C_{max} (ng·mL^{-1})	M	13.6	18.0	47.9	59.7	171	185
	F	11.1	23.6	37.1	74.1	108	182
NC_{max} (ng·mL^{-1})/(mg·kg^{-1})	M	34.1	44.9	39.9	49.8	53.5	57.7
	F	27.8	59.0	30.9	61.8	33.7	56.9
t_{max} (h)	M	7.0	4.0	7.0	4.0	7.0	7.0
	F	7.0	4.0	7.0	4.0	7.0	7.0
Parent/metabolite ratio[1)]	M	0.6	0.2	0.4	0.2	0.5	0.2
	F	0.7	0.2	0.6	0.2	0.6	0.2

SD = single dose, MD = multiple dose, M = male, F = female, [1)] : $AUC_{(0-7)}$ asenapine/ $AUC_{(0-24)}$ Org 30526

2.6.6.7 Local tolerance

Local tolerance was assessed in two studies in Beagle dogs: (1) Study No. SDG RR No. 4452, reviewed by Dr. Lois Freed (please see a copy of her review of the IND 51,641 of June 11, 2007 below) and (2) Study No. NL0052735 submitted to the NDA 22-117.

"1. A study of local tolerance after repeated sublingual administration of Org 5222 in Beagle dogs (SDGRR No. 4452, Dept of Drug Safety, RL-CERM, France, study date: 4/96 **GLP,** Vol 1.15). **Addendum: Characterization and stability (one month at 2-8°C) of Org 5222 (b) (4) tablets.**

This study was performed to determine the local toxicity of Org 5222 (batch DM2470, (b) (4) tablet) administered sublingually to female Beagle dogs (Harlan France; 4 received Org 5222, 2 received placebo). Org 5222 was administered at a total daily dose of 3.24 mg (4 tablets, or 4.5 mg/day of salt; two tablets placed on each side) for 7 days (~5 hr interval between daily doses). Observations included clinical signs, body weight, food consumption, and terminal studies [i.e., gross examination of the oral cavity, microscopic examinations of mandible and

tongue from each animal, 20 section of sublingual grooves (10 L, 10 R) were examined in all animals].

Analysis of the tablets indicated that the actual concentration per tablet were 100-143% of intended; disintegration time was < 10 sec.

There were no unscheduled deaths during the study. The only apparent clinical sign was slight salivation in 1 DTF. There were no drug-related effects on body weight, food consumption, or upon macroscopic or microscopic examination."

2. Study title: A seven days sublingual local tolerance study with Org 5222 in female Beagle dogs (Study No. NL0052735). Test facility: N.V. Organon, Schaijk, The Netherlands; Date of study initiation: December 23, 2003; GLP: yes

Key study findings: In the second study of local tolerance in dogs, sublingual administration of asenapine to female dogs at 15 mg b.i.d. (3 mg/kg/day) for seven days resulted in mild to moderate clinical signs of salivation, gnawing bars, panting, scratching, aggressive behavior, vomiting, tremors, reduced activity, incidental recumbency, pushing bars, shaking with the head, and active behavior. Food consumption was decreased in ¼ animals. There were no drug-related effects on body weight, gross anatomy, and histopathology at the site of treatment.

Methods: Asenapine (batch 21853ONB) was administered sublingually for 7 days to 4 female beagle dogs (8-10 kg at first dose; 45-53 months of age) at 15 mg active entity b.i.d. (the corresponding dose was ~3 mg/kg/day; interval 5-6 h). Tablets were placed in the left lateral sublingual grove near the salivary canal orifices and fixed for approximately 2 minutes. Parameters evaluated included mortality, clinical signs (observed daily), body weight determined once a week), food consumption (monitored daily), and macroscopy. Histopathological examination was performed on the site of the treatment (left side); the right side served as control site (not treated). The results were peer-reviewed.

Results: There were no deaths in this study. Clinical signs of salivation, gnawing bars, panting, scratching, aggressive behavior, vomiting, tremors, reduced activity, incidental recumbency, pushing bars, shaking with the head, and active behavior were observed. Food consumption was decreased in ¼ animals. No drug-related gross pathology findings were noted. The following histopathology effects were observed: foci of minimal mineralization (ductular, acinar) in salivary glands of 2/4 dogs, areas of slight submucosal mononuclear cell infiltration in tongue of 1/4 dogs, and areas of minimal granulocytic cell infiltration in oral cavity in ¼ dogs. These findings are considered to be within the background pathology in the Beagle dog.

2.6.6.8 Special toxicology studies

The following special toxicology studies were conducted with asenapine: (1) antigenicity study in guinea pigs (Study No. SDG RR 3292) submitted previously under the IND 51,641 and reviewed by Dr. Lois Freed (please see the following summary of this study taken directly from Dr. Freed's review of June 11, 1997); (2) phototoxicity 3T3 neutral

red uptake assay (Study No. 04230) submitted under the NDA 22-117, and (3) prolactin release study in male rats (Study No. INT00060363) using risperidone as comparator, submitted under the NDA 22-117. Moreover, genetic toxicology, general toxicity, and reproductive toxicity studies were conducted with impurities in the drug substance and drug product for qualification of these impurities.

1. "Assessment of the antigenicity of Org 5222 after oral and subcutaneous administration to the guinea-pig (NCL Study) [SDGRR No. 3292, (b) (4) (No. Org 237/9 11503), study dates: 8/91-20/91, GLP, Vol 1.15)

Org 5222 was tested in the PCA (passive cutaneous) and for active anaphylactic and delayed-type hypersensitization reactions in Dunkin-Hartley guinea pigs. In the PCA, Org 5222 (0.03-3 mg/kg p.o., s.c.) did not induce formation of IgG_{1a} during sensitization or of IgE/IgG_{lb} upon challenge. In the active anaphylaxis paradigm, animals sensitized with Org 5222 exhibited no signs of anaphylaxis when challenged with Org 5222/GPSA (i.e., as a conjugate of guinea pig serum albumin). However, in animals sensitized with Org 5222/HSA (1.5 mg/kg) given s.c. with FCA, all died of anaphylactic shock when challenged with Org 5222/GPSA and 2/5 died with challenged with Org 5222 alone and the remaining 3 showed "marked signs" of anaphylaxis. In the delayed-type hypersensitivity assay, Org 5222 produced erythema (at 24-72 hrs postdosing) in all animals when given at a dose of 0.45 mg/0.1 mL (but not at 0.03 and 0.15 mg/0.1 mL intradermal; once weekly for 4 wks) upon challenge; however, a similar effect was noted in control animals given vehicle. [It was not noted whether or not Org 5222 produced signs of primary irritation during the sensitization period.]" (Please see Dr. Freed's review of the IND 51,641 of June 11, 1997 for further details).

2. Study title: Determination of phototoxic potential of asenapine in the 3T3 neutral red uptake (NRU) phototoxicity assay (Study No. 04230). Test facility: Pfizer, PGRD Amboise, France; Study initiation date: March 2005; GLP: yes

Key study findings: Asenapine did not show evidence of phototoxicity in the 3T3 neutral red uptake phototoxicity assay *in vitro*.

Methods: The purpose of this study was to assess phototoxic potential of asenapine in the Balb/c 3T3 clone 31 mouse fibroblast cell line. Cells were treated with asenapine dissolved in EBSS for about 1 hour and then irradiated with 5 joules/cm^2 of UVA. The cell viability was measured approximately 24 hours after the irradiation. Treatment with the positive (Chloropromazine) and negative (Sodium Lauryl Sulfate) controls resulted in expected effects, indicating that this experiment was valid. The EBSS vehicle was not cytotoxic in the presence or absence of UVA light.

Results: Asenapine at concentrations up to 8000 µg/mL did not affect cellular viability either in the presence and absence of UVA light.

3. Study title: Asenapine: 4-week investigative toxicity study in the rat to assess prolactin levels using risperidone (Org 31196) as comparator (Study No. INT00060363). Test facility: (b) (4); Study initiation date: July 2007; GLP: yes

Key study findings: In a study designed to compare effects of daily subcutaneous administration of asenapine or oral administration of risperidone to male Sprague-Dawley rats on prolactin release after single and multiple dosing, the increases in prolactin release were similar in all treated groups. Therefore, the data did not provide an explanation for no increase in the incidence of prolactin-related tumors in carcinogenicity studies with asenapine and increased incidence of these tumors in carcinogenicity studies with risperidone.

Methods: 12 male Sprague-Dawley rats received asenapine (batch AX) by daily subcutaneous injection of 2.8 mg active entity/kg (volume: 4 mL/kg) for 4 weeks. The control group received subcutaneous injection of the vehicle at the same volume-dosage. The comparator group of 12 male rats received risperidone by daily oral gavage at a dose of 5 mg active entity/kg and a volume-dosage of 10 mL/kg for 4 weeks. Clinical signs, reaction to dosing, body weight, and gross pathology were examined. Blood was collected for prolactin measurements at the start and end of treatment.

Results: There were no treatment related mortalities and changes in the general condition of the animals. Animals receiving asenapine or risperidone showed partially or completely closed eyelids, and underactive or motionless behavior. The onset of signs was quicker in animals that had received a subcutaneous injection of asenapine than in animals treated orally with risperidone. The signs persisted to the final recording occasion each day; however animals generally appeared normal by the following day. Slight mean body weight loss was observed during the first week of treatment. The loss of 13 g and 3 g was recorded among animals receiving asenapine and risperidone, respectively. During the remaining three weeks of treatment, animals receiving asenapine or risperidone showed reduced mean body weight gain when compared with controls with a similar pattern of body weight gain recorded in both treated groups. Prolactin levels on day 1 of treatment were significantly higher than control among animals receiving asenapine or risperidone at 0.75, 1.5, and 3 hours after treatment (3.7 – 5.0 times the control value); the magnitude of the increase in mean prolactin levels was similar in both treated groups. On day 28 of treatment, mean prolactin levels were 4.4 – 5.2 times the control value at the 0.75 and 1.5 hours time-points (excluding values above upper limit of quantification of >200 ng/mL). At the 3 hour time-point, mean levels in the asenapine group were 7.7 times the control value and mean levels in the risperidone group were 6 times the control value. There were no macroscopic abnormalities detected at scheduled termination.

Impurities in the drug substance

Two impurities (b) (4) are present in the drug substance commercial size clinical/stability batches at (b) (4). The Sponsor proposed to set specification limits for these impurities in asenapine drug substance at (b) (4), thus above the ICH Q3A(R) qualification limit (b) (4)

Qualification of impurity (b) (4):

Studies conducted by the Sponsor to qualify the impurity (b) (4) directly included genotoxicity studies (Ames tests and chromosomal aberrations study in cultured human lymphocytes) and an intravenous 2-week toxicity study with (b) (4) in Wistar rats. Reproductive toxicology study (Segment II; embryofetal toxicity study in rats) recommended by the Agency for qualification of impurities was not conducted with (b) (4) . However, (b) (4) is considered to be qualified based on the presence of adequate amounts of this impurity in reproductive toxicology studies conducted with asenapine.

1. Study title: A mammalian/microsome mutagenicity test (Ames test) with Org 8444 in *Salmonella Typhimurium* and *Escherichia Coli.*

Key study findings: (b) did not induce changes in the number of revertants in any of the *Salmonella typhimurium* and *Escherichia coli* strains tested in the bacterial reverse mutation test in the presence or absence of metabolic activation. In conclusion, (b) (4) did not show mutagenic activity under conditions of this study.

Study no.: NL0005854
Volume #, and page #: electronic submission
Conducting laboratory and location: Department of Toxicology and Drug Disposition, N.V. Organon, Oss, location Schaijk, The Netherlands.
Date of study initiation: May 12, 1998
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: (b) (4), batch C, purity 99.5%
Formulation/vehicle: solution in DMSO

Methods
Doses: 8, 40, 200, 1000, 2500, and 5000 µg/plate.
Study design: (b) (4) was tested in triplicate in the presence and absence of metabolic activation system (mammalian microsomal fraction S9-mix) in *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537) and in *Escherichia coli* strain (WP2 uvrA pKM101). The following positive controls were used for assays in the absence of S9-mix: 4-Nitroquinoline-N-oxide for strains TA98, TA100, and WP2 uvrA pKM101, sodium azide for strain TA1535, and 9-aminoacridine for strain TA1537. In the presence of S9-mix, the positive controls were: 2-Acetylaminofluorene for strains TA98 and TA100, 2-Aminoanthracene for strains TA1535 and WP2 uvrA pKM101, and Benzo(a)pyrene for strain TA1537. A vehicle treated group served as a negative control. The number of revertant colonies was determined after incubation of agar plates for 48 hours.

Results
Stability data indicated that (b) (4) was stable under conditions of this study. Signs of cytotoxicity were observed in all strains tested at high concentration of (b) (4) i.e., in strains TA98 and TA100 at 1000 µg/plate and higher both in the presence and the absence of metabolic activation, in strain WP2 uvrA pKM101 at 200 µg/plate and higher the absence of metabolic activation and at 2500 µg/plate and higher in the presence of metabolic activation, and in strains TA1535 and TA1537 at 2500 µg/plate and higher

both in the presence and the absence of metabolic activation. All positive controls induced mutagenic responses indicating the validity of the test system. Mean number of revertants per plate for the negative control article and (b) (4) treated plates were within the historical control range both with and without metabolic activation.

Note: The Sponsor submitted "for completeness" two additional non-GLP Ames test reports (Studies No. 79R-01139 and 80R-00172), conducted in 1979. In the first study, (b) (4) caused a slight (up to 2.2 fold) increase in revertants in two tester strains TA98 and TA1538 at cytotoxic concentrations in the absence of metabolic activation. However, (b) (4) did not induce an increase in revertants in the second study. Therefore, it was concluded that under conditions of the second study, (b) (4) showed no mutagenic activity. The reviewer concluded that the adequacy of both studies is questionable based on the lack of adequate positive control response in experiments conducted in the absence of metabolic activation.

2. Study title: Evaluation of the ability of (b) (4) to induce chromosome aberrations in cultured peripheral human lymphocytes (with repeat experiment).

Key study findings: The ability of (b) (4) to induce chromosomal aberrations in cultured peripheral human lymphocytes evaluated in the presence and absence of the metabolic activation system indicated that (b) (4) is not clastogenic under conditions of this study.

