TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
death	all cause	4	8	6	4	2	5	7	3
adrenal	subcapsular hyperplasia								
	grade +/-	6/49	1/50	4/48	2/48	9/50	11/50	14/49	12/49
	grade +	8/49	6/50	5/48	11/48	28/50	20/50	23/49	19/49
	grade ++	1/49	1/50	2/48	6/48	4/50	6/50	3/49	9/49
	grade +++	0/49	0/50	0/48	0/48	1/50	0/50	0/49	1/49
	total	**15/49**	**8/50**	**11/48**	**19/48**	**42/50**	**37/50**	**40/49**	**41/49**
liver	vacuolation								
	grade +/-	6/50	9/50	3/50	0/50*	2/50	7/50	5/49	5/50
	grade +	6/50	1/50	0/50*	0/50*	2/50	1/50	3/49	3/50
	grade ++	2/50	0/50	0/50	0/50	0/50	0/50	0/49	0/50
	total	**14/50**	**10/50**	**3/50****	**0/50*****	**4/50**	**8/50**	**8/49**	**8/50**
	inflammation								
	grade +/-	9/50	7/50	4/50	12/50	4/50	12/50	12/49*	8/50
	grade +	3/50	7/50	4/50	4/50	7/50	4/50	5/49	4/50
	grade ++	0/50	0/50	1/50	3/50	1/50	2/50	0/49	0/50
	grade +++	0/50	0/50	0/50	0/50	1/50	0/50	0/49	0/50
	total	**12/50**	**14/50**	**9/50**	**19/50**	**13/50**	**18/50**	**17/49**	**12/50**
	hemorrhagic hepatocyte degeneration	0/50	0/50	0/50	0/50	1/50	4/50	5/49	4/50
	angiectasis	0/50	0/50	0/50	0/50	0/50	0/50	2/49	3/50
lung	congestion	2/50	3/50	10/50*	4/50	3/50	1/50	2/49	2/50
mammary gland	secretion present								
	grade +/-	0/48	0/50	0/49	0/49	24/49	12/49*	18/47	19/49
	grade +	0/48	0/50	0/49	0/49	5/49	3/49	4/47	15/49*
	total	**0/48**	**0/50**	**0/49**	**0/49**	**29/49**	**15/49****	**22/47**	**34/49**
thymus	atrophy	9/50	8/46	4/48	1/45*	6/48	2/48	0/48*	2/49
ovaries	interstitial cell hyperplasia								
	grade +/-					0/50	2/50	0/50	4/50
	grade +					3/50	2/50	1/50	2/50
	grade ++					1/50	2/50	2/50	1/50
	total					**4/50**	**7/50**	**4/50**	**8/50**
pancreas	focal acinar cell atrophy	0/50	0/50	0/50	0/49	0/50	0/50	0/49	2/50
prostate	arteritis	0/50	0/50	0/49	2/48				
stomach	gastric glandular hyperplasia								
	grade +/-	3/50	3/50	2/50	6/50	3/49	8/50	6/48	2/50
	grade +	5/50	3/50	3/50	2/50	2/49	2/50	2/48	6/50
	grade ++	0/50	5/50	2/50	0/50	0/49	1/50	2/48	1/50
	grade +++	0/50	0/50	1/50	0/50	0/49	1/50	1/48	0/50
	total	**8/50**	**11/50**	**8/50**	**8/50**	**5/49**	**12/50**	**11/48**	**9/50**
testes	tubular atrophy								
	grade +/-	5/50	0/50	0/50	4/50				
	grade +	2/50	2/50	2/50	0/50				
	grade ++	0/50	1/50	1/50	1/50				
	grade +++	0/50	0/50	3/50	1/50				
	total	**7/50**	**3/50**	**6/50**	**6/50**				
kidney	mineral deposits	6/50	3/50	6/50	11/50	0/50	0/50	0/49	0/50
urinary bladder	epithelial hyperplasia	0/50	1/49	0/50	0/49	4/50	0/49	0/48	10/48
	calculus	2/50	7/49	3/50	9/49*	0/50	0/49	0/48	0/48

*p<0.05, **p<0.01, ***p<0.001, grades: +/- (minimal), + (slight), ++ (moderate), +++ (marked)]

Tumor findings were not listed in separate tables by the sponsor. Therefore, they are summaried in the table below. The sponsor considered there to be no drug-related neoplastic findings.

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
liver	solitary hepatocellular carcinoma	1/50	0/50	2/50	0/50	1/50	0/50	0/49	0/50
	solitary hepatocellular adenoma	3/50	6/50	4/50	1/50	0/50	0/50	0/49	1/50
	multiple hepatocellular adenoma	0/50	0/50	1/50	0/50	0/50	0/50	0/49	0/50
	associated solitary hepatocellular adenoma	1/50	0/50	2/50	0/50	0/50	0/50	0/49	0/50
lung	solitary alveolar/bronchiolar adenoma	13/50	8/50	13/50	10/50	9/50	7/50	10/50	8/50
	multiple alveolar/bronchiolar adenoma	3/50	0/50	0/50	2/50	1/50	1/50	1/49	2/50
	associated alveolar/bronchiolar adenoma	2/50	1/50	1/50	0/50	0/50	0/50	0/49	0/50
	solitary alveolar/bronchiolar carcinoma	1/50	4/50	2/50	0/50	2/50	0/50	0/49	0/50
	metastisizing solitary alveolar/bronchiolar carcinoma	1/50	0/50	0/50	0/50	0/50	0/50	0/49	0/50
lymphoreticular/hemopoietic tissue	lymphoma, single site	0/2	1/1	0/1	0/2	0/7	0/4	1/4	1/6
	lymphoma, several sites	0/2	0/1	0/1	0/2	1/7	0/4	2/4	2/6
	lymphoma, generalized	2/2	0/1	1/1	2/2	6/7	4/4	1/4	2/6
	histiocytic sarcoma, generalized	0/2	0/1	0/1	0/2	0/7	0/4	0/4	1/6
kidney	metastasizing solitary tubular adenocarcinoma	1/50	0/50	0/50	0/50	0/50	0/50	0/49	0/50
lymph node, mesenteric	metastasis from primary in kidney	1/49	0/49	0/48	0/45	0/50	0/48	0/49	0/48
thyroid	solitary follicular adenoma	0/50	0/50	0/50	1/50	0/50	0/50	0/49	1/50
vascular system	hemangiosarcoma	0/1	--	0/1	1/3	0/1	--	--	1/2
	solitary hemangioma	1/1	--	0/1	0/3	0/1	--	--	1/2
uterus	polyp					3/50	0/50	0/49	2/50
	solitary leiomyoma					1/50	0/50	0/49	1/50
	solitary sarcoma					1/50	0/50	0/49	0/50
ovaries	unilateral luteoma					0/50	1/50	0/49	0/50
	solitary cystadenoma					2/50	0/50	0/49	0/50
mammary gland	solitary adenocarcinoma	0/50	0/50	0/49	0/49	2/49	0/49	0/47	1/49
vagina	solitary papilloma					--	--	--	1/2
pituitary	solitary adenoma (anterior lobe)	0/49	0/47	0/50	0/47	0/49	0/48	0/48	1/49
Harderian gland	solitary cystadenoma	--	1/1	0/2	--	--	0/1	--	--
	solitary papillary adenoma	--	0/1	2/2	--	--	0/1	--	--
skin/subcutis	solitary sarcoma	0/50	0/50	0/50	0/49	0/50	1/50	0/49	0/50
testis	rete testis adenoma	0/50	0/50	1/50	0/50				
pleura (thorax)	mesothelioma	--	--	1/1	--	--	0/1	--	--

The number of affected animals and the total number of tumors per grp are summarized in the following sponsor's Table 2:

TABLE 2

Selegiline Hydrochloride
78 Week Carcinogenicity Study in Mice with Administration by Diet
Histological Evaluation of Tissues from the Mouse Carcinogenicity Study -
Project No. 435664
Overall Incidence of Tumours: Males and Females

	TUMOUR TABLE							
	MALES				FEMALES			
TREATMENT	Grp 1 0 mg kg-1. day-1	Grp 2 3 mg kg-1. day-1	Grp 3 10 mg kg-1. day-1	Grp 4 30 mg kg-1. day-1	Grp 1 0 mg kg-1. day-1	Grp 2 3 mg Kg-1. Day-1	Grp 3 10 mg kg-1. day-1	Grp 4 30 mg kg-1. day-1
NUMBER OF ANIMALS:	50	50	50	50	50	50	50	50
NO. OF ANIMALS WITH TUMOURS	23	18	20	17	23	13	13	21
NO. OF ANIMALS WITH SINGLE TUMOURS	1	1	1	3	3	3	2	6
NO. OF ANIMALS WITH MULTIPLE TUMOURS	22	17	19	14	20	10	11	15
NO. OF ANIMALS WITH BENIGN TUMOURS	21	14	19	14	16	9	11	15
NO. OF ANIMALS WITH MALIGNANT TUMOURS	5	5	6	3	11	5	4	8
NO. OF ANIMALS WITH METASTASISING TUMOURS	1		1					
TOTAL NUMBER OF TUMOURS	52	40	55	33	51	24	26	46
TOTAL NUMBER OF BENIGN TUMOURS	42	31	44	29	32	18	22	35
TOTAL NUMBER OF MALIGNANT TUMOURS	10	9	11	4	19	6	4	11
TOTAL NUMBER OF METASTASISING TUMOURS	2		1					

Animals with more than one tumour type are recorded as having multiple tumours.

TABLE 2 (continued)

Overall Incidence of Tumours: Males and Females

	TUMOUR TABLE							
	MALES				FEMALES			
TREATMENT	Grp 1 0 mg kg-1. day-1	Grp 2 3 mg kg-1. day-1	Grp 3 10 mg kg-1. day-1	Grp 4 30 mg kg-1. day-1	Grp 1 0 mg kg-1. day-1	Grp 2 3 mg kg-1. day-1	Grp 3 10 mg kg-1. day-1	Grp 4 30 mg kg-1. day-1
% ANIMALS WITH TUMOURS	46	36	40	34	46	26	26	42
% ANIMALS WITH SINGLE TUMOUR	2	2	2	6	6	6	4	12
% ANIMALS WITH MULTIPLE TUMOURS	44	34	38	28	40	20	22	30
% ANIMALS WITH BENIGN TUMOURS	42	28	38	28	32	18	22	30
% ANIMALS WITH MALIGNANT TUMOURS	10	10	12	6	22	10	8	18
% ANIMALS WITH METASTASISING TUMOURS	2		2					

Animals with more than one tumour type are recorded as having multiple tumours.

It should be noted that the summary data for C and HD grps differ from those provided originally. For example, the total number of tumors were reported as 26 and 17 in CM and HDM, respectively, and as 29 and 26 in CF and HDF, respectively, in the original summary table. In the revised table, the total number of tumors were reported as 52 and 33 in CM and HDM, respectively, and as 51 and 46 in CF and HDF, respectively. This discrepancy would appear too large to be simply a result of a re-examination of 10% of C and HD animals. [The original overall incidence table is provided below.]