Study no.: NL0053577
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: December 10, 2003
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: (b) (4), batch E, purity 67.4%
Formulation/vehicle: solution or suspension in DMSO

Methods
Doses: First assay: with and without S9-mix: 100, 150, 200, 250, 300, and 333 µg/mL (3 h exposure time, 24 h fixation time). Dose levels selected for scoring: without S9-mix: 100, 200, and 250 µg/ml; with S9-mix: 200, 250, and 300 µg/mL.
Second assay: without S9-mix: 33, 42, 56, 75, and 100 µg/mL (24 h exposure time, 24 h fixation period); 3, 10, 15, 20, 25, and 33 µg/mL (48 h exposure time, 48 h fixation period); with S-9 mix: 200, 250, 275, 300, and 333 µg/mL (3 h exposure time, 48 h fixation period); Dose levels selected for scoring: without S-9 mix: 33, 42, and 75 µg/mL (24 h exposure time, 24 h fixation time); 10, 25, and 33 µg/ml(48 h exposure time, 48 h fixation time); In the presence of S-9 mix, no appropriate dose levels could be selected for scoring. Therefore, this part of experiment was repeated with the following concentrations: 33, 100, 125, 150, 175, 200, 250, and 275 µg/mL and 10, 33, 100, 150,

200, 250, 275, and 300 µg/mL (3 h exposure time 48 h fixation period). Dose levels selected for scoring: with S-9: 10, 100, and 250 µg/mL.

Study design: Cultured human lymphocytes from healthy male subjects were stimulated and exposed to (b) (4) in two independent cytogenetic assays as indicated above. Based on mitotic index of the dose range finding study and the first cytogenetic assay, appropriate dose levels were selected for the second assay. The mitotic index of each culture was determined by counting the number of metaphases per 1000 cells. At least three analyzable concentrations were used for scoring in the cytogenetic assay. In case of cytotoxicity, chromosomes of metaphase spreads were analyzed of those cultures with an inhibition of the mitotic index of 50% or greater (if available). Positive controls included mitomycin C and cyclophosphamide. Cell division was arrested in the metaphase according to standard methods and stained. 100 metaphase chromosome spreads were analyzed for the presence of structural chromosome aberrations such as breaks, gaps, minutes, dicentrics, and exchange figures.

Results
Appropriate cytotoxicity level was reached at high concentration of (b) (4). All positive controls induced clastogenic responses indicating the validity of the test system. (b) (4) did not induce a statistically significant or biologically relevant increase in the number of cells with chromosome aberrations in the absence or presence of metabolic activation in human lymphocytes in two independently repeated experiments.

3. Study title: An intravenous two-week toxicity study with (b) (4) in Wistar rats.

Key study findings: Intravenous administration of (b) (4) to Wistar rats for two weeks at dosages of 0, 0.2, 1, and 5 mg/kg/day resulted in mild to moderate miosis in all dosing groups, moderate increase in heart rate (up to 20%) observed immediately after dosing in all dosing groups (in most rats noted on the first day of dosing and at the end of dosing period), dose-related decrease in motor activity, and mild to moderate ptosis and lacrimation at the MD and HD, change in posture (mild to moderate abduction of the limbs) observed mainly during first week in HD males and females, moderate decrease in mean body weight gain in all male groups and LD females during the first week, slightly to moderately higher mean body weight gain in all female groups in the second week of treatment (overall changes in body weight gain were generally in agreement with changes in food consumption), slightly decreased cholesterol, phospholipids, and triglycerides in MD and HD males, slightly decreased cholesterol and phospholipids in HD females, and slightly increased mean adrenal gland weight in all (b) (4) treated male groups. There were no treatment-related gross or microscopic findings. The NOAEL was determined to be at the LD of 0.2 mg/kg/day for all drug-induced effects except the increase in the heart rate.

Study no.: NL0056094
Volume #, and page #: electronic submission
Conducting laboratory and location: N.V.Organon, Department of TDD, Nistelrooisebaan 3, NL-5374 RE Schaijk, The Netherlands

Date of study initiation: March 11, 2004
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: (b) (4)), batch E, purity 98.6%

Methods

Doses: 0 (control), 0.2, 1.0, and 5.0 mg/kg/day
Species/strain: rat/Wistar
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: intravenous, solution in cytric acid, sodium chloride and sodium hydroxide to pH 4.0, 5 mL/kg, 1 mL/min
Satellite groups used for toxicokinetics or recovery: none
Age: not provided
Weight: males: 238-279 g, females: 147-192 g
Sampling times: Animals were terminated following 2 weeks of treatment.
Unique study design or methodology (if any): none

Observations, times and results

Mortality: Animals were observed for morbidity and mortality daily. There were no deaths in this study.
Clinical signs: Animals were observed for clinical signs at least once a day. Mild to moderate miosis was observed within 1 min up to several hours after dosing at all dose levels. Dose-related decrease in motor activity and mild to moderate ptosis and lacrimation were observed at the MD and HD. Change in posture (mild to moderate abduction of the limbs) was observed mainly during first week in HD males and females.
Body weights: Body weights were determined and recorded at least once a week during the predosing period and two times a week during dosing. Mean values per group were reported weekly. Moderate decrease in mean body weight gain was observed during the first week in all male groups and LD females. Slightly to moderately higher mean body weight gain was observed in the second week of treatment in all female groups (overall changes in body weight gain were generally in agreement with changes in food consumption).
Food consumption: Food consumption was recorded weekly per cage. Slightly lower food consumption was noted in MD and HD males. Slightly higher food consumption was noted in MD and HD females.
Ophthalmoscopy: Not conducted
EKG: Recordings were made for 4 rats per sex per group during the predosing period, immediately after, and approximately 24 hours after the first treatment. At the end of the dosing period, procedures were repeated in the same animals immediately after and 24 hours after treatment. Conduction disturbance and heart rate were assessed. A moderate increase in heart rate (up to 20%) was observed immediately after dosing at all dose levels (in most rats noted on the first day of dosing and at the end of dosing period). The heart rate data are shown in the following Sponsor's tables:

Males : mean data (± standard deviation)

	Day-4	Day0	Day1	Day9	Day10
PL1	370 ± 32	413 ± 33	373 ± 28	400 ± 42	385 ± 10
DS1	398 ± 21	478 ± 33	370 ± 8	515 ± 24	420 ± 50
DS2	370 ± 27	500 ± 18	440 ± 82	508 ± 10	380 ± 42
DS3	383 ± 13	465 ± 27	373 ± 31	493 ± 10	415 ± 55

Females: mean data (+ standard deviation)

	Day-4	Day0	Day1	Day9	Day10
PL1	430 + 41	485 + 44	420 + 49	458 + 19	438 + 44
DS1	446 + 44	555 + 44	415 + 10	545 + 17	455 + 65
DS2	435 + 45	508 + 13	415 + 47	530 + 22	428 + 25
DS3	420 + 64	483 + 5	415 87	525 + 27	445 +27

Hematology: Blood samples were collected from all animals at the end of dosing period. The following parameters were determined: red blood cells, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, reticulocytes, white blood cells, lymphocytes, monocytes, neutrophilic granulocytes, eosinophilic granulocytes, basophilic granulocytes, platelets, mean platelet volume, platelet distribution width, and platelet packed cell volume. There were no test article related findings.
Clotting: Activated partial thromboplastin time and prothrombin time were assessed. Activated partial thromboplastin time was slightly decreased in MD and HD males.
Clinical chemistry: Blood samples were collected from all animals at the end of dosing period. The following parameters were determined: aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, creatinine kinase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, icterus index, triglycerides, cholesterol, HDL-cholesterol, phospholipids, lipemia index, creatinine, urea, glucose, total protein, albumin, globulin, albumin/globulin ratio, calcium, inorganic phosphorus, sodium, potassium, chloride, and hemolysis index. Slightly decreased cholesterol, phospholipids, and triglycerides were observed in MD and HD males. Slightly decreased cholesterol and phospholipids were observed in HD females.
Urinalysis: Urine was collected overnight prior to necropsy from animals placed in metabolism cages. The following parameters were determined: volume, osmolarity, pH, glucose, protein, N-acetyl-beta-D-glucosaminidase, creatinine, hemoglobin, ketones, sodium, potassium, and chloride. Slightly increased urine sodium was observed in HD males and females.
Gross pathology: Macroscopic evaluation of all cavities, tissues, and organs listed in histopathology section was conducted. There were no test article related findings.
Organ weights: The following organs were weighed at necropsy: adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, seminal vesicles, spleen, testes, thymus, thyroid and parathyroid glands, and uterus. Slightly increased mean adrenal gland weight was observed in all drug-treated male groups
Histopathology: Organs and tissues were embedded in paraffin, sectioned, and stained with H&E. Microscopic evaluation was conducted on organs and tissues from all animals

of the control and HD groups, and on the site of injection of all LD and MD animals. The following organs/tissues were examined microscopically: adrenal glands, aorta, bone marrow, brain, cecum, coagulating glands, colon, cranial cavity, diaphragm, duodenum, epididymides, esophagus, eyes, femur and joint, ileum, jejunum, kidneys, lacrimal gland, liver, lungs and bronchi, lymph node (mesenteric), lymph node (right poplietal), mammary gland, optic nerve, ovaries, pancreas, pituitary gland, preputial/clitorial gland, prostate gland, rectum, salivary glands, seminal vesicles, skeletal muscle, skin and subcutaneous tissues, spinal cord, spleen, sternum, stomach, testes, thoracic cavity, thymus, thyroid gland and parathyroid gland, tongue, trachea, urinary bladder, uterus, and vagina. There were no test article related findings.
Toxicokinetics: not conducted
Other: Bone marrow smears were prepared but not examined.

Qualification of impurity (b) (4):

Studies conducted by the Sponsor to qualify the impurity (b) (4) included direct testing in genotoxicity studies (Ames tests, mutations at HGPRT locus in CHO cells, mouse lymphoma assay, and *in vivo* rat micronucleus assay), 4-week oral toxicity studies in Wistar rats and Beagle dogs, and pilot embryofetal development study in Dutch rabbits.

1. Study title: A *Salmonella*/mammalian-microsome mutagenicity test (Ames test) with (b) (4) (Study No.79R-00021); Testing facility: Organon, Oss, The Netherlands; Study date: 1979; GLP: no

Key study findings: An equivocal mutagenic response was observed in the *Salmonella typhimurium* tester strain TA1538 in the presence of S-9 mix metabolic activation in Ames test under conditions of this study. No increase in the number of revertatnts was observed in strains TA98, TA100, and TA1535 or in the absence of metabolic activation in the strain TA1538.

Methods: (b) (4) (batch A) was tested for potential mutagenic effects in the study conducted at 8, 40, 200, and 500 µg per plate in the absence or presence of metabolic activation system (S-9 mix). Tester strains of bacteria included *Salmonella tymphimurium* TA98, TA100, TA1535, and TA1538. 500 µg (b) (4) concentration tested because of the solubility of the test article. A vehicle (aceton) treated group served as a negative control. 2-acetylaminofluorene and 2-aminoanthracene served as positive controls in the presence of S-9 mix. The plates were incubated at 37°C for 48 hours before counting. The experiment was repeated once.

Results: In the first experiment, (b) (4) induced a 2.3-fold and 2.2-fold increase in the number of revertants in the tester strain TA1538 in the absence and presence of metabolic activation, respectively, at a single concentration level of 500 µg/plate, when compared with the negative control plates. In the second experiment, minimal increases in the number of revertants of 1.9-fold without S-9 mix at 500 µg (b) (4) per plate as well as 1.4 and 1.5-fold with S-9 mix at 200 and 500 µg per plate were observed. However, at the

concentration of 500 µg (b) (4) the toxicity reached 95% in the absence of metabolic activation in a separate toxicity test. No toxicity was noted in plates incubated with S-9 mix at this dose level. No increase in the number of revertatnts was observed in strains TA98, TA100, and TA1535. The positive controls induced an increase in the number of revertants in the presence of S-9 mix. There were no adequate positive control responses in the absence of S-9 mix. The Sponsor concluded that a mutagenic effect was demonstrated in the tester strain TA1538 in this study. However, the reviewer concluded that the data indicate the equivocal mutagenic response in the tester strain TA1538, only in the presence of S-9 mix metabolic activation.

2. Study title: A *Salmonella*/mammalian-microsome mutagenicity test (Ames test) with (b) (4) (Study No.79R-01140); Testing Facility: N.V. Organon; Oss, The Netherlands; Study date: 1979; GLP: no.

Key study findings: An equivocal mutagenic response was observed in the tester strain TA1538 in the presence of S-9 mix metabolic activation based on the increase in the number of revertants greater than 2-fold noted only at one (the highest) concentration of 1000 µg/plate.

Methods: (b) (4) (batch A) was tested for potential mutagenic effects in the study conducted at 8, 40, 200, and 1000 µg per plate in the absence or presence of metabolic activation system (S-9 mix). Tester strains of bacteria included *Salmonella tymphimurium* TA98, TA100, TA1535, TA1537, and TA1538. 1000 µg (b) (4) per plate was the highest concentration tested causing 100% cytotoxicity. A vehicle (methanol) treated group served as a negative control. 2-acetylaminofluorene, 2-aminoanthracene, and benzo(a)pyrene served as positive controls in the presence of S-9 mix. The plates were incubated at 37°C for 48 hours before counting.

Results: (b) (4) induced 1.65-fold and 2.2-fold increase in the number of revertants in the tester strain TA1538 at concentrations of 200 and 1000 µg/plate, respectively, in the presence of metabolic activation when compared to the negative control plates. At the concentration of 1000 µg/plate, the toxicity reached 100% in the absence of metabolic activation in a separate toxicity test. However, no toxicity was seen in plates incubated in the test. No increase in the number of revertants was observed in strains TA98, TA100, TA1537, and TA1535. The positive controls induced an increase in the number of revertants in the presence of S-9 mix. There was no adequate positive control response in the absence of S-9 mix. The Sponsor concluded that a weak mutagenic effect was demonstrated in the tester strain TA1538 in this study. However, the reviewer concluded that the data indicate an equivocal mutagenic response in the tester strain TA1538 in the presence of S-9 mix metabolic activation because the increase in the number of revertants greater than 2-fold was noted only at one (the highest) concentration of 1000 µg.

3. Study title: A mammalian/microsome mutagenicity test (Ames test) with (b) (4) in *Salmonella typhimurium* and *Escherichia coli*. (Study No. NL0006414); Testing facility: N.V. Organon, Oss, The Netherlands; Study date: 1998; GLP: yes; QA: yes

Key study findings: (b) (4) did not induce a dose related increase in the number of revertants per plate in any of the bacterial strains tested. Therefore, (b) (4) did not show mutagenic activity in the bacterial mutagenicity test under conditions of this study**.**

(b) (4) (batch E; purity 99.6%) was tested for potential mutagenic effects in this study. Test concentrations chosen were 8, 40, 200, 1000, and 2500 µg (b) (4) per plate in the absence or presence of metabolic activation system (S-9 mix). Tester strains of bacteria included *Salmonella tymphimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2 uvrA pKM101. Bacterial death was observed at concentrations of 1000 µg (b) (4) per plate or higher. A vehicle (DMSO) treated group served as a negative control. Stability data indicated that (b) (4) was stable under conditions of this study. 4-nitroquinoline-N-oxide, sodium azide, and 9-aminoacridine served as positive controls in the absence of S-9 mix. 2-acetylaminofluorene, 2-aminoanthracene, and benzo(a)pyrene served as positive controls in the presence of S-9 mix. The plates were incubated at 37°C for 48 hours before counting. The positive controls induced an increase in the number of revertant colonies in the absence or presence of S-9 mix, indicating that the test system was working properly. (b) (4) did not induce a dose related increase in the number of revertants per plate in any of the bacterial strains tested.