TABLE 10

Selegiline Hydrochloride
78 Week Dietary Carcinogenicity Study in Mice
Overall Incidence of Tumours : Males and Females

	TUMOUR TABLE			
	MALES		FEMALES	
TREATMENT	Grp 1 0 mg kg-1. day-1	Grp 4 30 mg kg-1. day-1	Grp 1 0 mg kg-1. day-1	Grp 4 30 mg kg-1. day-1
NUMBER OF ANIMALS:	50	50	50	50
NO. OF ANIMALS WITH TUMOURS	23	16	23	21
NO. OF ANIMALS WITH SINGLE TUMOURS	20	15	17	16
NO. OF ANIMALS WITH MULTIPLE TUMOURS	3	1	6	5
NO. OF ANIMALS WITH BENIGN TUMOURS	19	13	16	16
NO. OF ANIMALS WITH MALIGNANT TUMOURS	5	3	11	8
NO. OF ANIMALS WITH METASTASISING TUMOURS	1			
TOTAL NUMBER OF TUMOURS	28	17	29	26
TOTAL NUMBER OF BENIGN TUMOURS	20	14	16	18
TOTAL NUMBER OF MALIGNANT TUMOURS	6	3	13	8
TOTAL NUMBER OF METASTASISING TUMOURS	2			
% ANIMALS WITH TUMOURS	46	32	46	42
% ANIMALS WITH SINGLE TUMOUR	40	30	34	32
% ANIMALS WITH MULTIPLE TUMOURS	6	2	12	10
% ANIMALS WITH BENIGN TUMOURS	38	26	32	30
% ANIMALS WITH MALIGNANT TUMOURS	10	6	22	16
% ANIMALS WITH METASTASISING TUMOURS	2			

4. Study Title: **Selegiline hydrochloride. 104 week dietary carcinogenicity study in rats: Histological evaluation of tissues from the rat carcinogenicity study** [— Project No. 435507/453725. Supplement to NDA 20-647, 4/20/98, Vol #3-4 of 4, Conducting laboratory and location: —. date of study initiation: 4/9/97, GLP.]

Methods: the purpose of this study was to provide microscopic data for the LD and MD grps of the 104-wk oral carcinogenicity study in Sprague-Dawley rats [— Project No. 435507]. In that study, selegline HCl was administered to 50/sex/grp as a drug-diet admixture at doses of 0, 0.7, 3.5, and 17.5 mg/kg/kg. [The doses were selected based on the results of a 13-wk dose-range finding study in which selegiline was administered to Sprague-Dawley rats at doses of 0, 10, 25, 60, and 90 mg/kg. Reduced body wt gain (17-54%) was noted at all doses in males and females. This effect was the sponsor's primary basis for dose-selection.] An additional 20/sex/grp were dosed for a 52-wk interim kill and analysis. Animals were housed 2/cage for the first 2 wks of dosing, and 5/cage thereafter. Diets were prepared fresh every 2 days to the end of Wk 7, then weekly thereafter. Drug concentrations of the drug-diet admixtures were found to be within 20% of intended values; however, the achieved doses were "theoretical" since animals were not housed individually. [Drug concentrations were adjusted for changes in body wt weekly during the first 13 wks, then once every 4 wks thereafter.] Observations in main-study animals consisted of the following: clinical signs, ophthalmology, differential blood count (C, HD during Wks 53, 78, 103), gross pathology, histopathology [in original report, C and HD only: adrenal, abnormal tissue, bladder, bone (sternum), brain (frontal cortex, basal ganglia, parietal cortex, thalamus, cerebellum, medulla oblongata), heart, intestine (ileum, colon), kidney, liver, lung, mammary gland, mesenteric lymph node, esophagus, ovaries, pancreas, pituitary, prostate, skin, spleen, stomach (glandular, non-glandular), submaxillary salivary gland, testes/epididymides, thymus, thyroid/parathyroid, trachea, uterus; kidney, liver, and lung were examined in all animals]. [The following tissues were not examined (unless gross lesions were detected): duodenum, jejunum, cecum, vagina/cervix, eye, fallopian tube, Harderian gland, lacrimal gland, larynx, cervical/mandibular lymph nodes, nasal cavity, optic nerve, sciatic nerve, pharynx, Zymbal gland.]

In the original study submission, microscopic findings in all tissues were provided only for C and HD grps. The present study report provides microscopic data for the LD and MD grps, based on examination of the following tissues [4-6 µm sections stained with H & E]: gross lesions, adrenal, bone (sternum), brain (forebrain, midbrain, cerebellum), GI (esophagus, stomach, ileum, colon), heart, mammary gland, mesenteric lymph node, ovary, pancreas, pituitary, prostate, skin, spleen, submaxillary salivary gland, testis, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus. All tissues for the entire study were peer reviewed; the peer review process included the following: (a) QA of the draft pathology report and pathology materials, (b) examination of all tissues from 10% of males and females in all grps [including C and HD grps], (c) examination of "...any target organs from all animals...[and]...of all neoplasms and hyperplasias". Statistical evaluations were conducted using Fisher's exact test (two-tailed).

Results [included are critical data from the original review that were not provided in this report].

Mortality: there were 110 unscheduled deaths, none of which were attributed to drug by the sponsor. The incidences per grp, clearly not dose-related, are summarized in the following sponsor's text table:

	Group/Dose Level (mg Selegiline Hydrochloride.kg^{-1}.day^{-1})			
Sex	1 (0)	2 (0.7)	3 (3.5)	4 (17.5)
♂	15/50	18/50	15/50	11/50
♀	12/50	14/50	14/50	11/50

Clinical signs: according to the sponsor, the only drug-related sign was an increase in excitability upon handling in "the majority" of HD animals; this behavior was observed only during the first 52 wks of dosing. In addition, there was a reduced incidence of palpable masses in HDM.

Body wt: overall, body wt gain was significantly reduced in MD and HD animals, in males by 13 and 30%, respectively, and in females by 23 and 37%, respectively. Final body wts in reduced by 10 and 22% in MDM and HDM, respectively, compared to CM, and by 16 and 28% in MDF and HDF, respectively, compared to CF. Changes were evident by Wks 24-28 at the MD and by Wks 1-2 at the HD. Body wt effects were illustrated in the following sponsor's Figures 3-4:

FIGURE 3

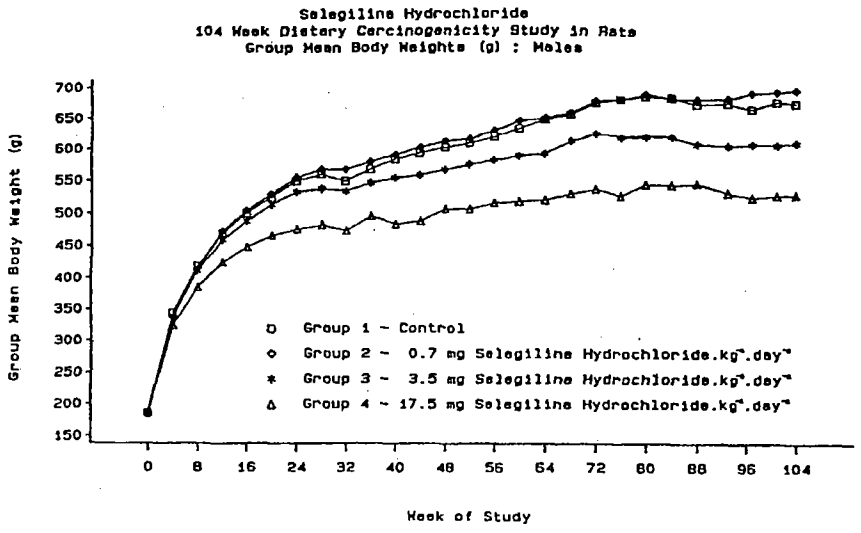
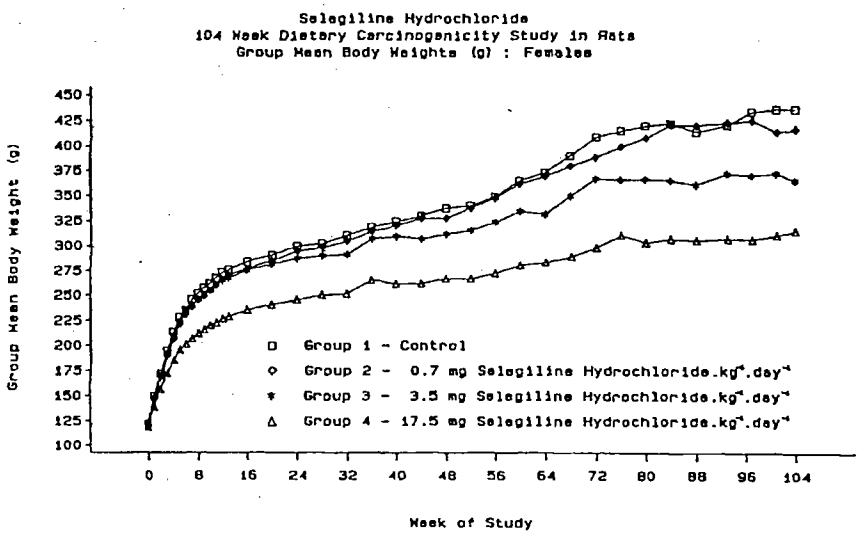


FIGURE 4



Food consumption: food consumption was reduced at the HD [14 and 7% in M and F, respectively] compared to Cs. It should be noted, however, that food intake was determined based on "cage consumption" and did not represent individual intakes. [Animals were housed 2/cage for the first 2 wks, then 5/cage thereafter by sex.]

Water consumption: according to the sponsor, no drug-related effects were noted.

Differential blood counts: in males, the % neutrophils was increased during Wks 78 and 103 at the HD (14-16%). According to the sponsor, 2/50 CM and 7/50 HDM were considered to exhibit "...a degree of neutrophilic leucocytosis; the criteria for this determination was not specified".

In females, an increase in the % neutrophils (23%) and a small decrease in the % lymphocytes (13%) was noted at the HD during Wk 78 only. According to the sponsor, neutrophilic leucocytosis was evident in 1/50 HDF.

Gross pathology: selected gross pathology findings are provided in the following table [the sponsor did not provide the total no. of animals examined per grp per tissue in the summary table]:

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
seminal vesicles	small horns	4	6	6	15				
subcutis	masses	10	13	11	4	14	13	16	11
dermal	masses	1	8	10	5	0	4	3	2
lumbar lymph nodes	enlarged	0	1	2	6	1	0	3	2
adrenal	enlarged	no effect				2	8	9	13
spleen	enlarged	4	4	1	8	no effect			
liver	pale	2	2	3	5	no effect			
kidney	pale	1	0	0	4	no effect			
lungs	pale	4	1	4	8	4	1	5	11

Histopathology: selected non-tumor findings are summarized in the table below. The sponsor did not appear to consider any non-tumor findings to be clearly drug-related.