4. Study title: Evaluation of the mutagenic activity of (b) (4) in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat) (Study No. NL0052683). Testing facility: Organon, Schaijk, The Netherlands; Study date: 2003/2004; GLP: yes.

Key study findings: (b) (4) did not induce a dose related increase in the number of revertants per plate in any of the bacterial strains tested. It is concluded that Org 5033 did not show mutagenic activity in the bacterial mutation test under conditions of this study.

(b) (4) (batch F; purity 69.9% on the basis of active entity) was tested for potential mutagenic effects in this study. In the first, second, and third experiment (b) (4) was tested at a concentration range of 3 to 1000 µg, 3 to 666 µg, and 10 to 1000 µg per plate, respectively, in the absence or presence of metabolic activation system (S-9 mix). Tester strains of bacteria included *Salmonella tymphimurium* strains TA98, TA100, TA1535, TA1537, TA1538, and *Escherichia coli* strain WP2 uvrA pKM101. Toxicity was observed in all tester strains at high concentrations of (b) (4) precipitated in the top agar at concentration of 666 and 1000 µg per plate. A vehicle (DMSO) treated group served as a negative control. 4-nitroquinoline-N-oxide, sodium azide, 9-aminoacridine, 2-nitrofluorene, daunomycin, and methylmethanesulfonate served as positive controls in the absence of S-9 mix. 2-aminoanthracene served as positive control in the presence of S-9 mix. The plates were incubated at 37°C for 48 hours before counting. The positive controls induced an increase in the number of revertant colonies in the absence or presence of S-9 mix, indicating that the test system was working properly. The negative and strain-specific positive control values were within the testing laboratory historical control data ranges. (b) (4) did not induce a dose related increase in the number of revertants per plate in any of the bacterial strains tested.

5. Study title: Report on a gene (HGPRT) mutation test in mammalian cells (CHO) *in vitro* with (b) (4) (Study no. 82R-01562); Testing facility: Organon, Schaijk, The Netherlands; Study date: 1982; GLP: no.

Key study findings: (b) (4) induced mutations at HGPRT locus in CHO cells under conditions of this study.

(b) (4) (batch E; purity: not provided) was examined for the ability to induce mutations at the hypoxantine-guanine-phosphoribosyl-transferase (HGPRT) locus of CHO cells. The test was carried out in the presence and absence of metabolic activation (S-9 mix) at concentrations of 25, 40, 55, and 70 µg (b) (4)/mL, selected based on a dose range finding study. Cultures were exposed to (b) (4) for 5 hours with S-9 mix or 24 hours without S-9 mix. Methanol served as the solvent (negative) control. Ethylmethylsulphonate (EMS) and dimethylnitrosamine (DMN) served as the positive controls. Only treatment with EMS resulted in mutagenic response. A relative plating efficiency was adequate. The mutagenic activity of (b) (4) was observed in the absence of S-9 mix at 55 and 70 µg (b) (4)mL, and in the presence of S9-mix at 55 µg (b) (4)/mL. The results are shown in the following Sponsor's table:

Culture No.	Test compound	Conc. µg/ml	S9-mix	Number of cells on day 1% (1)	Initial survival % (2)	Final survival % (plat. eff.)	No. of mutants per 10^6 survivors
1	Methanol (1%)	0	-	100	100 (3)	69	8
2	(b) (4)	25	-	77	94	72	3
3		40	-	53	100	71	3
4		55	-	53	98	74	29
5		70	-	21	61	68	31
6	EMS	400	-	96	33	60	1212
1	Methanol (1%)	0	+	100	100 (4)	67	12
2	(b) (4)	25	+	27	19	52	15
3		40	+	30	40	53	8
4		55	+	16	9	60	92
5		70	+	13	11	62	0
6	DMN	400	+	95	116	56	0

(1) expressed as a percentage of the control cultures 1 and 7
(2) defined as relative plating efficiency (control 100%)
(3) plating efficiency 56%
(4) plating efficiency 50%

6. Study title: Evaluation of the mutagenic activity of (b) (4) in an *in vitro* mammalian cell gene mutation test with L5178Y lymphoma cells (with independent repeat). (Study No. NL0053636); Test facility: (b) (4); Study date: 2004; GLP: yes; QA: yes

Key study findings: (b) (4) did not induce a significant increase in the mutant frequency in the presence or absence of metabolic activation. Therefore, (b) (4) was not mutagenic or clastogenic in the TK mutation test system under conditions of this study.

(b) (4) (batch F; purity: 98.3%) was examined in this study to evaluate its mutagenic activity and clastogenic potential in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells. The test was carried out in the presence and absence of metabolic activation (S-9 mix) at concentrations selected based on a dose range finding study. In the first experiment, (b) (4) was tested up to concentrations of 60 and 150 µg/ml in the absence or presence of metabolic activation S9-mix, respectively, up to cytotoxic levels of 83% and 95%, respectively. Incubation time was 3 hours. In the second experiment, (b) (4) was tested up to concentration of 130 µg/ml in the presence of metabolic activation S9-mix up to cytotoxic level of 98%. Incubation time was 3 hours. In the third experiment, (b) (4) was tested up to concentrations of 32.5 and 120 µg/mL in the absence or presence of metabolic activation S9-mix, respectively, up to cytotoxic levels of 80% and 94%, respectively. Incubation time was 24 and 3 hours in the absence or presence of metabolic activation, respectively. DMSO served as the solvent (negative) control. Methylmethanesulfonate and cyclophosphamide served as the positive controls in the absence or presence of metabolic activation, respectively. The study was conducted according to standard procedures. Mutant frequencies in cultures treated with positive controls were significantly increased, indicating the test system was functioning properly. (b) (4) did not induce a significant increase in the mutant frequency in the presence or absence of metabolic activation.

7. Study title: Micronucleus test with (b) (4) in rats. (Study No. 79R-01058); Testing facility: Organon, Oss, The Netherlands; Study date: 1979; GLP: no

Key study findings: No increase in the incidence of micronuclei was observed in the bone marrow smears of Sprague-Dawley rats administered (b) (4). It was concluded that (b) (4) was not clastogenic under conditions of this *in vivo* micronucleus test.

(b) (4) (batch a; purity: not provided) was examined for potential ability to induce chromosome aberrations. (b) (4) was administered orally by gavage at a volume of 2 ml/kg body weight to Sprague-Dawley rats (male and female, 6/sex/group) at 1, 10, or 100 mg/kg/day on two consecutive days. Vehicle (0.5% w/v gelatin and 5% w/v mannitol) was administered to the negative control group. Methylmethanesulphonate (MMS) administered intraperitoneally served as a positive control. There were no mortalities and no gross pathological changes. No other in-life observations were conducted. Therefore, the exposure to the test article was not demonstrated. Five hours after the second administration the rats were sacrificed, the femurs were removed, and bone marrow was rinsed out. Slides were prepared using standard procedures and examined for micronuclei per 1000 polychromatic erythrocytes. The bone marrow of MMS-treated animals showed a significant increase in the number of micronuclei in polychromatic erythrocytes. No increase in the incidences of micronuclei was observed in the bone marrow smears of rats administered (b) (4).

8. Study title: A four week toxicity study with (b) (4) in Wistar rats using the oral route.

Key study findings: Oral administration of (b) (4) to rats for 4 weeks at 12.5, 25, and 50 mg/kg/day resulted in transient clinical signs of decreased activity and reduced defecation in males and females at the MD and HD at the beginning of the study, transient slight decrease in body weight gain and body weight in LD and MD males and in animals of both sexes at the HD, transient decrease in food consumption in HD females and increased food consumption in all other groups of both sexes, slightly increased ALT values in 3 HD females and swollen and finely vacuolated hepatocytes in the same HD females (possibly due to enzyme induction), and hypertrophy of thyroid follicular epithelium in males at the HD. The NOAEL for (b) (4) in rats by oral route was 12.5 mg/kg/day in this study.

Study no.: 82R-01801
Volume #, and page #: electronic submission
Conducting laboratory and location: Scientific Development Group, Organon, Oss, The Netherlands
Date of study initiation: November 11, 1982 (report date)
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, and % purity: (b) (4), batch "e", purity: purity: 100.4%
Methods

Doses: 0, 12.5, 25, and 50 mg/kg/day once daily for four weeks
Species/strain: rat/Wistar
Number/sex/group or time point (main study): 8/sex/group
Route, formulation, volume, and infusion rate: oral (gavage), suspension in gelatin 0.5% m/v and mannitol 5.0% m/v, volume: 2 mL/kg body weight
Satellite groups used for toxicokinetics or recovery: none
Age: not provided
Weight: males: 135-164 g, females: 109-131 g
Sampling times: After 4 consecutive weeks of treatment rats were sacrificed and organs/tissues were dissected for further examinations.
Unique study design or methodology (if any): none

Observations, times and results
Mortality: One MD female and one MD male died during the study. The cause of death was not determined. Gavage error could not be excluded.
Clinical signs: Recorded daily. Transient clinical signs of decreased activity and reduced defecation were observed in males and females at the MD and HD for 3 days at the beginning of the study.
Body weights: Recorded weekly. Body weight gain and body weight were decreased in all male groups and in HD females in the first week of treatment. This effect was reversible.
Food consumption: Recorded weekly. Transient decrease in food consumption was observed in HD females, followed by increased food consumption. Food consumption was increased in all other test article treated groups.
Ophthalmoscopy: not conducted
EKG: not conducted

Hematology: Performed after 2 and 4 weeks of dosing. The following parameters were assessed: hemoglobin, packed cell volume, red blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, white blood cell count, differential white blood cell count, erythrocyte sedimentation rate, and platelets count. Coagulation parameters: prothrombin time and activated partial thromboplastin time. There were no test article-related findings.
Clinical chemistry: Performed after 2 and 4 weeks of dosing. The following parameters were assessed: ALT, AST, alkaline phosphatase, total bilirubin, glucose, urea nitrogen, sodium, potassium, cholesterol, triglycerides, free fatty acids, total protein, and total lipids. Triglycerides were increased in HD females after 2 and 4 weeks of dosing. Bilirubin was increased dose-dependently in LD, MD and HD females after 2 weeks of dosing, and in LD and HD males after 4 weeks of dosing. ALT was slightly increased in 3 HD females after 4 weeks of dosing. Swollen and vacuolated hepatocytes were observed in the same animals.
Urinalysis: Performed after 2 and 4 weeks of dosing. The following parameters were assessed: specific gravity, volume, pH, protein, reduced substances, glucose, ketones, bile pigment, and sediment. There were no test article-related findings.
Gross pathology: Conducted on all animals. There were no test article-related findings.
Organ weights. The following organs were weighed at necropsy: heart, spleen, thymus, liver, kidneys, testes, epididymis, prostate gland, seminal vesicles, ovaries, uterus, thyroids with parathyroids, adrenal glands, pituitary gland, and brain. Liver weights were increased in 4 LD, 5 MD and 8 HD females, and in 1 MD and 3 HD males.
Histopathology: The following organs were examined: skeletal muscle, diaphragm, heart, aortic arch, trachea, lungs, spleen, lymph node, thymus, bone marrow, tongue, liver, salivary gland, pancreas, esophagus, stomach, duodenum, jejunum and ileum, cecum and colon, kidneys, urinary bladder, testes, epididymides, prostate gland, seminal vesicles, ovaries, uterus, vagina, skin, mammary glands, thyroids and parathyroids, adrenal glands, pituitary gland, brain, spinal cord, eye, and optic nerve. Organs and tissues were stained with hematoxilin and eosin. Histopathology was conducted in all animals that died early in the study, all HD animals, and in 4 rats of either sex in the control group. Kidneys and livers were examined in all rats of the LD and MD groups. Swollen and finely vacuolated hepatocytes were observed in 3 HD females. Hypertrophy of thyroid follicular epithelium was observed in 2 HD males. There were no other test article related changes.
Toxicokinetics: not conducted
Other: none

9. Study title: A four weeks toxicity study with (b) (4) in Beagle dogs using the oral route.

Key study findings: Oral administration of (b) (4) to Beagle dogs for 4 weeks at 25, 50, and 75 mg/kg/day resulted in mortality in 2 HD males due to severe neurological effects leading to traumatic wounds. Test article-related clinical signs observed in surviving animals of all groups included intermittent nervous depression and excitation, forced movements, tremors of the head, gnawing, automutilation, abnormal behavior, salivation, soft defecation and miosis. In addition, sleepy appearance, restlessness, cycling movements, trembling, motor incoordination, diarrhea, and vomiting were

observed in MD and HD animals only. These signs developed after test article administration and lasted 6 to 23 hours after treatment. Overall physical condition of animals declined in a dose-dependent manner. Other findings included: decreased body weight in all treatment groups, increased incidence of biliary epithelial cell proliferation and slight peribiliary chronic inflammatory reaction in MD males and HD animals of both sexes, increase in GPT and alkaline phosphatase levels in MD and HD animals of both sexes, inhibition of spermatogenesis in HD males, decrease in size of the testes in one HD male, and increase in secretory activity of mammary glands in one MD female. The Sponsor considered the dose of 25 mg/kg/day as the NOAEL. However, based on severe neurological clinical signs observed at the LD, the reviewer concluded the NOAEL has not been determined in this study.