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
liver	vacuolation	8/49	10/49	4/49	1/49*	12/50	6/50	6/50	2/50**
	Kupffer cell proliferation	0/49	0/48	0/49	1/50	0/50	0/50	0/50	1/50
kidney	↑ pigment deposition	5/49	2/45	4/47	3/47	6/50	7/48	6/47	15/50*
	pyelitis	8/49	4/45	18/47*	14/47	2/50	3/48	5/47	8/50
	mineral deposits	5/49	4/45	1/47	2/47	23/50	19/48	34/47*	20/50
spleen	↑ hemosiderin	2/49	7/47	9/48*	2/49	11/50	18/48	10/49	9/50
adrenal	cortical atrophy	1/49	0/46	0/47	0/48	4/50	0/47	2/50	8/50
seminal vesicles	cyst(s)	1/7	3/7	7/9*	8/16				
	scant secretion	2/7	2/7	4/9	14/16*				
uterus	inflammation					1/49	3/49	2/50	7/50
	stromal sclerosis					12/49	13/49	22/50	18/50
thyroid	c-cell hyperplasia	11/49	11/46	10/45	10/47	24/49	15/47	12/46*	10/48*
	dilated/cystic follicles	10/49	13/46	12/45	14/47	39/49	31/47	28/46	27/48*
thymus	atrophy	37/47	30/44	27/42	35/45	31/45	27/46	20/46*	23/49
ovary	absence of recent CL					11/49	12/50	25/50**	17/50

Tumor findings were not summarized in a separate table. Therefore, they are summarized in the table below. The sponsor didn't consider any of the findings drug-related.

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
liver	cholangiocarcinoma	0/49	0/48	0/49	1/50	0/50	0/50	0/50	0/50
	hepatocellular carcinoma	0/49	3/48	0/49	0/50	0/50	0/50	0/50	0/50
	hepatocellular adenoma	0/49	1/48	1/48	1/50	1/50	1/50	0/50	0/50
heart	solitary sarcoma	0/50	0/50	0/50	1/49	0/50	0/49	0/49	0/50
kidney	metastasising solitary cortical tubular adenocarcioma	0/49	0/45	0/47	1/47	0/50	0/48	0/47	0/50
lung	soltary alveolar/bronchiolar carcinoma	0/49	0/48	1/50	0/50	0/50	0/49	0/49	0/50
	solitary alveolar/bronchiolar adenoma	0/49	0/48	0/50	0/50	1/50	0/49	0/49	0/50
adrenal	unilateral pheochromocytoma [M]	0/49	0/46	0/47	1/48	0/50	0/47	0/50	0/50
	unilateral pheochromocytoma [B]	10/49	13/46	5/47	5/48	4/50	2/47	0/50	1/50
	bilateral pheochromocytoma [B]	3/49	4/46	3/47	2/48	0/50	0/47	2/50	0/50
	bilateral cortical adenoma	0/49	0/46	0/47	0/48	0/50	1/47	0/50	0/50
	unilateral cortical adenoma	2/49	0/46	0/47	1/48	1/50	3/47	1/50	0/50
thymus	thyoma	0/47	1/44	1/42	0/45	0/45	0/46	0/46	1/49
testis	bilateral interstitial cell tumor [B]	2/50	2/49	1/50	1/50				
	unilateral interstitial cell tumor [B]	7/50	5/49	3/50	5/50				
seminal vesicle	adenoma	0/7	0/7	1/9	0/16				
ovary	unilateral granulosa/thecal cell tumor(s) [M]					0/49	1/50	0/50	0/50
	unilateral granulosa/thecal cell tumor(s) [B]					0/49	0/50	1/50	0/50
uterus	stromal sarcoma					1/49	1/49	0/50	2/50
	bilateral polyp					0/49	0/49	1/50	0/50
	polyp					8/49	7/49	3/50	4/50
brain	glioma	1/50	2/49	1/50	1/50	1/50	0/50	2/50	0/50
	reticulosis [M]	0/50	0/49	1/50	1/50	0/50	0/50	0/50	1/50
	solitary granular cell tumors(s) [B]	1/50	1/49	0/50	0/50	0/50	0/50	0/50	0/50
pancreas	islet adenocarcinoma	0/49	0/49	0/47	0/50	0/49	0/47	1/48	0/49
	islet cell adenoma	3/49	5/45	2/47	2/49	2/49	1/47	1/48	0/49
	exocrine adenoma	1/49	0/45	1/47	0/49	0/49	0/47	0/48	0/49

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
pituitary gland	locally invasive solitary adenocarcinoma	3/49	0/50	0/49	1/49	4/50	6/50	8/49	2/49
	solitary adenoma	3/49	7/50	9/49	1/49	13/50	18/50	22/49	15/49
	solitary adenoma, anterior lobe	30/49	29/50	20/49	23/49	25/50	16/50	12/49*	17/49
	solitary adenoma, intermediate lobe	2/49	1/50	0/49	1/49	0/50	0/50	0/49	0/49
skin/subcutis	solitary schwannoma	1/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50
	undifferentiated carcinoma	0/50	1/50	0/50	0/50	0/50	0/50	0/50	0/50
	solitary basal-cell carcinoma	1/50	1/50	0/50	0/50	0/50	0/50	0/50	0/50
	solitary undifferentiated sarcoma	0/50	0/50	1/50	1/50	0/50	1/50	0/50	0/50
	solitary fibrosarcoma	0/50	1/50	0/50	1/50	1/50	0/50	0/50	1/50
	intracutaneous cornifying epithelioma [B]	8/50	11/50	5/50	1/50*	0/50	3/50	1/50	0/50
	papilloma	0/50	1/50	2/50	0/50	0/50	0/50	0/50	0/50
	basal cell tumor [B]	0/50	1/50	1/50	0/50	0/50	0/50	0/50	0/50
	fibroma	5/50	4/50	6/50	1/50	1/50	2/50	0/50	1/50
	subcutaneous lipoma	3/50	1/50	4/50	2/50	0/50	1/50	1/50	0/50
	solitary myxoma [B]	0/50	1/50	0/50	0/50	0/50	0/50	0/50	0/50
	solitary sebaceous adenoma	0/50	0/50	1/50	0/50	0/50	0/50	0/50	0/50
mammary gland	adenocarcinoma	0/49	0/47	0/46	0/46	2/50	5/50	3/50	1/50
	fibroadenocarcinoma	0/49	0/47	0/46	0/46	4/50	6/50	9/50	2/50
	adeno/fibro sarcoma	0/49	0/47	0/46	0/46	0/50	0/50	0/50	1/50
	adenoma	0/49	0/47	0/46	1/46	0/50	0/50	0/50	1/50
	fibroadenoma	1/49	2/47	0/46	0/46	9/50	8/50	12/50	6/50
thyroid	follicular carcinoma	1/49	1/46	0/45	0/47	0/49	0/47	0/46	0/48
	c-cell carcinoma	2/49	1/46	2/45	1/47	0/49	0/47	0/46	0/48
	follicular adenoma	2/49	1/46	1/45	1/47	2/49	0/47	1/46	0/48
	c-cell adenoma	2/49	8/46*	4/45	1/47	2/49	4/47	1/46	4/48
parathyroid	adenoma	3/48	0/47	1/46	4/46	0/44	0/45	0/45	1/49
duodenum	solitary leiomyosarcoma	--	--	--	--	1/1	--	--	--
bone	metastasising solitary osteosarcoma	--	--	--	1/1	--	--	--	--
Zymbal's gland	solitary squamous-cell carcinoma	1/1	--	--	--	--	--	--	--
lymphoretic/hem opoietic tissue	histiocytic sarcoma	--	0/1	4/4	1/1	0/1	--	--	0/2
	lymphoma	--	1/1	0/4	0/1	1/1	--	--	1/2
vascular system	hemangiosarcoma	0/2	0/2	2/3	0/1	2/2	--	--	0/1
	hemangioma	1/2	0/2	0/3	1/1	0/2	--	--	0/1
head	ameloblastoma [M]	--	--	1/1	--	--	--	--	--

*p<0.05

The overall incidences of tumors are provided in the following sponsor's Table 2:

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TABLE 2

Selegiline Hydrochloride
104 Week Carcinogenicity Study in Rats
Histological Evaluation of Tissues from the Rat Carcinogenicity Study
[redacted] Project No. 435507
Overall Incidence of Tumours: Males and Females

	TUMOUR TABLE							
	MALES				FEMALES			
TREATMENT	Grp 1 0 mg kg-1. day-1	Grp 2 0.7 mg kg-1. day-1	Grp 3 3.5 mg kg-1. day-1	Grp 4 17.5mg kg-1. day-1	Grp 1 0 mg kg-1. day-1	Grp 2 0.7 mg kg-1. day-1	Grp 3 3.5 mg kg-1. day-1	Grp 4 17.5mg kg-1. day-1
NUMBER OF ANIMALS:	50	50	50	50	50	50	50	50
NO. OF ANIMALS WITH TUMOURS	44	47	44	40	45	44	44	40
NO. OF ANIMALS WITH SINGLE TUMOURS	14	14	13	21	17	16	17	26
NO. OF ANIMALS WITH MULTIPLE TUMOURS	30	33	31	19	28	28	27	14
NO. OF ANIMALS WITH BENIGN TUMOURS	43	46	40	36	41	41	40	38
NO. OF ANIMALS WITH MALIGNANT TUMOURS	10	10	13	12	15	16	18	9
NO. OF ANIMALS WITH METASTASISING TUMOURS	1		3	3		1	1	1
TOTAL NUMBER OF TUMOURS	99	110	85	67	87	87	85	62
TOTAL NUMBER OF BENIGN TUMOURS	89	99	72	55	69	67	59	51
TOTAL NUMBER OF MALIGNANT TUMOURS	10	11	13	12	18	20	26	11
TOTAL NUMBER OF METASTASISING TUMOURS	1		3	3		1	1	1

	TUMOUR TABLE							
	MALES				FEMALES			
TREATMENT	Grp 1 0 mg kg-1. day-1	Grp 2 0.7 mg kg-1. day-1	Grp 3 3.5 mg kg-1. day-1	Grp 4 17.5mg kg-1. day-1	Grp 1 0 mg kg-1. day-1	Grp 2 0.7 mg kg-1. day-1	Grp 3 3.5 mg kg-1. day-1	Grp 4 17.5mg kg-1. day-1
% ANIMALS WITH TUMOURS	88	94	88	80	90	88	88	80
% ANIMALS WITH SINGLE TUMOUR	28	28	26	42	34	32	34	52
% ANIMALS WITH MULTIPLE TUMOURS	60	66	62	38	56	56	54	28
% ANIMALS WITH BENIGN TUMOURS	86	92	80	72	82	82	80	76
% ANIMALS WITH MALIGNANT TUMOURS	20	20	26	24	30	32	36	18
% ANIMALS WITH METASTASISING TUMOURS	2		6	6		2	2	2

Carcinogenicity summary and conclusions: the sponsor conducted carcinogenicity studies in mouse (78-wk dietary study) and rat (104-wk dietary study). These studies were submitted in the original NDA for Eldepryl capsules (NDA 20-647, Somerset Pharmaceuticals Inc.). Additional data [i.e., histopathology for LD and MD grps] for both studies were submitted in a supplement to NDA 20-647. The sponsor is relying on these oral carcinogenicity studies to support the transdermal formulation.

Mouse carcinogenicity study: selegiline was administered in the diet to CD-1 mice (50/sex/grp) at doses of 0, 3, 10, and 30 mg/kg for 78 wks. The survival rate was high in all grps [76-90%], with no evidence of a dose-related effect. There were only 67 deaths [5-12/grp] during the study, probably due (at least in part) to the relatively short duration of the study. No drug-related clinical signs were evident. Mean body wt was reduced in MDM (8%), HDM (16%), LDF (7%), MDF (9%), and HDF (18%), compared to C grps. Drug-effects on body wt were evident from Wk 68 on at the LD, from Wk 44 on at the MD, and from Wk 8 on at the HD. These body wt effects were not accompanied by documented decreases in food consumption, suggesting a decreased efficacy of energy conversion at these doses. No gross lesions were considered drug-related by the sponsor [no summary tables were provided]. A complete battery of tissues was not examined microscopically. Notably, parts of the GI tract (duodenum, jejunum, cecum, rectum), eye, nasal cavity, peripheral nerve, seminal vesicles, skeletal muscle, and spinal cord) were not examined

microscopically (except when a gross lesion was detected). In those examined, the only non-tumor finding considered drug-related by the sponsor was a decrease in hepatocellular vacuolation in MDM and HDM. This was attributed to the reduced body wt (relative to CM) observed in these grps; however, similar reductions in body wt (relative to C) were observed in females, with no similar microscopic finding. Other non-tumor microscopic findings of note include the following: (a) a slight increase in severity of adrenal subcapsular hyperplasia in HDM and HDF (although the most severe effects were noted in 1/50 CF and 1/49 HDF), (b) a non-dose related increase in hemorrhagic hepatocyte degeneration in treated females, (c) an increase in lung congestion in MDM, (d) an increase in severity of mammary gland secretion in HDF, (e) a decrease in the incidence of thymic atrophy in MD and HD males, and at all doses in females, (f) pancreatic focal acinar cell atrophy only in 2/50 HDF, (g) a non-dose related increase in the severity of testicular tubular atrophy (particularly MDM), (h) a slight increase in renal mineral deposits in HDM, and (i) an increase in urinary bladder calculus in LDM and HDM [significant only at the HD] and an increase in epithelial hyperplasia in HDF. There were no drug-related increases in individual tumor findings, as analyzed by the sponsor and by FDA [cf. Statistical Review and Evaluation, Roswitha E. Kelly, Mathematical Statistician, HFD-710, 5/20/96] using only the C and HD data and by the sponsor taking into account the data for all grps. The original summary of overall tumor incidences indicated a decrease in tumors and affected animals at the HD [particularly evident in HDM]. In the revised summary of overall tumor incidences, the number of tumors and affected animals tended to be lower in treated grps; however, the incidences were not clearly dose-related. [As noted previously, the summary data for the C and HD grps differed substantially from those originally provided; the reason for the apparent discrepancy is unknown.]

In reviewing the original study report, there was concern that there may have been reduced sensitivity to tumor development due to the decrease in body wt in HDM and HDF relative to controls. Numerous published studies have demonstrated inhibition of spontaneous and chemically-induced tumor formation resulting from caloric restriction. Whether or not mechanisms underlying this effect were relevant to selegiline was uncertain since food intake was apparently not affected in selegiline-treated grps. [From literature reports, it is also unclear whether or not the magnitude of the body wt effect was great enough to result in inhibition of chemically-induced tumors.] However, the magnitude of the body wt effect at the HD and the tendency for tumor incidence to be reduced in HD grps suggested a possible decrease in assay sensitivity. Therefore, the sponsor was asked to provide microscopic data for the lower dose grps. The effect on body wt (the only sign of toxicity) was not excessive in MD grps, and there were no apparent increases in tumors in MD grps. [Electronic datasets have not been provided by the sponsor; therefore, the full dataset has not been statistically analyzed by FDA.]

The oral carcinogenicity was originally submitted to support oral selegiline for Parkinson's disease. The current application is for transdermal selegiline in patients with depression. In terms of overall acceptability, the deficiencies in the mouse study [i.e., relative short duration, lack of a complete battery of tissue examined microscopically] are of somewhat greater concern considering the relatively younger age of the patients and the available treatments. In terms of the difference in route (i.e., oral vs transdermal), the sponsor did not provide a bridging TK study in mice by which to estimate plasma exposure to selegiline in the dietary study. Since plasma exposure to selegiline in humans is markedly higher following transdermal application (compared to oral dosing), it is important to determine whether or not the mouse study provides a reasonable safety margin. Concern regarding these deficiencies is heightened by the positive genotoxicity findings [i.e., *in vitro* mouse lymphoma assay]. These issues cannot be entirely resolved if the sponsor provides adequate TK data; therefore, the sponsor should conduct additional assessment of carcinogenic potential in mouse. The sponsor may conduct either a 2-yr bioassay in mouse or an alternative assay (e.g., p53, neonatal mouse, TG.AC). [Please see Section IX, Appendix: Executive CAC meeting minutes; meeting held 1/8/02.] Justification should be provided for the assay selected and dosing regimen should be relevant to the transdermal formulation. It is this reviewer's opinion that the study should be conducted prior to approval; however, the sponsor has

previously been informed that such a study [the TG.AC assay was recommended] could be conducted Phase 4 [Division letter, 12/7/99].

Rat carcinogenicity study: selegiline was administered in the diet to Sprague-Dawley rats (50/sex/grp) at doses of 0, 0.7, 3.5, and 17.5 mg/kg. [Animals were housed 5/cage; therefore, individual food intake and achieved doses could not be accurately determined.] There were 110 unscheduled deaths during the study. The survival rate was high in all grps [64-78%], with no evidence of a clear dose-related effect. The only drug-related clinical signs were excitability upon handling in HD animals (observed only during the first 52 wks) and a decrease in the incidence of palpable masses in HDM. Mean body wt was reduced (compared to C grps) in MD and HD animals [males: 10 and 22%, respectively; females: 16 and 28%, respectively]. Body wt effects were evident by Wks 24-28 at the MD and by Wks 1-2 at the HD. "Cage" food intake was reduced at the HD in males (14%) and females (7%). There were no drug-related ophthalmology findings, according to the sponsor. An increase in the % neutrophils was evident in HDM (Wks 78, 103) and HDF (Wk 78). The following were noted at necropsy: (a) an increase in small horns in seminal vesicles in HDM, (b) a decrease in subcutaneous masses in HDM, (c) an increase in enlarged lumbar lymph nodes and spleen in HDM, (d) an increase in enlarged adrenals at all doses in females, an increases in pale liver, lung, and kidney in HDM and in pale lung in HDF. The sponsor didn't consider any of these drug-related. As in the mouse study, a complete battery of tissues was not examined (similar tissues were excluded). In those examined, non-tumor microscopic findings consisted of the following: (a) the incidence of liver vacuolation was reduced in a dose-related manner in males and females. This finding is consistent with the reduced body wt observed at the MD and HD. (b) increased incidence of pigment deposition in kidney in HDF; the nature of the pigment was not investigated, (c) increased renal pyelitis (i.e., inflammation of the renal pelvis) in MDM, HDM, and HDF. (d) increased mineral deposition in the kidney in MDF, (e) increased hemosiderin deposition in the spleen in MDM, (f) increased incidence of scant secretions in seminal vesicles in HDM, and to a lesser extent in MDM. (g) increased incidence of uterine inflammation in HDF, (h) reduced c-cell hyperplasia and dilated/cystic follicles in MDF and HDF. These findings may be associated with reduced body wt; however, they were not noted in males. The sponsor indicated that the incidences in treated grps were within historical control, whereas the incidence in CF was "...well outside" the HC range. (i) the incidence of thymic atrophy was reduced in MDF, and (j) absence of recent CL was increased in MDF, and to a lesser extent in HDF. There were no increases in the incidence of any tumor in selegiline-treated animals, as analyzed by the sponsor and by FDA [cf. Statistical Review and Evaluation, Roswitha E. Kelly, Mathematical Statistician, HFD-710, 5/20/96] using only the C and HD data and by the sponsor taking into account the data for all grps. There was a dose-related decrease in the incidence of intracutaneous cornifying epithelioma in males (MD, HD), which probably contributed to the decrease in palpable masses reported in HDM. The overall incidences of animals with multiple tumors and the total number of tumors were decreased at the HD. These parameters were not notably affected at the LD or MD; however, the total number of benign tumors was reduced at the MD in males and females.

As with the mouse study, there was concern that there may have been reduced sensitivity to tumor development due to the decrease in body wt observed in selegiline-treated animals. [No other dose-limiting toxicity was observed.] In the rat, the MD and HD were affected. The magnitude of the body wt effect at the HD clearly exceeded the ≈10% relative decrease in body wt considered acceptable in carcinogenicity studies. The body wt effect at the MD was sufficient to consider the MD an MTD, and there are apparently no increases in tumors at this dose in either males or females. [As noted, the FDA has not verified the statistical analyses performed by the sponsor on the full dataset.] Therefore, in terms of overall acceptability, one deficiency in the study is the lack of microscopic examination of a complete battery of tissues. Regarding the relevance of the oral study in assessing the carcinogenic potential of a transdermal formulation, the sponsor conducted a bridging TK study in rats in order to provide data by which plasma exposure to selegiline and metabolites in the oral study could be estimated. Estimates of plasma drug exposure in the carcinogenicity studies were considered of particular importance since mean

plasma levels of selegiline in humans following transdermal application are almost 7 times higher than those following oral dosing [$AUC_{(0\text{-}24\ hr)}$ = 4365.11 pg•hr/mL (%CV = 123) for oral, 29425.87 pg•hr/mL (%CV = 66) for transdermal]. Alternatively, plasma levels of the major circulating metabolites (in humans and animals), N-desmethylselegiline, L-amphetamine, and L-methamphetamine are 6, 9, and 8 times higher following oral dosing compared to transdermal application.