Study no.: 83R-00086
Volume #, and page #: electronic submission
Conducting laboratory and location: Scientific Development Group, Organon, Schaijk, The Netherlands
Date of study initiation: not provided; report date: 01/11/1983
GLP compliance: no; This study has been performed prior to implementation of GLP regulations. However, the design of this study is scientifically acceptable.
QA report: yes (x) no ( )
Drug, lot #, and % purity: (b) (4), batch E, purity: 100.4%

Methods

Doses: 0. 25, 50, and 75 mg/kg/day
Species/strain: dog/Beagle
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume: oral, tablets
Satellite groups used for toxicokinetics or recovery: none
Age: males: 19.5-20.5 months; females: 21.0-21.5 months
Weight: males: 9.8-14.4 kg; females: 9.5-12.0 kg
Sampling times: Animals were killed and tissues were collected for gross and histopathology examinations after four consecutive weeks of treatment.
Unique study design or methodology (if any): none

Observations, times, results:
Mortality: Animals were observed daily for mortality. Two HD male dogs were sacrificed on day 4 for humane reasons after showing signs of deteriorated physical condition, described by the Sponsor as a "serious nervous disorder". Reduced activity, forced movements (i.e., pushing the head against wall, severe licking and biting at different parts of the body, jumping) resulting in traumatic wounds, edema of head and oral cavity and signs of pain, vomiting, enlargement of the liver, and increased alkaline phosphatase (up to 4.7 fold) were observed in both dogs. Small areas of swollen vacuolated hepatocytes were noted in the liver of one dog.
Clinical signs: Clinical signs were observed daily. Physical examinations were performed prior to dosing and after 2 and 3 weeks of dosing. The following clinical signs were observed in LD animals: slight hypoactivity, less alert behavior, hyperreactivity

evidenced by forced movements, tremors of the head, gnawing, automutilation, abnormal behavior, salivation, soft defecation, and miosis. These signs developed after test article administration and lasted for several hours. However, the recovery was longer each week, with the duration of signs increasing from 6 hours after dosing in weeks 1 and 2 to 20-22 hours after dosing in weeks 3 and 4 of the treatment. In the MD and HD animals, the following clinical signs were observed in addition to those noted in the LD group: sleepy appearance, restlessness, cycling movements, trembling, motor incoordination, diarrhea, and vomiting. The recovery of MD and HD animals was seen 23 hours after treatment. Physical condition of animals declined in a dose-dependent manner. The Sponsor concluded that poor physical condition resulted form the superficial wounds and/or edema.

Neurological examinations: Performed prior to dosing and after 3 weeks of dosing. There were no treatment-related abnormal reflexes.

Body weights: Recorded weekly. Dose-related decrease in body weight was observed in all test-article treated groups during experimental period. This effect was most prominent during the first week of treatment and later decreased.

Food consumption: Recorded daily. Reduced food consumption was observed occasionally in female dogs. Clear relation to the treatment cannot be established.

Ophthalmoscopy: Performed prior to dosing and after 3 weeks of dosing. Redness of the conjunctiva and a diminished ability of pupil dilatation were noted in MD animals. This phenomenon was "not understood" by the Sponsor and is not dose-related.

EKG: Performed prior to dosing and after 3 weeks of dosing. There were no treatment-related obvious abnormal reflexes.

Hematology: Performed prior to dosing, on all HD animals on day 4, and on all animals after 2 and 3 weeks of dosing. The following parameters were assessed: hemoglobin, hematocrit, red blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, white blood cell count, differential white blood cell count, erythrocyte sedimentation rate, and platelets count. Coagulation parameters: prothrombin time and activated partial thromboplastin time. Decreased hemoglobin, hematocrit and red blood cell count were observed in some HD animals after 2 and 3 weeks of dosing. Other changes are most likely related to traumatic wounds.

Clinical chemistry: Performed prior to dosing, on all HD animals on day 4, and on all animals after 2 and 3 weeks of dosing. The following parameters were assessed: ALT, AST, alkaline phosphatase, total bilirubin, glucose, blood urea nitrogen, sodium, potassium, cholesterol, triglycerides, free fatty acids, total protein, total lipids, glucose tolerance test. Increased ALT levels were observed in 1 male and 1 female in each of MD and HD groups after 2 and 3 weeks of dosing. Alkaline phosphatase was increased in 1 MD male, 2 MD females and in all HD animals after 3 days and 2 and 3 weeks of dosing.

Urinalysis: Performed prior to dosing, on all HD animals on day 4, and on all animals after 2 and 3 weeks of dosing. The following parameters were assessed: specific gravity, volume, pH, protein, reduced substances, glucose, ketones, bile pigment, and sediment. There were no treatment related changes.

Gross pathology: Performed on all animals. Liver was enlarged in HD males and 3 HD females. Testes were decreased in HD males.

Organ weights: The following organs were weighed at necropsy: heart, spleen, thymus, liver, pancreas, kidneys, testes, epididymis, prostate gland, ovaries, uterus, thyroids with

parathyroids, adrenal glands, pituitary gland, and brain. Liver weight was increased in 2 LD males, 2 LD females, 2 MD males, 2 MD females, and in all HD animals. Testes and epididymides weight was decreased in HD males. Thymus weight was decreased in most MD and HD animals.
Histopathology: The following tissues and organs from all animals were examined: skeletal muscle, diaphragm, heart, aortic arch, trachea, lungs, spleen, lymph node, thymus, bone marrow, tongue, liver, gall bladder, salivary gland, pancreas, esophagus, stomach, duodenum, jejunum and ileum, cecum and colon, kidneys, urinary bladder, testes, epididymides, prostate gland, ovaries, uterus, vagina, skin, mammary glands, thyroids and parathyroids, adrenal glands, pituitary gland, brain, spinal cord, eye, and optic nerve. Frozen sections of the liver and kidneys were stained for fat and glycogen. Frozen sections of the liver were stained for lipofuscin, bile pigments and haemosiderin pigments. Bone marrow smears were prepared and examined. The following treatment-related effects were noted: involution of thymus in some MD and HD animals; swollen and vacuolated hepatocytes in MD and HD animals of both sexes, and to a lesser extent in LD females; increased incidence of biliary epithelial cell proliferation and slight peribiliary chronic inflammatory reaction in MD males and HD animals of both sexes; inhibition of spermatogenesis in HD males; increased secretory activity of mammary glands in one MD female.
Toxicokinetics: not conducted
Other: none

10. Study title: Pilot embryotoxicity study with (b) (4) and Org 5882 in rabbits (Study No. 80R-00224) (Note: the reviewer did not evaluate Org 5882 data)

Key study findings: Oral administration of (b) (4) to Dutch rabbits from day 6 to day 18 of pregnancy at a single dose of 80 mg/kg/day resulted in embryotoxic effects (2-fold increase in post-implantation loss) and teratogenic effects (9-fold increase in the incidence of malformations) at levels not causing maternal toxicity. The NOAEL has not been identified for these effects.

Study no.: 80R-9224
Volume #, and page #: electronic submission
Conducting laboratory and location: Scientific Development Group, Organon, Oss, The Netherlands
Date of study initiation: November 14, 1979 (report date)
GLP compliance: No
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: (b) (4) batch C, purity: not provided

Methods

Doses: 0, 80 mg/kg/day
Species/strain: rabbit/Dutch
Number/sex/group: 12 pregnant females/group
Route, formulation, volume, and infusion rate: oral (gavage), suspension in 0.5% (w/v) gelatine containing 5% (w/v) mannitol, volume 2 mL/kg

Satellite groups used for toxicokinetics: none
Study design: (b) (4) was administered orally by gavage to female Dutch rabbits during the period of organogenesis from day 6 to day 18 of pregnancy at a single dose of 80 mg/kg/day. Control group was administered the vehicle. On day 29 of pregnancy, the female rabbits were sacrificed, dissected and examined.
Parameters and endpoints evaluated: Mortality, clinical signs, food consumption, body weight and body weight gain, number of implantation sites, number and distribution of live fetuses, number and distribution of intra-uterine dead embryos/fetuses, external malformations of the fetuses, ovarian weights and number of corpora lutea, fetal and placental weights, signs of gross visceral changes in the doe, and visceral and skeletal malformations of all fetuses from each litter.

Results
Mortality (dams): Two female rabbits died during pregnancy likely due to gavage error.
Clinical signs (dams): Recorded on each occasion of animal handling. There were no test article-related findings.
Body weight (dams): Recorded on days 0, 6, 12, 19, 24 and 29 of pregnancy. There were no test article-related findings.
Food consumption (dams): Recorded daily. There were no test article-related findings.
Toxicokinetics: not conducted
Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): All embryos were dead in two rabbits administered (b) (4). No effects on the mean ovarian weight, pre-implantation loss, mean fetal weight, and placental weight were observed. Post-implantation loss was increased 2-fold in the (b) (4) group when compared to the vehicle group.
Offspring (malformations, variations, etc.): Ossification was delayed and the incidence of malformations was increased in the (b) (4) group. Malformations were observed in 1/3 of the (b) (4)-treated litters. "The major defect index" was significantly higher in the test article-treated group compared to the vehicle group.

The litter data and skeletal and visceral malformations are summarized in the following table:

Treatment	vehicle	(b) (4) 80 mg/kg
Litter data:		
No. of pregnant dams	10	11
No. of dams with live fetuses	10	9
No. of dams with intra-uterine dead embryos only	0	2
No. of dams with live fetuses and also intra-uterine dead embryos	6	9
Mean number of intra-uterine dead embryos per dam with live fetuses	1.1	3.6
Post implantation loss	**20.7%**	**53.9%**
Fetal abnormalities:		
No. of fetuses examined	53	34
No. of fetuses with one or more abnormality	1	6
Percentage of fetuses with 1 or more abnormalities	**1.9%**	**17.6%**
Number of fetuses with 1 type of abnormality		4

Number of fetuses with 2 types of abnormalities		2
Number of fetuses with 3 types of abnormalities	1	
Total number of different major defects	3	7
Skeletal malformations:		
Spinal cord flexed	1 (1.9%)	-
Forelimbs severely flexed	1 (1.9%)	-
Tibia left + right shortened		1 (2.9%)
Fibula left + right shortened		1 (2.9%)
Visceral malformations:		
Fetus - Dixon's giant		1 (2.9%)
Fetus - Dixon's runt		2 (5.9%)
Brain - Hydrocephaly interna	1 (1.9%)	1 (2.9%)
Tongue - cleft		1 (2.9%)
Truncus arteriosus dilated		1 (2.9%)

Degradants in drug product:

The following degradation products have been identified in asenapine tablets: (b) (4) the N-oxide of asenapine, is a metabolite found in abundance in animals but not in humans. This degradant is qualified with respect to its general toxicity, reproductive toxicity and genotoxicity based on its presence as the metabolite in laboratory animals. (b) (4) is present at levels less than (b) (4) in 5 mg and 10 mg asenapine tablets. Therefore, this degradation product does not exceed a toxicology-based specification limit. The chemical structures of both degradants (b) (4) do not carry structural alerts for genotoxicity. A battery of *in vitro* and *in vivo* genotoxicity studies was 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 of (b) (4). However, current data indicate it is unlikely these degradants would reach levels higher than (b) (4) at the end of shelf life. Since full reviews of the genonotoxicity studies with degradants (b) (4) are not available in the DARRTS, these studies have been reviewed by the NDA 22-117 reviewer:

Genotoxicity studies with (b) (4):

1. Study title: (b) (4): Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. (Study No. INT00009335); Test facility: (b) (4) Study initiation date: February 20, 2006; GLP: yes; QA: yes

Key study findings: (b) (4) did not show mutagenic activity in the bacterial reverse mutation test (Ames test) in *Salmonella tymphimurium* strains TA98, TA100, TA1535, TA1537, and TA102.

Methods

Doses: Experiment 1 (all strains) 1.6, 8, 40, 200, 1000, and 5000 µg/plate; Experiment 2 with strains TA98, TA100 (without S9), and TA1535: 156.25, 312.5, 625, 1250, 2500,

and 5000 µg/plate; Experiment 2 with strains TA100 (with S9), TA1537, and TA102: 31.25, 62.5, 125, 250, 500, and 1000 µg/plate.

Study design: (b) (4) (batch No. C; purity 92.5%) was tested for potential mutagenic effects in tester *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102. Treatment of all strains in two experiments was conducted covering a broad range of concentrations up to the maximum recommended level of 5000 µg (b) (4) per plate in the absence or presence of metabolic activation system (induced rat liver postmitochondrial fraction S-9 mix). Triplicate plates without and with S-9 mix were used. A vehicle (DMSO) treated group served as a negative control. 4-nitrofluorene (for TA98), sodium azide (for TA100, TA1535), 9-aminoacridine (for TA1537), and mitomycin C (for TA102) served as positive controls in the absence of S-9 mix. 2-aminoanthracene (for TA100, TA1535, TA1537, and TA102) and benzo(a)pyrene (for TA98) served as positive controls in the presence of S-9 mix. The plates were incubated at 37°C for 3 days before counting. Stability of (b) (4) was assessed by the Sponsor in DMSO and phosphate buffer (b) (4) was considered stable in DMSO when tested at concentrations of 1, 10 and, 50 mg/mL, when stressed for 6 hours at ambient temperature and in daylight. (b) (4) degraded in phosphate buffer pH 7.4 at concentrations 0.002, 0.05, 0.5, and 1 mg/mL when stressed for 48 hours at 40°C in the dark. Maximum degradation of (b) (4) was observed at 0.05 mg/mL.

Results: Evidence of toxicity was observed at the highest one or two test concentrations both in the absence or presence of metabolic activation. Precipitation of the test article was observed on all test plates treated at 2500 µg/plate and above in the presence of S-9 and solely at 5000 µg/plate in the absence of S-9. As the results of the first experiment were negative, treatments in the presence of S-9 in second experiment included a 1 hour preincubation step to increase the range of mutagenic effects that could potentially be detected. The mean number of revertants on negative control plates fell within acceptable ranges. The positive controls induced an increase in the number of revertant colonies in the absence or presence of S-9 mix, indicating that the test system was working properly. (b) (4) did not induce statistically significant, dose related or reproducible increases in the number of revertants per plate in any of the bacterial strains tested in the absence or presence of matabolic activation.

2. Study title: A chromosome aberration test in cultured human peripheral blood lymphocytes. (Study No. INT00010385); Test facility: (b) (4) Study initiation date: February 10, 2006; GLP: yes; QA: yes

Key study findings: (b) (4) induced reproducible 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. Significant increase in the frequency of cells with numerical aberrations (polyploidy cells) was also observed in cultures treated with (b) (4) in the absence of metabolic activation.

Methods

Doses: The following doses were selected for chromosome analysis: Experiment 1: 3 h treatment + 17 h recovery, - S-9: 160.0, 170.0, and 190.0 µg/mL (mitotic index 57%); 3 h treatment + 17 h recovery, + S-9: 70.0, 100.0, and 110.0 µg/mL (mitotic index 44%); Experiment 2: 20 h treatment + 0 h recovery, - S-9: 50.0, 80.0, and 90.0 µg/mL (mitotic index 53%); 3 h treatment + 17 h recovery, + S-9: 40.0, 50.0, and 60.0 µg/mL (mitotic index 56%); Experiment 3: 3 h treatment + 17 h recovery, + S-9: 30.0, 40.0, and 50.0 µg/mL (mitotic index 44%).

Study design: (b) (4) (batch No. C; purity 92.5%) was tested in an *in vitro* chromosomal aberrations assay using duplicate human lymphocyte cultures prepared from the pooled blood of three male donors in two or three experiments. Experiments were conducted using standard methods. The highest concentration used in the main experiments was determined following a preliminary cytotoxicity range-finding experiment. Duplicate cultures were treated with a broad range of (b) (4) concentrations in the absence or presence of metabolic activation (S-9 mix). The test article concentrations for chromosome analysis were chosen based on mitotic index. (b) (4) was dissolved in DMSO that served as a negative control. 4-nitroquinoline 1-oxide and cyclophosphamide served as positive control in the absence or presence of metabolic activation, respectively. Where possible, 100 metaphases from each slide were analyzed for aberrations. Stability data indicated that (b) (4) degraded in phosphate buffer when stressed for 48 hours at 40 °C. Maximum degradation of (b) (4) was observed at 0.05 mg/mL.

Results: Treatment with (b) (4) for 3 hours in Experiment 1 increased the frequencies of cells with structural aberrations (excluding gaps) at two dose levels of 190.0 and 170.0 µg/mL in the absence of S-9 mix. Treatment for 3 hours with (b) (4) in Experiments 2 and 3 resulted in increases in the frequencies of cells with structural aberrations (excluding gaps) at all dose levels and at the highest dose level, respectively, in the presence of S-9 mix. Structural aberrations of a chromatid type (deletions and some exchanges) were predominantly observed in all three experiments. There was no increase in cells with structural aberrations following treatment for 20 hours. Significant increase in the frequency of cells with numerical aberrations (polyploidy cells) was also observed in cultures treated with (b) (4) following 3 hours treatment in the absence of S-9 mix at the highest two concentrations analyzed. The positive results from the Experiments 1 and 2 are shown in the following Sponsor's tables:

3 hour treatment -S-9, 17 hour recovery (3+17), Experiment 1
Donor sex: male

Treatment (μg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Solvent	A	100	1	1		
	B	100	0	0		
	Totals	200	1	1		-
160.0	A	100	3	3		
	B	100	4	4		
	Totals	200	7	7	$p \leq 0.05$	13
170.0	A	94	**16**	**16**		
	B	100	**11**	**11**		
	Totals	194	27	27	$p \leq 0.001$	32
190.0	A	53	**22**	**20**		
	B	73	**15**	**15**		
	Totals	126	37	35	$p \leq 0.001$	57
NQO, 2.50	A	39	**21**	**20**		
	B	44	**20**	**20**		
	Totals	83	41	40	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 7.14, not significant
§ Statistical significance (Appendix 5)
Numbers highlighted exceed historical negative control range (Appendix 6)

3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2
Donor sex: male

Treatment (μg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Solvent	A	100	0	0		
	B	100	0	0		
	Totals	200	0	0		-
40.00	A	100	**9**	**9**		
	B	100	**9**	**8**		
	Totals	200	18	17	$p \leq 0.001$	0
50.00	A	100	**7**	**7**		
	B	100	**9**	**8**		
	Totals	200	16	15	$p \leq 0.001$	22
60.00	A	100	**10**	**9**		
	B	100	**13**	**13**		
	Totals	200	23	22	$p \leq 0.001$	56
CPA, 12.5	A	30	**20**	**20**		
	B	41	**24**	**20**		
	Totals	71	44	40	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 0.95, not significant
§ Statistical significance (Appendix 5)
Numbers highlighted exceed historical negative control range (Appendix 6)

3. Study title: Org 43474: Induction of micronuclei in the bone marrow of treated rats. (Study no. INT00021012); Test facility: **(b) (4)** Study initiation date: June 26, 2006; GLP: yes; QA: yes

Key study findings: (b) (4) did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of rats treated up to 100 mg/kg/day, the maximum feasible dose, for two days.

Methods
Doses: 25, 50, and 100 mg/kg/day
Study design: (b) (4) (batch No. D; purity 92.1%) was tested *in vivo* in a rat bone marrow micronucleus test at three dose levels selected based on an initial range-finding study conducted at 100 mg/mL. (b) (4), formulated in 10% (v/v) ethanol in 1% (v/v) Tween 80, was administered to rats (6/sex/group) at a dose volume of 10 mL/kg once daily on two consecutive days via subcutaneous injection in both the range-finding and definitive study. The highest dose tested (100 mg/kg) was the maximum feasible dose. Cyclophosphamide administered via subcutaneous injection served as a positive control. Group of 4 male and 4 female satellite animals were dosed at the same dose levels for 2 days to demonstrate the systemic exposure to the test article. Blood samples were taken and plasma isolated at 0, 1, 2, 4, 8, and 24 hours after the second administration. Animals were observed daily for clinical signs or overt toxicity. Animals were killed 24 hours after the second administration, bone marrow was isolated and slides were prepared. At least 2000 polychromatic erythrocytes (PCE) per animal were examined for micronuclei. The ratio of PCE to normochromatic erythrocytes for each animal and group were calculated

Results:
Clinical signs of piloerection, lethargy, and palpebral closure were observed in male and female rats administered 100 mg/kg/day in the range-finding study but not in the definitive study. There was no evidence of any test-article induced toxicity to the bone marrow. However, the results of plasma analysis confirmed that animals were systemically exposed to (b) (4). Negative control rats demonstrated normal frequencies of polychromatic erythrocytes (PCE) which fell within historical control range. Cyclophosphamide administered as a positive control induced expected positive response. The frequencies of micronucleated PCE observed in animals treated with (b) (4) were not significantly different to that of the vehicle control group.

4. Study title: (b) (4): Detection of DNA damage in liver, stomach and duodenum of treated rats using the Comet assay. (Study no. INT00022157); Test facility: (b) (4); Study initiation date: June 21, 2006; GLP: yes; QA: yes

Key study findings: (b) (4) did not induce DNA damage in the liver, stomach, and duodenum of rats treated at 100 mg/kg/day, the maximum practical dose when analyzed 3 hours after the second dose administration.

Methods
Doses: 50 and 100 mg/kg/day
Study design: (b) (4) (batch No. D; purity 92.1%) was tested in the single cell gel electrophoresis assay (Comet assay) for the potential to induce DNA strand breaks or

alkali labile sites by the degree of DNA damage in the liver, stomach, and duodenum of treated rats. The dose levels were selected based on range-finding study in which 100 mg (b) (4)/kg/day (the maximum practicable dose) was administered to 3 male and 3 female rats via oral gavage once daily for two days. There were no adverse effects or sex differences in the range-finding study. Therefore, the definitive assay was conducted at 50 and 100 mg/kg/day. In the definitive assay, 6 male rats were administered (b) (4) in 10% (v/v) ethanol in 1% (v/v) Tween 80 in water via oral gavage for two days at a volume of 10 mL/kg. The vehicle was administered to 6 rats in the negative control group. Ethyl methanesulfonate (EMS) dissolved in water was administered as a single dose of positive control to 6 rats. Animals in both control groups were killed 3 hours after dose administration. Animals in (b) (4) group were killed 3 hours after the second dose. The liver, stomach and duodenum of the treated rats were analyzed for DNA damage in all groups. Single cell suspensions were prepared from these tissues, slides were prepared and the DNA was allowed to unwind. The slides were then electrophoresed and used for Comet analysis. Measurements of tail moment and tail intensity (% DNA in tail) were obtained from 100 cells per animal per tissue. Each slide was also examined for cytotoxicity.

Results: No adverse effects on clinical signs were observed in animals. EMS induced an increase in tail moment and tail intensity confirming the validity of the study. For the liver and stomach, the mean tail moment and tail intensity of the vehicle control group fell within the historical control range. No historical data were available for the duodenum. The mean tail moment and tail intensity for the test article groups were similar to that of the negative control, demonstrating that (b) (4) did not induce DNA damage in the liver, stomach, and duodenum in this study.

Genotoxicity studies with (b) (4):

1. Study title: (b) (4): Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. (Study No. INT00009333); Test facility: (b) (4) Study initiation date: February 20, 2006; GLP: yes; QA: yes

Key study findings: (b) (4) did not show mutagenic activity in the bacterial reverse mutation test (Ames test) in *Salmonella tymphimurium* strains TA98, TA100, TA1535, TA1537, and TA102.

Methods

Doses: Experiment 1 (all strains) 1.6, 8, 40, 200, 1000, and 5000 µg/plate; Experiment 2 with strains TA98, TA100, TA1535, and TA1537 (without S-9): 156.25, 312.5, 625, 1250, 2500, and 5000 µg/plate; Experiment 2 with strains TA1537 (with S9), and TA102: 31.25, 62.5, 125, 250, 500, and 1000 µg/plate.

Study design: (b) (4) (batch No. C; purity 93.8%) was tested for potential mutagenic effects in tester *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102. Treatment of all strains in two experiments was conducted covering a broad

range of concentrations up to the maximum recommended level of 5000 µg (b) (4) per plate in the absence or presence of metabolic activation system (induced rat liver post-mitochondrial fraction S-9). Triplicate plates without and with S-9 mix were used. The plates were incubated at 37°C for 3 days before counting. A vehicle (DMSO) treated group served as a negative control. 4-nitrofluorene (for TA98), sodium azide (for TA100, TA1535, 9-aminoacridine (for TA1537), and mitomycin C (for TA102) served as positive controls in the absence of S-9 mix. 2-aminoanthracene (for TA100, TA1535, TA1537, and TA102) and benzo(a)pyrene (for TA98) served as positive controls in the presence of S-9 mix. Stability of (b) (4) was assessed by the Sponsor in DMSO and phosphate buffer. (b) (4) was shown to degrade in DMSO at concentrations of 0.1, 1, 10, and 51 mg/mL, when stressed for 6 hours at ambient temperature and in daylight. Maximum degradation (10%) occurred at the lowest concentration tested (0.1 mg/ml). (b) (4) degraded in phosphate buffer pH 7.4 at concentrations 0.002, 0.05, and 0.5, and 1 mg/mL when stressed for 48 hours at 40°C. Maximum degradation of (b) (4) was observed at 0.05 mg/mL.

Results: Evidence of toxicity was observed in all strains at the highest one or two test concentrations both in the absence or presence of metabolic activation. Precipitation of the test article was observed on all test plates treated at 2500 µg/plate and above in the presence of S-9 and solely at 5000 µg/plate in the absence of S-9. As the results of the first experiment were negative, treatments in the presence of S-9 in second experiment included preincubation step to increase the range of mutagenic effects that could potentially be detected. The mean number of revertants on negative control plates fell within acceptable ranges. The positive controls induced an increase in the number of revertant colonies in the absence or presence of S-9 mix, indicating that the test system was working properly. Slight increases in revertant numbers observed in the Experiment 1 in strain TA102 and in the Experiment 2 in strain TA100 were no dose related; therefore these changes were not considered test article-related. A 2-fold increase in revertants at single dose level in the Experiment 1 in strain TA98 was statistically significant; however this increase was not reproduced in the Experiment 2. (b) (4) did not induce any other statistically significant, dose related or reproducible increases in the number of revertants per plate in any of the bacterial strains tested in the absence or presence of metabolic activation.

2. Study title: (b) (4): A chromosome aberration test in cultured human peripheral blood lymphocytes. (Study no. INT00010354); Test facility: (b) (4) Study initiation date: February 10, 2006; GLP: yes; QA: yes.

Key study findings: (b) (4) induced reproducible 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. Sporadic increases in the frequency of cells with numerical aberrations (polyploid cells) were not consistently reproduced and were considered to be of no toxicological relevance.

Methods

Doses: The following doses were selected for chromosome analysis: Experiment 1: 3 h treatment + 17 h recovery, - S-9: 130.0, 155.0, 167.5, 180.0, and 192.5 µg/mL (mitotic index 50%); 3 h treatment + 17 h recovery, + S-9: 85.0, 100.0, and 115.0 µg/mL (mitotic index 61%); Experiment 2: 20 h treatment + 0 h recovery, - S-9: 10.0, 25.0, and 50.0 µg/mL (mitotic index 51%); 3 h treatment + 17 h recovery, + S-9: 120.0, 142.2, and 167.3 µg/mL (mitotic index 46%); Experiment 3: 3 h treatment + 17 h recovery, - S-9: 190.0, 205.0, and 220.0 µg/mL (mitotic index 56%).

Study design: (b) (4) (batch No. C; purity 93.8%) was tested in an in vitro chromosomal aberrations assay using duplicate human lymphocyte cultures prepared from the pooled blood of three male donors in three experiments. Experiments were conducted using standard methods. The highest concentration used in the main experiments was determined following a preliminary cytotoxicity range-finding experiment. Duplicate cultures were treated with a broad range of (b) (4) concentrations in the absence or presence of metabolic activation (S-9 mix). The test article concentrations for chromosome analysis were chosen based on mitotic index. (b) (4) was dissolved in DMSO that served as a negative control. 4-nitroquinoline 1-oxide and cyclophosphamide served as positive control in the absence or presence of metabolic activation, respectively. Where possible, 100 metaphases from each slide were analyzed for aberrations. Stability of (b) (4) was assessed by the Sponsor in DMSO and phosphate buffer. (b) (4) was shown to degrade in DMSO at concentrations of 0.1, 1, 10, and 51 mg/mL, when stressed for 6 hours at ambient temperature and in daylight. Maximum degradation (10%) occurred at the lowest concentration tested (0.1 mg/ml). (b) (4) degraded in phosphate buffer pH 7.4 at concentrations 0.002, 0.05, 0.5, and 1 mg/mL when stressed for 48 hours at 40 °C. Maximum degradation of (b) (4) was observed at 0.05 mg/mL.