The TK bridging study was conducted reproducing the original methodology as closely as possible. According to the sponsor, there were two differences in methodology between the 2-yr dietary carcinogenicity and the 14-day dietary TK study: (a) a different strain of rat (the original strain was no longer available) and (b) a different diet. One additional difference was that in the 2-yr study animals were housed 5/grp (by sex) throughout most of the dosing period, whereas in the bridging study animals were housed individually. In the bridging study, selegiline was administered to Sprague-Dawley rats (65/sex) as a drug-diet admixture at the HD used in the 2-yr study [i.e., 17.5 mg/kg/day] for 15 consecutive days. [The $t_{1/2}$ of selegiline in Sprague-Dawley rat following transdermal and i.v. 24-hr infusion dosing is 3-5 hrs; therefore, measurements taken after 2-wks of dosing should reflect "steady-state".] Blood samples were collected from 5/sex/time point on Days 15-16. Plasma levels of selegiline and metabolites, N-desmethylselegiline, amphetamine, and methamphetamine, were quantitated in plasma. Plasma levels of all metabolites were higher than plasma selegiline levels, with amphetamine being the major circulating material [14-fold higher than selegiline] in males and methamphetamine being the major circulating material [10-fold higher than selegiline] in females. The mean AUC for selegiline was >4-fold higher in females than in males. The plasma AUCs for selegiline in male and female rats were ≈1 and 4 times, respectively, the mean AUC in humans at the proposed daily dose. The major factor affecting the validity of these estimates is the difference in housing between the 2-yr and the TK studies in rat, with the primary effect probably being the variability in plasma exposure (i.e., greater in the 2-yr study). One other factor to consider is that plasma exposure at the lower doses was not quantitated in the bridging study. Therefore, there are no data on the plasma exposure achieved at the "MTD". Assuming linearity, the plasma AUCs for selegiline in males and females at the MD could be estimated as 5.8 ng•hr/mL in males and 25.2 ng•hr/mL in females. These values are 0.2 and ≈1 times the AUC in humans at the proposed clinical dose.

Therefore, the primary deficiencies in the 2-yr rat study are the lack of a complete histopathology battery and no safety margin (based on plasma AUC for selegiline). In addition, as noted previously, there is additional concern regarding the carcinogenic potential of selegiline based on the positive genotoxicity findings (i.e., *in vitro* mouse lymphoma assay). The 2-yr rat study does, however, provide some value in that the MD provided a reasonable assessment of carcinogen potential for circulating metabolites and, at least in female rat, for selegiline. A repeat oral study would probably be of little value due to the dose-limiting body wt effects already observed, except that a complete battery of tissues could be microscopically evaluated. A repeat study could be conducted using a different route of administration; however, there is some question as to the feasibility of conducting a transdermal study (e.g., problems with maintaining patch placement). The results of the 6-mo study do suggest that higher plasma levels of selegiline could be achieved with transdermal application, without accompanying drug-related body wt effects. [At a dose of 120 mg/kg/day, there were no drug-related deaths and mean body wt at the end of the 6-mo dosing period was only 9% lower than placebo controls and was only transiently affected at 60 mg/kg.] However, body wt in placebo controls were 27% lower than in untreated controls. Plasma AUCs at 30, 60, and 120 mg/kg td were 13, 33, and 70 times the plasma AUC for selegiline in humans at the proposed clinical dose.

Considering the potential difficulty in conducting a 2-yr transdermal study, it would seem that a reasonable approach would be for the sponsor to first conduct a carcinogenicity (2-yr or alternative) study in mouse (as discussed previously). Based on the results of that study, the issue of whether or not additional assessment of carcinogenic potential is needed in rat could be reconsidered.

Additional comments: the sponsor did not provide the data for either carcinogenicity study in a format that would allow for an independent review. The sponsor has been asked to provide datasets in the format described in the draft guidance, Draft Guidance for Industry -- Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (Division of Biometrics; CDER; May 2001); the sponsor has committed to providing the datasets [Telecon, 1/30/02]. A final decision as to the need for additional assessment of carcinogenic potential will be made following a complete analysis of these data. However, if the sponsor decides to pursue higher clinical doses of selegiline STS, the resulting higher plasma exposure to selegiline, in particular, would not be adequately covered by the existing (oral) carcinogenicity study in rat. No TK bridging study was conducted in mouse, therefore, there are no data upon which to base an estimate of the plasma exposures achieved in the oral mouse study. One additional factor is the potential for tumorigenic effects at the application site, an effect not assessed in the oral studies. There was not consistent evidence of drug-related changes at the application site. A dose-related increase in epidermal hyperplasia was observed in the 6-mo toxicity study in rat, but in the 9-mo dog study, no clear drug-related effects were observed at the application site. Regardless of the potential for a drug effect, the patch itself was consistently found to be irritating in both rat and dog. Therefore, the possibility of local effects should be considered when determining the need for additional carcinogenicity assessment.

Labeling Recommendations: none

Addendum/appendix listing:
Exe-CAC report

VI. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

1.Study Type: a non-GLP feasibility study to determine the logistics of conducting a transdermal reproduction study.
Study Title: **Selegiline transdermal system: a non-regulated rat feasibility study, final letter report** (Somerset Study No: TOX-536-98B, Vol #1.031, Conducting laboratory and location: [redacted], date of study initiation: 6/24/98, GLP, QA)

Methods: this study was conducted in male and female Sprague-Dawley rats. Selegiline was not administered. Grp 1 (10/sex/grp) was untreated; Grp 2 (20/sex/grp) received placebo patches, 2 plus wrappings [shoulder harness secured with self-adhesive tape placed on shaved skin] per 24-hr period. [Patches were replaced daily.] Grp 2 animals received placebo patches for 2 wks prior to mating and during mating. On Day 5 of the mating period, the patches were removed from the females overnight. Males were treated for 1 wk following confirmed pregnancy in the female, then sacrificed with no examination. Females received the patches through gestation and lactation, except for the period between Gestation Day 22 and Day 0 of lactation. On Day 1 of lactation, modified placebo patches were applied ["...a small slit was cut into the ventral side of the wrapping...to facilitate nesting and nursing"]. Observations in the F_0 generation consisted of mortality, clinical signs, body wts, and "An abbreviated gross necropsy" in any animal that died or was sacrificed prior to study termination and on non-pregnant females.

Results: no notable clinical signs were observed. An initial mean body wt loss was noted in males and females receiving the placebo patch [-41 gm in M, -34 gm in F]; mean body wt loss was also noted in males during Wk 2. During Wk 3, mean body wt gain was noted in both males and females [30-31 gm]. Presence of the placebo patches resulted in a delay in mating. In untreated Cs, all females had mated during the first 4 days of the mating period, whereas only 20% of placebo-treated females had mated. However, removal of the patches overnight overcame this effect and by the end of the mating period copulation and fertility indices were "within normal limits" in both grps.

Mean body wt in placebo-treated females was lower compared to untreated females by the end of the first wk of gestation. However, mean body wt gain was not affected thereafter. Gestation length and pup survival were unaffected. Pup body wt and body wt gain were reduced in placebo-treated dams during the lactation period (from Day 4 on). At the end of the lactation period, mean pup body wt was 27% lower in the placebo grp as compared to the untreated grp.

The sponsor concluded that, since the effect on pup body wt/body wt gain could adversely impact on developmental parameters, "Appropriate placebo and untreated control groups should be used, and the time lines for the tests modified, if needed, to accommodate the growth pattern seen in placebo treated animals".

2.Study Type: Segment I (mating and fertility study)
Study Title: **Selegiline transdermal system: a reproduction and fertility (Segment I) study in Sprague-Dawley rats** (Somerset Study No: TOX-539-98B, Vol #1.031, Conducting laboratory and location: [redacted] date of study initiation: 3/1/99, GLP, QA)

Methods
Dosing
species/strain: Sprague-Dawley rat [redacted]
#/sex/group or time point: 25/sex/grp
age: ≈8 wks for males and ≈10 wks for females
weight: 186-282 gm for males, 175-235 gm for females

satellite groups used for toxicokinetics or recovery:
dosage groups in administered units: 0 (placebo), 0 (untreated), 10, 30, 75 mg/kg.
route, form, volume, and infusion rate: transdermal

Drug, lot#, and % purity: STS, lots 26E007D and 26E007N, purity of drug substance

Formulation/vehicle: 20 cm^2 20 mg/unit transdermal patch [patches were cut to provide accurate dose to nearest 2.5 mg]. Patches were held in place with binding "...wrapped around the thoracoabdominal area with a shoulder strap and secured with athletic tape". Skin was clipped, but the application area was not abraded. Patch placement was checked twice daily.

Duration of dosing: patches were applied daily in males from 4 wks prior to mating, during cohabitation ("for a minimum of 6 hours daily"), and until sacrificed [i.e., ≈70 days]. Patches were applied daily in females from 2 wks prior to mating, during cohabitation (for at least 6 hrs/day), and until gestation Day 6. [The mating period was a maximum of 14 days in duration.] Patches were removed "...during the evening hours of the cohabitation period until positive evidence of copulation was observed in order to avoid disturbing the mating process". [Day 0 of gestation = day copulation was confirmed (copulatory plug in vagina or sperm positive vaginal smear)]. Females with no evidence of copulation were dosed for 6 days following removal from males, then sacrificed 15 days following removal.

Dose Justification: doses based on results of a feasibility study and a dose-range finding Segment II study in rats.

Observations and times:

Clinical signs: animals were examined daily; "detailed clinical observations" were performed weekly. Application sites were examined twice weekly in males and at sacrifice. In females, application sites were examined twice weekly until evidence of mating, and on Gestation Days 0, 3, 7, 12, and 15. Observations were scored according to the "Macroscopic Dermal Grading System". Vaginal smears were examined daily to determine estrus stage, beginning 10 days prior to start of dosing and continuing until evidence of copulation was obtained or until the end of the mating period. [Estrus data were not summarized or discussed.]

Body weights: in males, body wts were recorded weekly during the study and at scheduled sacrifice. In females, body wts were recorded until evidence of mating was obtained, then on Gestation Days 0, 3, 7, 12, and 15.

Food consumption: food consumption was recorded on the same days body wts were recorded, except that food intake was not measured during the mating period. Data were expressed as "gm/animal/day".

Ophthalmoscopy: n/a

ECG: n/a

Hematology: n/a

Clinical chemistry: n/a

Urinalysis: n/a

Gross pathology: a necropsy was performed on all F_0 animals [males: Days 71-75; females: Gestation Day 15]. In males, testes [Bouin's fixative], and internal gross lesions, epididymides, prostate, and seminal vesicles [10% neutral buffered formalin] were retained for possible future microscopic examination. Sperm ct, motility, and morphology were evaluated.

In females, the uterus, ovaries, and gross lesions [10% neutral buffered formalin] were retained for possible future microscopic examination. Uteri from pregnant females were examined; the following parameters were recorded: no. of live/dead fetuses, early resorptions, no. of corpora lutea. Uteri from apparently non-pregnant females were prepared for examination of embryolethality [10% aqueous ammonium sulfide].

Organ weights: the following organs were weighed in males: brain, left cauda epididymis,

prostate, testes, epididymides, seminal vesicles. No organs were weighed in females.

Histopathology: n/a

Toxicokinetics: n/a

Results

Clinical signs: there were no drug-related deaths; one female was sacrificed on Day 15 postmating due to the lack of evidence that mating had occurred. Selected clinical signs are summarized in the following table [data expressed as "no. occurrences/no. affected animals]:

SIGN	MALES					FEMALES				
	CP	UC	LD	MD	HD	CP	UC	LD	MD	HD
scab-left forelimb	1/1	--	4/2	5/2	12/6	1/1	--	1/1	0/0	22/6
scab-right forelimb	0/0	--	6/2	2/1	3/3	0/0	--	0/0	4/2	21/6
scab-abdominal area	19/10	--	9/6	15/8	23/10	0/0	--	8/1	3/1	11/6
hairloss	22/11	5/1	65/14	23/7	46/15	17/4	19/4	96/8	78/10	158/12
urine stain	1/1	0/0	0/0	1/1	1/1	4/1	0/0	0/0	2/1	14/6
dk material around nose	9/5	2/1	9/6	32/12	39/14	12/6	5/3	19/7	11/9	32/14
dk material around mouth	0/0	0/0	0/0	0/0	5/3	0/0	0/0	0/0	0/0	1/1
scab-nose	0/0	0/0	0/0	1/1	5/3	2/1	0/0	0/0	21/6	8/2
patch found off										
0.125	3/3		48/15	20/10	10/9	1/1		5/4	6/5	1/1
0.25	1/1	--	7/5	21/8	8/6	2/2	--	0/0	9/6	6/6
0.5	12/7		0/0	30/15	8/5	22/10		0/0	0/0	15/10
whole patch found off	109/21	1/1*	0/0	0/0	102/23	3/2	--	0/0	0/0	1/1
patch(es) replaced	57/20	--	21/13	25/12	42/15	4/4	--	0/0	3/3	10/5

*the untreated control was not to have received clipping, dosing, or wrapping; there was also no listing for this observation in the individual line listings. It is unclear to what this refers.

An examination of individual data indicated that the number of occurrences of ≥0.5 of the patch found to be off or damaged was none or low in individual females; therefore, dosing was probably not seriously compromised in females. In males, individual LD and MD animals were not notably affected; however, at the HD, ≥0.5 of the patch was found to be off or damaged on >10% of the dosing days in 3/25 animals. In HDM #42, 22/75 (29%) days were affected.

Signs of dermal irritation (i.e., erythema, edema, desquamation, eschar) were observed in C and treated grps, with no notable difference in incidence or severity.

Body weights: mean body wt was not affected in males when treated grps were compared to CP; however, mean body wt in CP was lower (15-27%) than in CU throughout the dosing period.

In females (as in males), mean body wt was lower in CP (6-10%) than in CU throughout the pre-mating period. In addition, mean body wt was reduced in MDF and HDF. In MDF, mean body wt was reduced on pre-mating Day 22, by 4% compared to CP and by 13% compared to CU. In HDF, mean body wt was reduced on Days 15 and 22, by 6-5% compared to CP and by 16-14% compared to UC. Mean body wt gain was increased in UC (compared to CP) on premating Days 1-8 and 8-15; mean body wt loss was observed in MDF and HDF during Days 8-15 compared to CP and UC, with the effect being dose-related [statistically significant only at the HD]. During Days 15-22, mean body wt gain was fairly similar among grps in females. During gestation, mean body wt was reduced in CP (5-11%) compared to CU. Mean body wt was reduced in MDF [Day 0 of gestation only] and HDF compared to CP. At the HD, mean body wt was significantly reduced on Days 0, 7, and 12 [6-8% compared to CP, 11-13% compared to CU]. Mean body wt gain

was not adversely affected during the first few days of gestation [mean body wt was significantly greater at the MD and HD compared to CP], but was reduced (50%) in HDF during Gestation Days 3-7. Body wt gain was increased [≈50%] at all doses during Days 12-15 of gestation (compared to CP). Mean body wt gain in CU was not consistently higher compared to other grps during the gestation period.

Food consumption: there were no clear drug-related effects in males (although food intake was significantly reduced in MDM and HDM during Days 36-43); however, food intake in UC was higher (10-15%) than in CP.

In females, food intake was not consistently higher in CU compared to CP during the premating period. However, food intake was significantly reduced in CU (compared to CP) during the gestation period. Compared to CP, food intake was reduced in MDF and HDF during the premating period [10 and 20%, respectively, during Days 8-15, and 9% at both doses during Days 15-22] and in HDF during the gestation period [12-7% on Days 3-7 and Days 7-12].

Organ Weights: there were differences in mean wts of certain organs [e.g., brain, prostate, seminal vesicles, and/or epididymides] between CU and CP grps due (at least in part) to differences in body wt. Prostate wt (absolute and relative) was reduced in HDM compared to CP (24-27%) and CU (45-26%). Relative, but not absolute, epididymidus wt was reduced in HDM compared to CP (8%).

Gross pathology: the only gross findings of note were (1) liver "lesion" in males [4/25, 0/25, 4/25, 3/25, and 3/25 in CP, CU, LD, MD, and HD grps, respectively] and (2) hair loss in females [2/25, 1/25, 6/25, 3/24, and 9/25 in CP, CU, LD, MD, and HD grps, respectively].

Semen analysis: there were no significant differences in sperm parameters between the 2 control grps. Sperm concentration and total sperm ct were reduced in HDM [25 and 17%, respectively] compared to CP; sperm motility and morphology were not affected. [The sponsor noted that the data for sperm concentration and total ct were within the range of HC data.]

Pregnancy parameters: the data were summarized in the following sponsor's tables:

TABLE 19

STUDY NO.: 3477.8 SELEGILINE TRANSDERMAL SYSTEM: A SEGMENT I STUDY IN RATS PAGE 1
CLIENT: SOMERSET PHARMACEUTICALS SUMMARY OF COPULATION AND FERTILITY DATA
CLIENT NO.: TOX-539-98B

GROUP:	1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):	0	0	10	30	75
TEST ARTICLE:	PLACEBO	UNTREATED	SELEGILINE	SELEGILINE	SELEGILINE
COPULATION INDEX					
NO. OF ANIMALS PAIRED	25/25	25/25	25/25	24/25	25/25
PERCENT	100.0	100.0	100.0	96.0	100.0
FERTILITY INDEX					
NO. OF ANIMALS PAIRED	25/25	25/25	22/25	23/24	22/25
PERCENT	100.0	100.0	88.0	95.8	88.0
PRECOITAL INTERVAL (DAYS)					
MEAN	2.9	2.7	3.0	2.9	3.5
S.D.	1.2	1.1	1.6	2.5	2.8
N	25	25	25	24	25

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL
NOTE: COPULATION INDEX = NO. OF ANIMALS PAIRED WITH SUCCESSFUL COPULATION / NO. OF MATED ANIMALS X 100.
FERTILITY INDEX = NO. OF GRAVID FEMALES / NO. OF ANIMALS PAIRED WITH SUCCESSFUL COPULATION X 100.

TABLE 20

STUDY NO.: 3477.8 SELEGILINE TRANSDERMAL SYSTEM: A SEGMENT I STUDY IN RATS PAGE 1
CLIENT: SOMERSET PHARMACEUTICALS SUMMARY OF PREGNANCY STATUS
CLIENT NO.: TOX-539-98B

GROUP:	1		2		3		4		5	
TARGET LEVEL (MG/KG/DAY):	0		0		10		30		75	
TEST ARTICLE:	PLACEBO		UNTREATED		SELEGILINE		SELEGILINE		SELEGILINE	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	25		25		25		25		25	
FEMALES EUTHANIZED POST-BREEDING DAY 15	0	0.0	0	0.0	0	0.0	1	4.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0
GRAVID	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES EXAMINED AT SCHEDULED NECROPSY	25	100.0	25	100.0	25	100.0	24	96.0	25	100.0
NONGRAVID	0	0.0	0	0.0	3	12.0	1	4.2	3	12.0
GRAVID	25	100.0	25	100.0	22	88.0	23	95.8	22	88.0
WITH RESORPTIONS ONLY	0	0.0	0	0.0	0	0.0	0	0.0	1	4.5
WITH VIABLE FETUSES	25	100.0	25	100.0	22	100.0	23	100.0	21	95.5
TOTAL FEMALES GRAVID	25	100.0	25	100.0	22	88.0	23	92.0	22	88.0

TABLE 21

STUDY NO.: 3477.8 SELEGILINE TRANSDERMAL SYSTEM: A SEGMENT I STUDY IN RATS PAGE 1
CLIENT: SOMERSET PHARMACEUTICALS SUMMARY OF CESAREAN SECTION DATA
CLIENT NO.: TOX-539-98B

GROUP:		1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):		0	0	10	30	75
TEST ARTICLE:		PLACEBO	UNTREATED	SELEGILINE	SELEGILINE	SELEGILINE
FEMALES GRAVID		25	25	22	23	22
CORPORA LUTEA	TOTAL	404	438	346	356	341
	MEAN	16.2	17.5	15.7	15.5	15.5
	S.D.	2.6	2.1	2.6	2.1	2.4
IMPLANTATION SITES	TOTAL	363	392	325	315	295
	MEAN	14.5	15.7	14.8	13.7	13.4
	S.D.	3.2	2.6	2.6	3.6	3.2
PRE-IMPLANTATION LOSS	TOTAL	41	46	21	41	46
	MEAN	1.6	1.8	1.0	1.8	2.1
	S.D.	2.4	2.9	1.5	3.4	3.9
VIABLE FETUSES	TOTAL	331	362	294	293	268
	MEAN	13.2	14.5	13.4	12.7	12.2
	S.D.	3.3	2.8	3.1	4.1	3.9
DEAD FETUSES	TOTAL	0	0	0	0	0
LATE RESORPTIONS	TOTAL	0	0	0	0	0
EARLY RESORPTIONS	TOTAL	32	30	31	22	27
	MEAN	1.3	1.2	1.4	1.0	1.2
	S.D.	1.4	1.2	1.6	1.8	1.6
POST-IMPLANTATION LOSS	TOTAL	32	30	31	22	27
	MEAN	1.3	1.2	1.4	1.0	1.2
	S.D.	1.4	1.2	1.6	1.8	1.6

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL

There were no clear drug-related effects on reproductive parameters. Estrus cycle data were provided only as individual data; no drug-related effects were evident. Mean precoital interval was only slightly increased at the HD; an examination of individual data indicated that the precoital interval was prolonged in 1/25 LDF [8 days], 1 MDF [13 days], and 2 HDF [9 and 14 days] compared to the maximum interval in C grps [i.e., 4-5 days]. One gravid HDF had no viable fetuses at necropsy.

3.Study Type: dose-range finding study for Segment II study in Sprague-Dawley rat.
Study Title: **Selegiline Transdermal System: a range-finding developmental study (Segment II) in Sprague-Dawley rats** [Somerset Study No: TOX-540-98B, Vol #1.032, Conducting laboratory and location: [redacted] date of study initiation: 12/15/98, GLP, QA]

Methods
Dosing

species/strain: female Sprague-Dawley rat [redacted]
#/sex/group or time point: 7/grp
age: ≈89-99 days
weight: 201-297 gm
satellite groups used for toxicokinetics or recovery: 8/grp for TK
dosage groups in administered units: 0 (CP), 0 (CU), 12.5, 25, 50, 75 mg/kg
route, form, volume, and infusion rate: transdermal. Skin was clipped free of hair.
duration: patches were replaced daily from Gestation Day 6 to Day 17. All animals were sacrificed on Day 20 of gestation.