Results*:* Treatment with (b) (4) for 3 hours in Experiment 1 increased the frequencies of cells with structural aberrations (excluding gaps) at 3 dose levels of 167.5, 180.0, and 192.5 µg/mL in the absence of S-9 mix. Similar increases were noted in Experiment 3 in the absence of S-9 mix. Treatment for 3 hours with (b) (4) in Experiment 2 resulted in increases in the frequencies of cells with structural aberrations (excluding gaps) at all dose levels and at the highest dose level, respectively, in the presence of S-9 mix. Structural aberrations of a chromatid type (deletions and some exchanges) were predominantly observed in all three experiments. There was no increase in cells with structural aberrations following treatment for 20 hours. Sporadic increases in the frequency of cells with numerical aberrations (polyploid cells) were not consistently reproduced between experiments. The positive results from the Experiments 1 and 2 are shown in the following Sponsor's tables:

3 hour treatment -S-9, 17 hour recovery (3+17), Experiment 1
Donor sex: male

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Solvent	A	100	1	0		
	B	100	4	2		
	Totals	200	5	2		-
130.0	A	100	3	1		
	B	100	2	1		
	Totals	200	5	2	NS	24
155.0	A	100	1	1		
	B	100	5	3		
	Totals	200	6	4	NS	33
167.5	A	100	8	6		
	B	100	7	6		
	Totals	200	15	12	$p \leq 0.01$	42
180.0	A	100	10	6		
	B	100	11	8		
	Totals	200	21	14	$p \leq 0.001$	48
192.5	A	100	20	16		
	B	100	18	17		
	Totals	200	38	33	$p \leq 0.001$	50
NQO, 5.00	A	100	8	5		
	B	100	20	15		
	Totals	200	28	20	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 3.38, NS
§ Statistical significance (Appendix 5)
NS =not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2
Donor sex: male

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Solvent	A	100	2	1		
	B	100	0	0		
	Totals	200	2	1	-	-
120.9	A	100	7	6		
	B	100	1	1		
	Totals	200	8	7	$p \leq 0.05$	0
142.2	A	100	9	9		
	B	100	3	2		
	Totals	200	12	11	$p \leq 0.01$	25
167.3	A	100	7	7		
	B	100	5	5		
	Totals	200	12	12	$p \leq 0.001$	46
CPA, 12.5	A	61	23	20		
	B	49	20	20		
	Totals	110	43	40	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 9.77, $p \leq 0.05$
§ Statistical significance (Appendix 5)
Numbers highlighted exceed historical negative control range (Appendix 6)

3. Study title: [(b) (4)]: Induction of micronuclei in the bone marrow of treated rats. (Study no. INT00012699); Test facility: [(b) (4)] Study initiation date: May 10, 2006; GLP: yes; QA: yes

Key study findings: [(b) (4)] did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of rats treated up to 70 mg/kg/day, the estimated maximum tolerated dose, for two days.

Methods
Doses: 17.5, 35, and 70 mg/kg/day
Study design: [(b) (4)] (batch No. D; purity 93.4%) was tested *in vivo* in a rat bone marrow micronucleus test at three dose levels selected based on an initial range-finding study conducted at 50, 70, and 100 mg/mL. [(b) (4)], formulated in 10% (v/v) ethanol in 1% (v/v) Tween 80, was administered to rats (6/sex/group) at a dose volume of 10 mL/kg once daily on two consecutive days via subcutaneous injection in both the range-finding and definitive study. Cyclophosphamide administered via subcutaneous injection served as a positive control. Group of 4 male and 4 female satellite animals were dosed at the same dose levels for 2 days to demonstrate the systemic exposure to the test article. Blood samples were taken and plasma isolated at 0, 1, 2, 4, 8, and 24 hours after the second administration. Animals were observed daily for clinical signs or overt toxicity. Animals were killed 24 hours after the second administration, bone marrow was isolated and slides were prepared. At least 2000 polychromatic erythrocytes (PCE) per animal were examined for micronuclei. The ratio of PCE to normochromatic erythrocytes for each animal and group were calculated.

Results:
Clinical signs of piloerection, decreased activity and palpebral closure were observed in male and female rats administered 100 mg/kg/day in the range-finding study but not in the definitive study. All female animals in the range-finding study at 100 mg/kg/day were terminated in extremis and further dosing of males was stopped due to excessive weight loss. 70 mg/kg was the highest non-lethal dose. There was no evidence of any test-article induced toxicity to the bone marrow. However, the results of plasma analysis confirmed that animals were systemically exposed to [(b) (4)]. Negative control rats demonstrated normal frequencies of polychromatic erythrocytes (PCE) which fell within historical control range. Cyclophosphamide administered as a positive control induced expected positive response. The frequencies of micronucleated PCE observed in animals treated with [(b) (4)] were not significantly different to that of the vehicle control group.

4. Study title: [(b) (4)]: Detection of DNA damage in liver, stomach and duodenum of treated rats using the comet assay. (Study no. INT00022602); Test facility: [(b) (4)] ; Study initiation date: May 10, 2006; GLP: yes; QA: yes

Key study findings: [(b) (4)] did not induce DNA damage in the liver, stomach, and duodenum of rats treated at 100 mg/kg/day, the maximum practical dose when analyzed 3 hours after the second dose administration.

Methods
Doses: 50 and 100 mg/kg/day
Study design: (b) (4) (batch No. D; purity 93.4%) was tested in the single cell gel electrophoresis assay (Comet assay) for the potential to induce DNA strand breaks or alkali labile sites by the degree of DNA damage in the liver, stomach, and duodenum of treated rats. The dose levels were selected based on range-finding study in which 100 mg (b) (4)/kg/day (the maximum practicable dose) was administered to 3 male and 3 female rats via oral gavage once daily for two days. There were no adverse effects or sex differences in the range-finding study. Therefore, the definitive assay was conducted at 50 and 100 mg/kg/day in 6 male rats administered (b) (4) in 10% (v/v) ethanol in 1% (v/v) Tween 80 in water via oral gavage for two days at a volume of 10 mL/kg. The vehicle was administered to 6 rats in the negative control group. Ethyl methanesulfonate (EMS) dissolved in water was administered as a single dose of positive control to 6 rats. Animals in both control groups were killed 3 hours after dose administration. Animals in (b) (4) group were killed 3 hours after the second dose. The liver, stomach and duodenum of the treated rats were analyzed for DNA damage in all groups. Single cell suspensions were prepared from these tissues, slides were prepared and the DNA was allowed to unwind. The slides were then electrophoresed and used for Comet analysis. Measurements of tail moment and tail intensity (% DNA in tail) were obtained from 100 cells per animal per tissue. Each slide was also examined for cytotoxicity.

Results: No adverse effects on clinical signs were observed in animals. EMS induced an increase in tail moment and tail intensity confirming the validity of the study. For the liver, stomach, and duodenum, the mean tail moment and tail intensity of the vehicle control group fell within the historical control range. The mean tail moment and tail intensity for the test article groups were similar to that of the negative control, demonstrating that (b) (4) did not induce DNA damage in the liver, stomach, and duodenum in this study.

2.6.6.9 Discussion and Conclusions

Toxicological profile of asenapine was investigated in 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 two impurities (b) (4) in the asenapine drug substance.

Moreover, the *in vitro* and *in vivo* genotoxicity studies were conducted for two structurally related degradants in drug product (b) (4) present in the commercial batches at levels (b) (4) Since the overall metabolic profile in humans and laboratory animal species is similar, individual metabolites or enantiomers have not been tested toxicologically.

General toxicity: Toxic effects of asenapine were investigated in the single and repeated dose toxicity studies conducted in rat, dogs, and mice. The following single dose toxicity studies were conducted with asenapine: oral studies in Wistar rats (at doses of 0, 75, 115, 160, 200, 225, 315, 440, and 625 mg/kg/day) and Sprague-Dawley rats (at doses of 0, 50, 100, and 200 mg/kg/day), intravenous study in Sprague-Dawley rats (at doses of 0, 9, 15, and 21 mg/kg/day), and oral study in Beagle dogs (at doses of 0, 50, 100, and 200 mg/kg/day). Oral studies in rats demonstrated the LD_{50} values in the range of 110 to 176 mg/kg. The highest doses not associated with deaths in these studies were 50 and 100 mg/kg in males and females, respectively. Clinical signs were observed at all dose levels and consisted of labored respiration, decreased reaction to stimuli, ptosis, sedation, tremors, and clonic convulsions. Death-preceding symptoms in rats were predominantly CNS-related clinical signs of convulsion, motor incoordination, reduced activity/ventral recumbency, ptosis, and piloerection. Gross anatomy of animals found dead showed mucoid and/bloody discharge around mouth and nostrils, and enlarged adrenal glands. 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. No specific cause of death could be determined. Intravenous administration of asenapine to rats caused no deaths up to the maximum achievable dose of 21 mg/kg (limited by solubility). Drug-related clinical signs included sedation and ataxia at all doses and convulsions at the MD and HD. The LD_{50} value had not been established for male dogs since the animals tolerated high doses up to 200 mg/kg. Observations in dogs included miosis and reduced activity at all doses, and ataxia, trembling and stereotype behavior at the MD and HD. These data indicate a wide safety margin in case of human overdose.

Repeated dose: 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 oral study in dog, the MTD has not been reached. Therefore, a 39-week study has been performed in dogs by the intravenous administration. All pivotal general toxicology studies are listed below:

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	p.o. escalating dose	escalating dose	NL0001154

	3.6, 5.4, 7.2, 10.8 b.i.d. fixed dose part 10.8 b.i.d.	3-5 days fixed dose part 4 weeks	3 males, 3 females fixed dose 3 males, 3 females	
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

The subchronic and chronic toxicity studies in rats and dogs have been submitted previously to the INDs 51,641 and 70,329 and have been reviewed by Drs. Lois Freed and Sonia Tabacova. These reviews are available in the Division's file and DARRTS.

Toxicologically important observations in the repeated oral toxicity studies in rats consisted of palpebral ptosis, reduced motor activity, miosis, decreased body weight and decrease in food consumption in males, decreased body weight and increase in food consumption in females, deteriorating overall body condition and death, slight reductions in blood glucose, cholesterol and triglycerides, slight increases in blood urea nitrogen, creatinine values and urinary volume (indicating renal effect but without histopathological findings in kidneys), disturbance in the estrus cycle, and stimulation of the mammary glands. The effects on the CNS and these caused by deregulation of prolactin release by interference with dopaminergic and serotonergic regulation of the hypothalamus-pituitary axis were considered related to the pharmacologic activity of asenapine. Such effects were not considered adverse by the Sponsor unless the degree was such that it led to severe deterioration of the health of animals.

Toxicologically important observations in dogs in repeated oral toxicity studies consisted of miosis, reduced activity, motor incoordination, tremors, abnormal behavior, increased heart rate, reduced body weight and food consumption (in some animals at doses $\geq$ 20 mg/kg/day), inhibition of spermatogenesis in males, disturbance of the estrus cycle and increased secretory activity of mammary glands in females, and an increased liver enzyme activity in blood accompanied by various degrees of liver cell damage and inflammatory cell reactions. Moreover, accumulation of grayish-brownish intrahepatocellular pigment was noted in dogs in the 52-week study. However, this effect was not accompanied by morphological lesions or liver dysfunction in this study. In the 13- and 39-week intravenous toxicity studies no hepatotoxicity was observed.

Toxicological effects of asenapine administered by two routes (p.o. and i.v.) were compared in rats following treatment for two weeks. In these studies asenapine 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/group in the p.o. study and 8/sex/group 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 week 1 of dosing. 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 red blood cell parameters) were noted only with i.v. dosing, as were increases in lung (male) and heart (female) weight. 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% (male-female) higher following i.v. than p.o. dosing; level 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.

The reversibility of asenapine-induced effects was investigated in the oral 13 and 52-week studies. Most effects were partially reversible. In adult and juvenile rats, hyperactivity had been observed after the treatment with asenapine was stopped, followed by a gradual decrease over 8 weeks in adult animals and no decrease in young animals during a 4-week recovery period.

Genetic toxicology: Asenapine was tested for the genotoxic potential in a battery of *in vitro* and *in vivo* tests including *in vitro* bacterial reverse mutation (Ames) tests, *in vitro* chromosomal aberration assay in human lymphocytes, *in vitro* mouse lymphoma assay, *in vitro* sister chromatid exchange test in rabbit lymphocytes, and *in vivo* micronucleus assay in rats. Three separate bacterial mutagenicity studies were submitted under the NDA 22-117. Two of them (SDG RR No. 2283 and 2963) were originally reviewed by Dr. Lois Freed under the IND 51,641 (please see Dr. Freed's review of June 11, 1997 for further details). She concluded that the Ames test conducted in *Salmonella typhimurium* strains was not validated in the absence of metabolic activation since only indirect-acting positive controls were included. Therefore, the Division recommended this study be repeated using strain appropriate direct-acting positive controls (Letter to the Sponsor, June 24, 1997). In response, the Sponsor conducted an additional Ames study (Study SDG RR No. 5097). The repeat study was acceptable. There were no increases in revertants at any test article concentrations with any tester strains. Therefore, asenapine was negative for genotoxic potential in the bacterial mutation test under conditions of these studies. Asenapine 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. Although 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 close to the historical mean value for the negative control mutant frequency and this result was not reproduced in the second experiment. Therefore, it can be concluded that asenapine was not mutagenic in the mouse lymphoma assay under conditions of this study. Asenapine

did not induce sister chromatid exchange when tested in the non-mutational DNA damage test in cultured rabbit lymphocytes. There was a small, statistically significant increase in the number of human peripheral lymphocytes with structural chromosomal aberrations following the 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 one concentration tested in the absence of metabolic activation. Moreover, the mean number of cells with the structural chromosomal aberrations either in the presence or absence of metabolic was within the normal historical range. Therefore, the effect of asenapine on chromosomal aberrations is considered equivocal in this study. Asenapine did not produce chromosomal or spindle damage leading to the formation of micronuclei in the immature erythrocytes in the *in vivo* micronucleus assay conducted in rats. 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. Overall, no evidence of genotoxic potential of asenapine was identified.

Carcinogenicity: Carcinogenicity studies were conducted in rats and mice using subcutaneous route of administration. In the rat carcinogenicity study, subcutaneous administration of asenapine maleate at 0.3, 1.2, and 3.0/5.0 mg/kg/day in pH-adjusted citric acid monohydrate to Sprague-Dawley female rats for 99 weeks and to male rats for 105 weeks did not demonstrate any organ specific, systemic or local tumorigenic potential of the test article. Although the Executive CAC did not concur with the doses, the reason for nonconcurrence was that it had been thought that the doses were not high enough. However, the doses turned out to be sufficiently high, and it was noted that the MTD was exceeded. Due to high morbidity/mortality in the female vehicle control group, the female study was terminated in weeks 100 to 102 after obtaining the Agency's concurrence. 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. The reduction in body weight did not correlate 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. The LD and MD groups were not routinely examined.

Since it is known that a significant decrease in body weight can lead to a decrease in tumor development, the Sponsor should conduct a full histopathologic examination of the lower dose groups when an excessive decrease in body weight (or survival) is observed in the examined dose group. Because full histological evaluation was conducted only on tissues from the two control groups (vehicle and untreated) and the high dose group in both genders, the rat carcinogenicity study cannot yet be evaluated for either male or female rats. The Sponsor should be asked to perform a full histopathology evaluation of the low dose and mid dose males and females and submit the findings to the Division, for an additional evaluation.

In the mouse carcinogenicity study, asenapine maleate was administered subcutaneously at 0.5, 1.5, and 5.0/4.0 (HD in males) or 7.5/5.0 (HD in females) mg/kg/day in pH-adjusted citric acid monohydrate to CD-1 mice. The dosages selected by the Sponsor were not previously concurred with by the Executive CAC due to the lack of sufficient information at the time. 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. The study was terminated early (males in Weeks 89/90; females in Weeks 98/99) due to increased, generally dose-dependent mortality among all asenapine-treated groups. The incidence of pleomorphic malignant lymphomas and all combined lymphomas in the hemolymphoreticular system was statistically significantly increased in the female mice at the HD compared to the vehicle control (7/57 and 20/60 in the vehicle control and HD group, respectively). However, the incidence of this tumor in the female mice at the HD 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. The LD and MD groups were not routinely examined.