Drug, lot#, and % purity: STS, lot no. 26E007D, purity of drug substance: [redacted]

Formulation/vehicle: transdermal patch [20 mg/20 cm^2]. Patches were held in place with ——— binding using a sholder strap and athletic tape. Animals in the CU (untreated control) grp were not clipped, dosed, or bound. Patches were checked twice daily, and reapplied or replaced if necessary.

Observations and times:

Clinical signs: animals were observed daily. Local irritation was assessed on Gestation Days 0, 6, 9, 12/13, 15, 18, and 20 using the Macroscopic Dermal Grading System.

Body weights: body wts were recorded on Gestation Days 0, 6, 9, 12/13, 15, 18, and 20.

Food consumption: n/a

Ophthalmoscopy: n/a

ECG: n/a

Hematology: n/a

Clinical chemistry: n/a

Urinalysis: n/a

Gross pathology: a necropsy was performed on each animal on Day 20 of gestation. The following reproductive parameters were assessed: (a) viable/nonviable fetuses, early/late resorptions, no. of corpora lutea. In females with no evidence of pregnancy, the uteri were "...placed in 10% aqueous ammonium sulfide..." for examination of embryolethality.

Organ weights: only the uterus was weighed, and for the purpose of calculating corrected body wts.

Fetal examination: each viable fetus was examined for external findings, sex, length [crown-to-rump], and weighed.

Histopathology: n/a

Toxicokinetics: blood samples were collected from the satellite-TK animals according to the schedule indicated in the sponsor's table below. Frozen plasma samples were stored, then shipped to ——— for analysis. [It was noted in the study report that "Animals assigned to the toxicokinetic (TK) phase were utilized exclusively for generatng TK data..."; however, body wt, gross pathology, and reproductive data were provided for these animals.]

Gestation Day	Post-Dose Collection Interval	Set of Animals	Number of Animals for Blood Collection				
			Group 7	Group 8	Group 9	Group 10	Group 11
6	2 hour	SET 1	4	4	4	4	*
	6 hour	SET 2	4	4	4	4	
	12 hour	SET 1	4	4	4	4	
	23 hour	SET 2	4	4	4	4	
12	23 hour	SET 2	4	4	4	4	*
17	23 hour	SET 2	4	4	4	4	*

*Blood samples from 8 untreated animals on gestation days 6, 12 and 17 at 23 hours post-dose were used for validation purposes.

Results

Mortality: there were no unscheduled deaths.

Clinical signs: clinical signs are summarized in the following table [data expressed as "no. of occurrences/no. of affected animals]:

SIGN	DOSE (mg/kg)					
	0 (CP)	0(CU)	12.5	25	50	75
reddish vaginal discharge	0/0	0/0	0/0	0/0	2/2	2/2
mucoid vaginal discharge	0/0	0/0	0/0	0/0	0/0	1/1
small feces	0/0	0/0	1/1	0/0	2/1	5/3
urine stain	0/0	0/0	0/0	1/1	2/1	2/1
dk material around eyes	1/1	0/0	1/1	0/0	0/0	3/2
dk material around nose	1/1	0/0	2/1	10/2	19/5	21/3
dk material around mouth	0/0	0/0	2/1	1/1	1/1	2/1
patch off						
0.25	0/0	0/0	3/3	4/3	2/1	0/0
0.50	0/0	0/0	0/0	0/0	1/1	0/0
whole	4/3	0/0	0/0	0/0	0/0	0/0

Local irritation [expressed as "no. of occurrences/no. of animals affected] was detected in all grps receiving patches; however, the severity of erythema was greater at the MD and HD [1/1 in CP and at 12.5 and 25 mg/kg, 6/4 at 50 mg/kg and 5/4 at 75 mg/kg] and exfoliation eschar was observed only in treated animals (although the incidences were not dose-related).

Body weights: mean body wt tended to be higher in CU than in CP throughout the dosing period. There were no clear dose-related effects on mean body wt. The primary drug-related effect was body wt loss (greatest at the HD) during the first few days of dosing (Gestation Days 6-9) at the 25, 50, and 75 mg/kg. Mean corrected maternal body wt was fairly similar among grps.

Gross pathology: there were no apparent drug-related findings. There were also no apparent drug-related effects on reproductive parameters, including live/dead fetuses, fetal wt, early/late resorptions, pre/postimplantation loss.

Fetal Examinations: **malformations** [i.e., micrognathia (maxillary or mandibular) and microstomia] were observed **only in 1 HD fetus**.

TK: the data were summarized in the sponsor table (provided below). Blood samples were collected via the orbital plexus under anesthesia. Two of the satellite animals were found dead [1-50 mg/kg, 1-75 mg/kg]. All survivors were pregnant. Mean body wt was lower in all treated grps compared to CU; final mean body wts were 7, 17, 10 and 14% lower (than CU) at 12.5, 25, 50, and 75 mg/kg, respectively. The primary effect on body wt gain was noted in the HD grp (i.e., mean body wt loss) during the first few days of dosing.

Table 1. Mean ± SD concentrations of selegiline and its metabolites in pregnant rats

Analyte	Mean ± SD (N=4) Analyte Concentration (ng/mL) in Maternal Plasma		
	23 hr post dose GD 6	23 hr post dose GD 12	23 hr post dose GD 17
25 mg/kg/day targeted (actual mean dose applied 19.6 – 20.7 mg/kg/day)			
Selegiline	9.87 ± 1.72	13.8 ± 2.5	15.7 ± 8.2
N-Desmethylselegiline	2.36 ± 0.51	3.19 ± 0.13	2.43 ± 1.33
Amphetamine	3.72 ± 1.80	5.54 ± 1.04	4.37 ± 2.68
Methamphetamine	4.71 ± 1.84	6.98 ± 0.89	6.23 ± 3.55
50 mg/kg/day targeted (actual mean dose applied 52.1 – 54.6 mg/kg/day)			
Selegiline	27.7 ± 5.9	29.6 ± 18.2	22.3 ± 9.6[a]
N-Desmethylselegiline	6.41 ± 1.15	7.48 ± 4.10	6.54 ± 1.77[a]
Amphetamine	10.3 ± 4.0	17.3 ± 9.9	14.0 ± 3.0[a]
Methamphetamine	17.6 ± 3.7	23.1 ± 13.4	20.8 ± 3.3[a]
75 mg/kg/day targeted (actual mean dose applied 73.2 – 74.6 mg/kg/day)			
Selegiline	42.3 ± 9.2[a]	49.3 ± 18.6	39.1 ± 6.3
N-Desmethylselegiline	10.0 ± 4.5[a]	9.49 ± 4.49	8.10 ± 3.12
Amphetamine	12.5 ± 4.7[a]	23.9 ± 12.2	17.7 ± 4.9
Methamphetamine	23.8 ± 11.1[a]	33.1 ± 20.7	24.2 ± 6.7

[a] N=3

Gross findings in the two animals that died were as follows: (1) dark material on the haircoat and skin scabbing in the 50-mg/kg animal; this animal was pregnant but early resorptions were detected, (2) hairloss, wet haircoat, eye opacity, dark red areas on the lungs, abnormal oral cavity contents, and skin scabbing; this animal was not pregnant.

In survivors, there were no apparent drug-related effects.

4.Study Type: Segment II (embryofetal development) study
Study Title: **Selegiline transdermal system: a developmental toxicity (Segment II) study in Sprague-Dawley rats** (Somerset Study No: TOX-541-98B, Vol #1.031, Conducting laboratory and location: , date of study initiation: 3/1/99, GLP, QA)

Methods
Dosing

species/strain: female Sprague-Dawley CD®(SD)IGS(BR) rat
#/sex/group or time point: 25/grp
age: 89-125 days
weight: 223-320 gm
satellite groups used for toxicokinetics or recovery: 6/grp for TK
dosage groups in administered units: 0 (placebo patch), 0 (untreated), 10, 30, 75 mg/kg
route, form, volume, and infusion rate: transdermal, 20 mg/20 cm^2 patch
duration: patches were applied daily from Gestation Days 6 through 17

Drug, lot#, and % purity: STS, lot no.'s 26E006N and 26E007N (selegiline lot no. 10027), purity of drug substance:
Formulation/vehicle: STS; patches were held in place on clipped skin with '...with a shoulder strap and secured with athletic tape". Untreated C (CU) were not clipped, wrapped, or bound. Patch placement was checked twice daily, and patches were reapplied or replaced as necessary.
Dose Justification: data from dose-range finding and feasibility studies in rats
Observations and times:

Clinical signs: animals were observed twice daily. "Application sites were examined on gestation

days 6, 9, 12, 15, 18, and 20..." using the Macroscopic Dermal Grading System.

Body weights: body wts were recorded on Gestation Days 0, 6, 9, 12, 15, 18, and 20. Corrected maternal wts were calculated on Day 20.

Food consumption: food intake was recorded on Gestation Days 0, 6, 9, 12, 15, 18, and 20. Data were expressed as gm/animal/day.

Ophthalmoscopy: n/a

ECG: n/a

Hematology: n/a

Clinical chemistry: n/a

Urinalysis: n/a

Gross pathology: necropsies were performed on Gestation Day 20. The uterus was removed and the follow parameters were recorded: uterus wt, live/dead fetuses, early/late resorption, no. of corpora lutea. Those uteri with no visible evidence of pregnancy were placed in 10% aqueous ammonium sulfide for evaluation of embryolethality.

Organ weights: uterus only.

Fetal examination: all fetuses were examined externally and the following parameters were recorded: sex, wt, crown-rump length, external findings. Visceral examinations were performed on ½ of the fetuses per litter using Bouin's solution and Wilson's dissection technique. Skeletal examinations were conducted on the remaining fetuses in each litter, using 1-2% aqueous potassium hydroxide, Alizarin Red S, and glycerin.

Histopathology: n/a

Toxicokinetics: blood samples were collected from satellite animals on Days 7, 12, 18, and 20 [i.e., 23 hrs after patch application on Gestation Days 6, 12, 17, and 19] for quantitation of selegiline and metabolites. Fetal blood and amniotic fluid samples were collected at necropsy [Day 20; 23 hrs after patch application on Gestation Day 19.] Assays were conducted by ; using GC/MS. The LLOQ for all compounds was g/mL.

Results:

Mortality: there were no unscheduled deaths.

Clinical signs: selected findings are summarized in the following table:

SIGN	DOSE (mg/kg)				
	0 (CP)	0 (CU)	10	30	75
limping-R hindlimb	0/0	0/0	0/0	0/0	9/1
swelling-R hindlimb	0/0	0/0	0/0	0/0	9/1
few feces	0/0	0/0	0/0	0/0	9/7
soft stool	0/0	0/0	0/0	0/0	1/1
reddish vaginal discharge	0/0	0/0	0/0	0/0	1/1
urine stain	3/1	0/0	1/1	0/0	61/10
scab(s)-abdominal area	0/0	0/0	0/0	0/0	8/2
dk material around nose	2/2	2/2	7/4	13/7	67/20
dk material around mouth	0/0	0/0	1/1	1/1	7/7
patch off					
0.125	0/0	0/0	0/0	0/0	1/1
0.25	0/0	0/0	0/0	0/0	1/1
0.5	0/0	0/0	1/1	0/0	0/0
patch replaced	0/0	0/0	1/1	0/0	0/0

Local irritation [i.e., edema, erythema, desquamation, eschar] was observed in all grps receiving patches, with no clear drug-related effect.