The incidence of malignant lymphoma in high dose females was significantly increased compared to the vehicle control but not to the untreated control. The Sponsor should be asked for an explanation for the large difference in the incidence of lymphomas between vehicle and untreated female controls. Furthermore, full histopathology of the low-dose and mid-dose female groups should be performed. The final evaluation of the lymphomas will be made after the additional data are received.

Safety margins for the carcinigenicity studies are shown in the following Sponsor's table:

Safety margins for the rodent carcinogenicity studies

Species		Dose ($mg.kg^{-1}$)	Safety margin			
			$mg.kg^{-1}$-based	AUC-based	C_{max}-based	$mg.m^{-2}$ based
Mouse	M	4 (2.8 AE)[a]	8.6	4	47	0.7
	F	5 (3.5 AE)[b]	11	5	59	0.9
Rat	M & F	5 (3.5 AE)	11	8	47 (38[c])	1.7

[a]: reduced from 5 (3.5 AE) $mg.kg^{-1}$ (0.9 times the MRHD based on $mg.m^{-2}$) in week 25 of dosing
[b]: reduced from 7.5 (5.3 AE) $mg.kg^{-1}$ (1.3 times the MRHD based on $mg.m^{-2}$) in week 25 of dosing
[c]: estimated from the carcinogenicity study

Reproductive toxicology: The following reproductive studies were submitted: pilot (p.o.) and definitive (p.o.) fertility and early embryonic development (Segment I) studies in rats, pilot (p.o.) and two definitive (p.o. and i.v.) embryofetal development (Segment II) studies in rats, pilot (p.o.) and two definitive (p.o. and i.v.) embryofetal development (Segment II) studies in rabbits, three pilot (p.o., i.v. and i.v.) and definitive (i.v.) prenatal and postnatal development (Segment III) studies in rats.

In the pilot fertility study, oral administration of asenapine at 30 mg/kg/day to male Sprague-Dawley rats in 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 oral study in rats.

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. to males and females before and during mating and gestation to termination on day 21 of gestation or day 21 after birth. 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.

In a pilot study designed to assess effects of asenapine at 30 mg/kg/day on pregnancy and embryofetal development, oral administration to pregnant female Sprague-Dawley rats during the period of organogenesis induced slight transient reduction in mean body weight during pregnancy. The abnormal litter ratio and the malformation rate were increased, although the differences were not statistically significant. The degree of ossification of various skeletal elements was also decreased in fetuses of asenapine treated females.

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 and increased post-implantation loss. The mean fetal body weight was also slightly reduced (by 8%) in this group. 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 NOAEL for maternal toxicity was considered to be below the LD. The NOAEL for the reproduction and F1 parameters was the MD. However, it is unclear whether the external and visceral malformations were properly examined. Only one malformed fetus was reported upon external or visceral examinations in the LD group. It appears extremely unlikely that no spontaneous external or visceral findings were detected in any fetus in all other groups. Therefore, evaluation of external and visceral teratogenic effects in this study is considered inadequate. This study was also reviewed by Dr. Lois Freed under the IND 51,641. She concluded that "the lack of specific findings suggests reduced sensitivity to detect soft tissue abnormalities, variants, etc. Unless data can be provided that adequately document the sensitivity of the methods used to assess fetal effects, the studies may need to be repeated". Please see Dr. Freed's review dated January 9, 1998 for further details. Her conclusions were conveyed to the Sponsor in a letter dated February 11, 1998. The Sponsor did not address these concerns directly in the NDA 22-117. However, an i.v. 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 in 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. There were no test article-related external or visceral findings in fetuses at any dose level. 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 from the HD dam no. 85. Macroscopic observations indicated a mass in the chest wall region of this dam, which was considered an incidental occurrence. Therefore, findings in the litter from the dam no. 85 can be excluded from the assessment of teratogenic effects. In conclusion,

findings at the HD are not considered drug-related. The NOAEL for maternal toxicity and for fetal and skeletal abnormalities is the HD of 1.5 mg/kg/day AE (2.11 mg/kg/day expressed as the maleate).

In the pilot embryotoxicity study with asenapine in Dutch rabbits, oral administration of asenapine to pregnant females during the period of organogenesis (from day 6 to day 18 of pregnancy) at 30 mg/kg/day did not induce any maternal and/or embryotoxic or teratogenic effects that could be attributed to treatment. However, the trunks were not examined for visceral malformations. Nevertheless, the dose of 30 mg/kg/day was chosen as the HD for the definitive study in rabbits.

An oral embryotoxicity/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: No. 62 (day 10 of gestation, day 5 of dosing) and No. 52 (day 15 of gestation, day 10 of dosing). In female No. 62 dyspnea and ventral recumbency were observed prior to deaths. These symptoms started about 20 minutes after the first daily administration in the morning. No clinical signs were observed in female No. 52. At necropsy, reddened and incompletely collapsed lungs were noted in female No. 52. Both deaths are considered to be drug related. Clinical sign of dyspnea and ventral recumbency were observed also in HD female No. 54 on days 18 and 19 of pregnancy. 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 by 17% (male and female fetuses combined). Therefore, the NOAEL for the maternal and fetal toxicities was the MD. 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. This study was also reviewed by Dr. Lois Freed under the IND 51,641. She concluded that “the lack of specific findings suggests reduced sensitivity to detect soft tissue abnormalities, variants, etc. Unless data can be provided that adequately document the sensitivity of the methods used to assess fetal effects, the studies may need to be repeated”. Please see Dr. Freed’s review dated January 9, 1998 for further details. Her conclusions were conveyed to the Sponsor in a letter dated February 11, 1998. The Sponsor did not address these concerns directly in the NDA 22-117 submission. However, an i.v. embryofetal developmental study in the rabbits, designed to characterize teratogenic effects of asenapine, was submitted under this NDA.

In the second pivotal embryofetal development study, New Zealand White rabbits were administered asenapine intravenously at 0, 0.025, 0.125, and 0.625 mg/kg/day (0.018,

0.088, and 0.444 mg/kg/day AE) from day 6 to day 18 of gestation. There were two drug-related unscheduled deaths. An abdominal subcutaneous hematoma was detected at autopsy in the MD female that died on day 24. Polypnea, ptosis, and many red foci on the surface of all lobes of the lungs were noted at necropsy in the HD female that died on day 9. Clinical signs of polypnea (all animals), occasional motor incoordination (18/26 animals), occasional ptosis (all animals), and occasional hyperactivity (8/26 animals) were observed at HD usually from 5 to 30 minutes after dosing and lasted up to 2 hours after dosing. 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).

This study was also reviewed by Dr. Lois Freed under the IND 51,641. She concluded in her review of January 9, 1998 that "Due to technical problems, data from a number fetuses could not be used (11 C, 5 LD, 6 MD, 13 HD fetuses). Unfortunately, individual line listings were provided only for those fetuses that could not be used. Due to the lack of adequate fetal examination, the data from this study cannot be considered to have adequately assessed the teratogenic potential of Org 5222". Therefore, the individual line listings for all fetuses included in the final analysis, with each fetus identified by number and litter, were requested from the Sponsor at that time. The requested data were submitted to the NDA 22-117. Although 6.2% and 7.9% of the total number of fetuses were not available for skeletal examinations in this study, the overall number of fetuses examined is sufficient for an adequate study.

In the intravenous prenatal and postnatal development study in Sprague-Dawley rats administration of asenapine i.v. at 0.3, 0.9, and 1.5 mg active entity/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. These effects may have resulted from impaired nursing ability of parental animals. 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 No. NL0052638 was a result of the effects on the mother or a toxicological effect on offspring. All parental animals treated with asenapine exhibited strongly reduced motor activity and increased muscle tone. The mean body weight gain was 6% lower during gestation in dosed females. Post implantation loss was slightly increased in animals administered asenapine. At first litter check after parturition, 23 dead pups were noted in the group 2 administered asenapine compared to one dead pup in the control group 1. Postnatal pup loss was increased up to 19%-26% up to day 4 of lactation in all groups breeding litters from asenapine treated dams (V/HD, HD/HD, and HD/Control). The number of cannibalized (missing) pups was highest in the group of vehicle-treated nursing animals (V/HD). During lactation days 1 to 10, suckling of individual pups had not occurred at all (or was low) in the HD/HD and HD Control groups. The results indicate that peri- and postnatal pup loses after prenatal 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.

Juvenile animal studies: 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.

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

Special toxicology: 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 of 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:

Impurity (b) (4). Drug substance impurity (b) (4) has been present in the drug substance commercial size clinical/stability batches at (b) (4). However, the Sponsor proposed to set a specification limit for this impurity in asenapine drug substance at (b) (4), thus above the ICH Q3A(R) qualification limit of (b) (4). The content of (b) (4) 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 of (b) (4) 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 (b) (4) 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) (b) (4) 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 (b) (4) 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 (b) (4) in the rabbit to qualify this impurity during phase IV or reduce the specification of (b) (4) to the ICH Q3A(R) qualification limit of (b) (4).

Other studies conducted to qualify (b) (4) 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 (b) (4) is considered non-genotoxic and qualified for general toxicity.

Impurity (b) (4). Studies conducted to qualify the impurity (b) (4) directly included genotoxicity studies (Ames tests and chromosomal aberrations study in cultured human lymphocytes) and an intravenous 2-week toxicity study with (b) (4) in Wistar rats. Reproductive toxicology study (embryofetal toxicity study in rats) recommended by the Agency for qualification of impurities was not conducted with (b) (4). However, (b) (4) is considered to be qualified based on the presence of adequate amounts of this impurity in reproductive toxicology studies conducted with asenapine.

Qualification of degradants in drug product:

The following degradation products have been identified in asenapine tablets: (b) (4)

(b) (4), 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, (b) (4) is an aliphatic N-oxide (usually aromatic N-oxides are known for structural alerts for mutagenicity). (b) (4) 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 (b) (4) achieved in rats and dogs following long term treatment (oral and intravenous administration) is similar to or exceeds that to 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 (b) (4) will significantly increase with time up to (b) (4) respectively. It is concluded that (b) (4) is qualified with respect to its general toxicity, reproductive toxicity and genotoxicity based on its presence as the metabolite in laboratory animals.

(b) (4)**:** As indicated by the reviewing chemist Dr. Tele**,** (b) (4) is present at levels less than 0.5% in 5 mg and 10 mg asenapine tablets. Therefore, this degradation product does not exceed a toxicology-based specification limit set in the ICH Q3B (R) guidance.

(b) (4)**:** 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 of (b) (4). 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 (b) (4) 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 (b) (4) 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.

Exposure comparisons:

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 the 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. The following is the summary of the exposure data and conclusions taken directly from Dr. Tabacova's review of June 21, 2007:

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)

The exposure in pivotal toxicology studies at the NOAEL and safety margins calculated by the Sponsor relative to human exposure at the MHRD of 10 mg b.i.d. are shown in the Sponsor's table below:

Study	Dose*	Toxicological endpoint	AUC ($ng.h.mL^{-1}$) at NOAEL	Cmax ($ng.mL^{-1}$) at NOAEL	Safety margin human AUC[a]	Safety margin human C_{max}
52-week p.o. rat	1: 0.6 - 3.6 - 21.6 2: 0.4 - 2.6 - 15.3 3: 0.2 - 1.2 - 7.5	Detoriated bodily condition at 3.6 $mg.kg^{-1}$	42	15	1.0	2.3
39-week i.v. dog	1: 0.1 - 0.4 - 1.6 2: 0.07 - 0.3 - 1.1 3: 0.11 - 0.46 - 1.8	Severe CNS effects at 1.6 $mg.kg^{-1}$	122	68	1.4	10
Segment I p.o. rat	1: 1 - 5 - 30 2: 0.7 - 3.6 -21 3: 0.35 - 1.7 - 10	Prolonged pre-coital interval /embryotoxicity at 30 $mg.kg^{-1}$	131[b]	25[b]	3.0	4
Segment II p.o. rat	1: 1 - 5 - 30 2: 0.7 - 3.6 -21 3: 0.35 - 1.7 - 10	Embryotoxicity at 30 $mg.kg^{-1}$	131[b]	25[b]	3.0	4
Segment II I.v. rat	1 not applicable 2: 0.3 - 0.9 - 1.5 3: 0.15 - 0.44 - 0.73	Embryotoxicity at 1.5 $mg.kg^{-1}$	212	147	2.4	22
Segment III i.v. rat	1 not applicable 2: 0.3 - 0.9 - 1.5 3: 0.15 - 0.44 - 0.73	Embryotoxicity at 1.5 $mg.kg^{-1}$				
Segment II p.o. rabbit	1: 1 - 5 - 30 2: 0.7 - 3.6 -21 3: 0.69 - 3.5 - 21	Embryotoxicity at 30 $mg.kg^{-1}$	nd	nd		
Segment II i.v. rabbit	1: 0.025 - 0.125 - 0. 625 2: 0.02 - 0.09 - 0.44 3: 0.02 - 0.09 - 0.43	No effects at highest dose tested	179	232[c]	2.1	35

*: 1. Dose ($mg.kg^{-1}$) (NOAEL) <> 2. Dose ($mg.kg^{-1}$ AE) <> 3. Multiples of MHRD on $mg.m^{-2}$ basis
NOAEL: No Observed Adverse Effect Level <> **MHRD**: Maximum Human Recommended Dose (20 mg, 12.3 $mg.m^{-2}$) <> **AE**: Active Entity <> **nd**: Not determined
[a]: Relative to a human AUC_{0-12} of 43.4 $ng.h.mL^{-1}$ for b.i.d. dosed animals and 86.8 $ng.h.mL^{-1}$ for qd dosed animals
[b]: Extrapolated from kinetic data from the 52-week p.o. rat study (3.6 $mg.kg^{-1}$ dose)
[c]: Average plasma concentrations at the first sampling time (5 min)

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

It is recommended the NDA 22-117 for asenapine maleate not be approved until the issues concerning the rat and mouse carcinogenicity studies described below have been resolved.

Unresolved toxicology issues (if any):

- Final evaluation of carcinogenic potential of asenapine will be possible after an additional data are received from the Sponsor (see below).

- The reviewing pharmacologist has not received requested information regarding major circulating asenapine metabolites in humans from the Clinical Pharmacology and Biopharmaceutics Review Team. There is a possibility that additional major human metabolites are identified that have not been qualified to date in preclinical toxicity studies. Therefore, the pharmacology/toxicology final recommendations may require modification depending on resolution of this issue.

Recommendations for nonclinical studies

1. Carcinogenicity studies: Based on review of the carcinogenicity studies, entitled "104 week subcutaneous administration oncogenicity study with Org 5222 in the rat" and "104 week subcutaneous administration oncogenicity study with Org 5222 in the mouse" it is concluded that both studies are inadequate and further information from the Sponsor is needed to complete evaluation of the carcinogenic potential of asenapine.

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, the Sponsor should conduct a full histopathologic examination of the low and mid dose males and females.

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. The Sponsor should provide an explanation for the large difference in the incidence of lymphomas between vehicle and untreated female controls. 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.

2. Qualification of impurity (b) (4): Drug substance impurity (b) (4) has been present in the drug substance commercial size clinical/stability batches at (b) (4). However, the Sponsor proposed to set a specification limit for this impurity in asenapine drug substance at (b) (4), thus above the ICH Q3A(R) qualification limit of (b) (4). The content of (b) (4) 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 of (b) (4) 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 (b) (4) 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) (b) (4) 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 (b) (4) 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 (b) (4) in the rabbit to qualify this impurity during phase IV or reduce the specification of (b) (4) to the ICH Q3A(R) qualification limit of (b) (4).

Suggested labeling:

Mechanism of Action: "The mechanism of action of asenapine, as with other drugs having efficacy in schizophrenia and bipolar disorder, is unknown. Nonetheless, it has been proposed that the efficacy of asenapine is mediated through a combination of antagonist activity at D_2 and 5-HT_{2A} receptors. Actions at other receptors e.g., 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, 5-HT_6, 5-HT_7, D_3, and α_2-adrenergic receptors, may contribute to the clinical effects of asenapine".
Pharmacodynamics: "Asenapine exhibits high affinity for dopamine D_2, D_3, D_4, and D_1 receptors (Ki values of 1.3, 0.42, 1.1, and 1.4 nM), serotonin 5-HT_{1A}, 5-HT_{1B} , 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_5, 5-HT_6 and 5-HT_7 receptors (Ki values of 2.5, 4.0, 0.06, 0.16, 0.03, 1.6, 0.25, and 0.13 nM), α_1 and α_2-adrenergic receptors (Ki values of 1.2 and 1.2 nM), and H_1 receptors (Ki value 1.0 nM), and moderate affinity for H_2 receptors (Ki value of 6.2 nM). In *in vitro* assays asenapine acts as an antagonist at these receptors. Asenapine has no appreciable affinity for muscarinic cholinergic receptors (e.g., Ki value of 8128 nM for M_1)".
Carcinogenesis: No recommendations can be provided at this time due to the lack of adequate carcinogenicity studies.
Mutagenesis: "The genotoxic potential of asenapine was tested in the *in vitro* bacterial reverse mutation assay, the *in vitro* forward gene mutation assay in mouse lymphoma cells, the *in vitro* chromosomal aberration assay in human lymphocytes, the *in vitro* sister chromatid exchange assay in rabbit lymphocytes, and the *in vivo* micronucleus assay in rats. Asenapine was negative in these assays, except for an equivocal response in the chromosomal aberration assay. The weight of evidence suggests that asenapine lacks genotoxic potential".
Impairment of Fertility: "Asenapine did not impair fertility in rats when tested at doses up to 15 mg/kg b.i.d. given orally. This dose is 15 times the maximum recommended human dose of 10 mg b.i.d. given sublingually".
Pregnancy: Pregnancy Category C
"Asenapine was not teratogenic in reproduction studies in rats and rabbits at i.v. doses up to 1.5 mg/kg in rats and 0.44 mg/kg in rabbits. These doses are 0.73 and 0.43 times, respectively, the maximum recommended human dose (MRHD) of 10 mg b.i.d. given sublingually on a mg/m^2 basis. Plasma level of asenapine was measured in the rabbit

study, and the AUC at the highest dose tested was 2 times that in humans receiving the MRHD.
In a study in which rats were treated from day 6 of gestation through day 21 postpartum with i.v. doses of asenapine of 0.3, 0.9, and 1.5 mg/kg/day (0.15, 0.44, and 0.73 times the MRHD of 10 mg b.i.d. given sublingually on a mg/m^2 basis), increases in post-implantation loss and early pup deaths were seen at all doses, and decreases in subsequent pup survival and weight gain were seen at the two higher doses. A cross-fostering study indicated that the decreases in pup survival were largely due to prenatal drug effects. Increases in post-implantation loss and decreases in pup weight and survival were also seen when pregnant rats were dosed orally with asenapine.
There are no adequate and well-controlled studies in pregnant women. Sycrest should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus".

Signatures:

Reviewer Signature: Elzbieta Chalecka-Franaszek, Ph.D., Pharmacologist

{see appended electronic signature page}

Supervisor Signature: Barry Rosloff, Ph.D. Team leader

{see appended electronic signature page}

Concurrence Yes _X__ No ___

APPENDIX/ATTACHMENTS

Attachment I
Executive Carcinogenicity Assessment Committee Meeting Minutes April 4, 2000

Attachment II:
Executive Carcinogenicity Assessment Committee Meeting Minutes March 25, 2008

Attachment III:
Draft letter to the Sponsor regarding inadequate carcinogenicity studies

Attachment I

Executive CAC
Date: 4/4/00

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Charles Resnick, HFD-110, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Team Leader
Lois M. Freed, Ph.D., HFD-120, Presenting Reviewer

Author of Draft: Lois M. Freed, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND #51,641
Drug Name: Org 5222
Sponsor: Organon Inc.

Mouse Dose Selection: the doses selected by the sponsor for the mouse carcinogenicity study were 0, 0.5, 1.4, and 4 mg/kg s.c. in males and females. No study protocol was submitted. Doses were based on the results of previous studies (one 2-wk, two 13-wk s.c.) in CD-1 mouse. The strain to be used in the carcinogenicity study was not specified, but was assumed to be the CD-1 mouse. The HD selected for the carcinogenicity study is expected to result in the following (as described by the sponsor): (1) transient hypoactivity, (2) reduced body weight gain (males), (3) increased heart weight, (4) reduced wbc count (males), and (5) no significant injection site effects. The sponsor also pointed out that the plasma exposure (to the parent compound) at the HD is expected to be ≥25 times that predicted in humans at the maximum therapeutic dose.

The reviewer could not concur with the doses selected by the sponsor for the following reasons: (1) there was a discrepancy between the text and the summary table for one of the 13-week studies making it impossible to determine the doses associated with death in males and (2) there were no clear dose-limiting toxicities identified in females. In addition, the sponsor did not provide sufficient information to allow for selection of a HD based on the 25-fold exposure (i.e., PK) criterion. There was no discussion of the metabolic profile in mouse or human, no data on major metabolites, no serum protein binding data for mouse, or any discussion of the genotoxicity data for Org 5222.

Rat Dose Selection: the doses selected by the sponsor for the rat carcinogenicity study were 0, 0.3, 1, and 3 mg/kg s.c. in males and females. No study protocol was submitted. Doses were based on the results of previous studies (one 2-wk, two 13-wk s.c.). The 2-wk and one of the 13-wk studies were conducted in Wistar rat; the second 13-wk study was conducted in Sprague-Dawley rat. The strain to be used in the carcinogenicity study was not specified. The HD selected for the carcinogenicity study is expected to result in the following (as described by the sponsor): (1) a >10% reduction in body weight gain (males), (2) hypoactivity, (3) mild hematological effects, (4) prolongation of diestrus, (5) decreased uterus weight, (6) lobular development/secretory activity of mammary glands (females), (7) increased adrenal gland weight (males), (8) no significant local injection site effects. The sponsor also pointed out that the plasma exposure (to the parent compound) at the HD is expected to be 21-27 times that predicted in humans at the maximum therapeutic dose.

The reviewer could not concur with the doses selected by the sponsor for the following reasons: (1) the strain of rat to be used in the carcinogenicity study was not specified [the studies used to support the doses were conducted in Wistar and Sprague-Dawley rat] and (2) there were no clear dose-limiting

toxicities identified in females. In addition, the sponsor did not provide sufficient information to allow for selection of a HD based on the 25-fold exposure (i.e., PK) criterion. There was no discussion of the metabolic profile in rat or human, no data on major metabolites, no discussion of the genotoxicity data for Org 5222. Most importantly, the plasma exposure data provided for the proposed HD were extrapolated from data at lower doses but were not assessed in the 13-wk study in which the proposed HD was tested.

For both the mouse and rat dose-selections, the summary tables did not provide information sufficient to evaluate drug-related effects. Actual data (and/or percentages) should have been given instead of designations such as "d", "i", etc.

Executive CAC Recommendations and Conclusions:

The Exe-CAC could not concur with the doses selected by the sponsor for the 2-yr carcinogenicity studies in mouse or rat for the reasons given by the reviewer. The sponsor should provide full study reports for those studies that have not previously been submitted to the Division or protocols and summary data tables and line listings. The data specifically used to support dose-selection should be provided in summary tables as actual data, not as designations such as "d", "i", etc.

The Committee expressed concern that daily s.c. dosing would not be well tolerated in either mouse or rat for a 2 year period and suggested consideration of a different route or less frequent dosing (e.g., 3-4 dose/wk) for the carcinogenicity studies. Appropriate dose ranging data would be needed for any change in dosing regimen.

04/07/00

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\
HFD-120
/Division File
/GFitzgerald
/LMFreed
/SHardeman
HFD-024
/ASeifried

Attachment II

Executive CAC
Date of Meeting: March 25, 2008

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
William Taylor, Ph.D., DSPTP, Alternate Member
Barry Rosloff, Ph.D., DPP, Team Leader
Elzbieta Chalecka-Franaszek, Ph.D., DPP, Presenting Reviewer

Author of Draft: Elzbieta Chalecka-Franaszek, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #22-117
Drug Name: Asenapine maleate
Sponsor: Organon USA Inc.

Background: Asenapine maleate (Org 5222) is a psychopharmacologic agent belonging to the group of dibenzoxepinopyrrolidine compounds with high affinity for blocking serotonin, dopamine, adrenergic and histamine receptors. Asenapine is being developed for the treatment of schizophrenia and treatment of acute manic or mixed episodes associated with Bipolar I Disorder.

Rat Carcinogenicity Study: 104 week subcutaneous administration oncogenicity study with Org 5222 in the rat

Subcutaneous administration of asenapine maleate at 0.3 (LD), 1.2 (MD) and 3.0/5.0 (HD) mg/kg/day in pH-adjusted citric acid monohydrate to Sprague-Dawley female rats for 99 weeks and to male rats for 105 weeks did not demonstrate any organ specific, systemic or local tumorigenic potential of the test article. The dosages selected by the sponsor were not previously concurred with by the Executive CAC due to the lack of sufficient information at the time. Due to high morbidity/mortality in the female vehicle control group, the female study was terminated in weeks 100 to 102 after obtaining the Agency's concurrence. 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. The reduction in body weight did not correlate 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. The LD and MD groups were not routinely examined.

Mouse Carcinogenicity Study: 104 week subcutaneous administration oncogenicity study with Org 5222 in the mouse

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Asenapine maleate was administered subcutaneously at 0.5 (LD), 1.5 (MD) and 5.0/4.0 (HD in males) or 7.5/5.0 (HD in females) mg/kg/day in pH-adjusted citric acid monohydrate to CD-1 mice. The dosages selected by the sponsor were not previously concurred with by the Executive CAC due to the lack of sufficient information at the time. 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. The study was terminated early (males in Weeks 89/90; females in Weeks 98/99) due to increased, generally dose-dependent mortality among all asenapine-treated groups.
The incidence of pleomorphic malignant lymphomas and all combined lymphomas in the hemolymphoreticular system was statistically significantly increased in the female mice at the HD compared to the vehicle control (7/57 and 22/60 in the vehicle control and HD group, respectively). However, the incidence of this tumor in the female mice at the HD 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. The LD and MD groups were not routinely examined.

Executive CAC Recommendations and Conclusions:

Rat:

Although the exec-CAC did not concur with the doses, the reason for non-concurrence was that it had been thought that the doses were not high enough. However, the doses turned out to be sufficiently high, and it was noted that the MTD was exceeded.

Since it is known that a significant decrease in body weight can lead to a decrease in tumor development, the sponsor should conduct a full histopathologic examination of the lower dose groups when an excessive decrease in body weight (or survival) is observed in the examined dose group. Because full histological evaluation was conducted only on tissues from the two control groups (vehicle and untreated) and the high dose group in both genders, the rat carcinogenicity study cannot yet be evaluated for either male or female rats.

- The sponsor should be asked to perform a full histopathology evaluation of the low dose and mid dose males and females and submit the findings to the Division, as soon as possible.

Mouse:

- Although the exec-CAC did not concur with the doses, the reason for non-concurrence was that it had been thought that the doses were not high enough. However, the doses turned out to be sufficiently high, and it was noted that the MTD was exceeded.

2

- The Committee concurred that the incidence of malignant lymphoma in high dose females was significantly increased compared to the vehicle control but not to the untreated control.

- The Sponsor should be asked for an explanation for the large difference in the incidence of lymphomas between vehicle and untreated female controls. Furthermore, full histopathology of the low-dose and mid-dose female groups should be performed.

- The final evaluation of the lymphomas will be made after the additional data are received.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DPP
/Barry Rosloff, Ph.D., Team Leader, DPP
/Elzbieta Chalecka-Franaszek, Ph.D., Reviewer, DPP
/Keith Kiedrow, Pharm. D., Project Manager, DPP
/ASeifried, OND IO

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

David Jacobson-Kram
3/31/2008 01:50:39 PM

Attachment III

NDA #22-117
Drug Name: Asenapine maleate
Sponsor: Organon USA Inc.

We have completed our review of your carcinogenicity studies, entitled "104 week subcutaneous administration oncogenicity study with Org 5222 in the rat" and "104 week subcutaneous administration oncogenicity study with Org 5222 in the mouse", and have concluded that further information is needed as discussed below.

Rat carcinogenicity study:

The MTD (maximum tolerated dose) was clearly exceeded in this study 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, you should conduct a full histopathologic examination of the low and mid dose males and females.

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 22/60 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.

You should provide an explanation for the large difference in the incidence of lymphomas between vehicle and untreated female controls. 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.

In addition, we recommend that slides from all groups in the rat study and the female groups in the mouse study, including the slides from previously fully evaluated groups, be examined simultaneously by one study pathologist. Peer review should also be conducted for all of these groups.

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/s/

Elzbieta Chalecka-Franaszek
4/30/2008 05:20:39 PM
PHARMACOLOGIST

Barry Rosloff
4/30/2008 05:28:57 PM
PHARMACOLOGIST
I agree with the conclusions and recommendations in Dr. Chalecka-Franaszek's excellent and comprehensive review.