Body weight: mean body wt was lower in CP (11%, corrected wt) than in CU. Compared to the CP, mean body wt in HDF was consistently lower; however, the greatest effect (10%) was noted during the first few days of dosing. Final mean corrected body wt in HDF was

5% lower than in CP. Mean body wt loss was noted during the first few days of dosing at the MD and HD; overall mean daily body wt gain was highest in CU (96 gm) and significantly lower in MDF (61 gm) and HDF (59 gm) than in CP (81 gm).

Food consumption: food intake was reduced in MDF and HDF primarily during the first few days of dosing. Overall mean daily food intake was highest in CU (29 gm) and significantly lower in MDF and HDF (23 gm) compared to CP (26 gm).

Gross pathology (dams): the following notable findings were observed: (a) dk material on the haircoat, particularly in HDF, (b) hairloss, observed in 1/25 CPF and CUF, 5/25 LDF, 4/25 MDF, and 4/5 HDF.

Reproductive parameters: there were no apparent drug-related effects on the following parameters: corpora lutea, implantation sites, pre-implantation loss, live/dead fetuses. There were, however, slight increases in early and late resorptions, post-implantation loss, and a significant reduction in fetal wt at the HD. The data were summarized in the following sponsor's table:

TABLE 8

STUDY NO.: 3477.9 SELEGILINE TRANSDERMAL SYSTEM: A SEGMENT II STUDY IN RATS PAGE 1
CLIENT: SOMERSET PHARMACEUTICALS SUMMARY OF CESAREAN SECTION DATA
CLIENT NO.: TOX-541-98B

GROUP:		1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):		0	0	10	30	75
TEST ARTICLE:		PLACEBO	UNTREATED	SELEGILINE	SELEGILINE	SELEGILINE
FEMALES GRAVID		24	24	23	25	25
CORPORA LUTEA	TOTAL	408	411	377	409	450
	MEAN	17.0	17.1	16.4	16.4	18.0
	S.D.	2.5	3.7	2.8	3.3	4.2
IMPLANTATION SITES	TOTAL	369	367	357	379	410
	MEAN	15.4	15.3	15.5	15.2	16.4
	S.D.	4.3	4.3	2.5	3.0	1.8
PRE-IMPLANTATION LOSS	TOTAL	39	44	20	30	40
	MEAN	1.6	1.8	0.9	1.2	1.6
	S.D.	3.1	2.7	1.5	1.8	3.0
VIABLE FETUSES	TOTAL	342	333	331	346	362
	MEAN	14.3	13.9	14.4	13.8	14.5
	S.D.	4.2	4.2	2.5	3.1	1.8
DEAD FETUSES	TOTAL	0	0	0	0	0
LATE RESORPTIONS	TOTAL	1	0	0	0	3
	MEAN	0.0	0.0	0.0	0.0	0.1
	S.D.	0.2	0.0	0.0	0.0	0.3
EARLY RESORPTIONS	TOTAL	26	34	26	33	45
	MEAN	1.1	1.4	1.1	1.3	1.8
	S.D.	1.4	1.3	1.4	1.1	2.3
POST-IMPLANTATION LOSS	TOTAL	27	34	26	33	48
	MEAN	1.1	1.4	1.1	1.3	1.9
	S.D.	1.5	1.3	1.4	1.1	2.3

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL

TABLE 8

STUDY NO.: 3477.9 SELEGILINE TRANSDERMAL SYSTEM: A SEGMENT II STUDY IN RATS PAGE 2
CLIENT: SOMERSET PHARMACEUTICALS SUMMARY OF CESAREAN SECTION DATA
CLIENT NO.: TOX-541-98B

GROUP:		1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):		0	0	10	30	75
TEST ARTICLE:		PLACEBO	UNTREATED	SELEGILINE	SELEGILINE	SELEGILINE
SEX M / F	TOTAL	177 165	171 162	166 165	163 183	175 187
	MEAN	7.4 6.9	7.1 6.8	7.2 7.2	6.5 7.3	7.0 7.5
	S.D.	2.9 2.7	3.0 2.7	2.3 2.1	2.2 2.6	1.8 1.6
GRAVID UTERUS WEIGHT (G)	MEAN	79.9	80.7	82.2	77.1	77.7
	S.D.	23.8	23.4	14.3	15.0	9.5
FETAL WEIGHT (G)	MEAN	3.5	3.7	3.6	3.5	3.3**
	S.D.	0.4	0.2	0.3	0.2	0.3

SIGNIFICANTLY DIFFERENT FROM CONTROL: ** = P<0.01

Fetal examinations: the data on malformations were summarized in the following sponsor's table:

TABLE 9

STUDY NO.: 3477.9 SELEGILINE TRANSDERMAL SYSTEM: A SEGMENT II STUDY IN RATS PAGE 1
CLIENT: SOMERSET PHARMACEUTICALS SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS
CLIENT NO.: TOX-541-98B

	FETUSES					LITTERS				
GROUP:	1	2	3	4	5	1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):	0	0	10	30	75	0	0	10	30	75
NUMBER EXAMINED EXTERNALLY	342	333	331	346	362	24	24	23	25	25
GASTROSCHISIS	1	0	0	0	0	1	0	0	0	0
DOMED HEAD	2	0	0	0	0	2	0	0	0	0
MICROSTOMIA	0	0	1	0	0	0	0	1	0	0
FACIAL PAPILLA(E) ANOMALY	0	0	1	0	0	0	0	1	0	0
MICROGNATHIA (MAXILLARY OR MANDIBULAR)	0	0	1	1	1	0	0	1	1	1
MULTIPLE HEAD ANOMALIES	0	0	0	0	1	0	0	0	0	1
ABSENT TAIL	1	0	0	0	0	1	0	0	0	0
OMPHALOCELE	0	0	0	0	1	0	0	0	0	1
ANOPHTHALMIA AND/OR MICROPHTHALMIA	1	0	1	0	2	1	0	1	0	2
MICROGLOSSIA	0	0	0	0	1	0	0	0	0	1
NUMBER EXAMINED VISCERALLY	172	168	166	169	181	24	24	23	25	25
DIAPHRAGMATIC HERNIA	0	0	0	0	2	0	0	0	0	2
SITUS INVERSUS	0	0	0	0	1	0	0	0	0	1
NUMBER EXAMINED SKELETALLY	170	165	165	177	181	24	23	23	25	25
CERVICAL VERTEBRAE ANOMALY	0	0	1	0	2	0	0	1	0	2
THORACIC VERTEBRAE MALALIGNED	0	0	0	0	1	0	0	0	0	1
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	0	0	1	0	0	0	0	1	0	0
ATLAS-OCCIPITAL DEFECT	0	0	0	0	1	0	0	0	0	1
SKULL ANOMALY	0	0	1	0	1	0	0	1	0	1
TOTAL MALFORMATIONS										
NUMBER WITH EXTERNAL MALFORMATIONS	3	0	2	1	4	3	0	2	1	3
NUMBER WITH SOFT TISSUE MALFORMATIONS	0	0	0	0	3	0	0	0	0	3
NUMBER WITH SKELETAL MALFORMATIONS	0	0	2	0	2	0	0	2	0	2
TOTAL NUMBER WITH MALFORMATIONS	3	0	2	1	8	3	0	2	1	7

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL
NOTE: GROUP 1 RECEIVED THE PLACEBO, GROUP 2 WAS UNTREATED AND GROUPS 3, 4 AND 5 RECEIVED SELEGILINE.

The sponsor did not consider the data indicative of a teratogenic effect based on the following: (a) malformations were "...dissimilar in nature and they were not significantly different from the placebo patch control", (b) there was no "...pattern which would indicate a relationship to treatment", and (c) they were associated with maternal toxicity "...coupled with the stress of 24-hour transdermal exposure".

The malformation findings in individual affected fetuses are summarized in the following table:

DOSE GRP	DAM	FETUS	MALFORMATION	
			TYPE*	DESCRIPTION
CP	22434	12	E	gastroschisis [liver, stomach, intestines protrubing through midline closure defect], domed head, anophthalmia and/or microphthalmia [anophthalmia (bilateral) noted upon visceral examination]
	22345	5	E	domed head
LD	22312	4	E	micrognathia (mandibular)
			S	skull anomaly [basisphenoid and pterygoid processes misshapen, tympanic rings malpositioned vetnrally and straighter than normal, small bone positioned between aspects of tympanic rings, hyoid absent; no evidence of cartilaginous anlage]
	22390	4	E	anopthalmia and/or microphthalmia [microphthalmia (right), noted upon skeletal examination]
			S	vertebral anomaly w/wo associated rib anomaly, extra arch between #9 and #10 (left) fused to #10, extra rib between #9 and #10 (left), with associated malalignment of vertical arches #7-11 (left), hemicentra at #9 and #11, malaligned centra at #7, #8, and #10; cervical vertebrae anomaly [six arches present on right, 4 arches present on left, arch #2 misshapen (ventral aspect longer than normal]
MD	22375	1	E	micrognathia (maxillary and mandibular)
HD	22353	9	E	multiple head anomalies [rinocephaly, exencephaly, anophthalmia (bilateral), malpositioned pinnae, astomia, aglossia]
			V	situs inversus (complete)
	22448	7	E	omphalocele [several loops of intestine protruding through opening at base on umbilicus]
		16	E	anophthalmia and/or microphthalmia [microphthalmia (left) noted upon visceral examination]
	22409	17	E	micrognathia (mandibular), microphthalmia (left), microglossia
	22408	3	V	diaphragmatic hernia [large portion of liver, stomach, spleen, pancreas extending into thoracic cavity]
	22310	9	V	diaphragmatic hernia [small portion of liver lobe protruding into thoracic cavity]
	22429	3	S	cervical vertebral anomaly [#4 and #5 (right), thread-like fusion of lateral aspects, dorsal aspects of these arches are slightly reduced in ossification with a medial fusion of the cartilage models; thoracic vertebrae malaligned [arches #11 and #12 (right) malpositioned, centra #11 and #13 malaligned (slight)]
	22456	15	S	atlas-occipital defect (bilateral), cervical vertebrae anomaly [medial aspect of #6 and #7 fused (left), arches reduced in ossification (slight, right: #2-#7; left: #3, and #5-7); skull anomaly [pterygoid processes (bilatera) misshapen, basisphenoid also appears misshapen (possibly due to reduction in ossification)]

*E = external, V = visceral, S = skeletal

GRP	DAM	BODY WEIGHT GAIN (gm)						
		D0-6	D6-9	D9-12	D12-15	D15-18	D18-20	D6-18
CP	22434							
	22345							
	mean	2	9	19	18	35	45	81
	range							
CU	mean	32	21	17	19	39	37	96
	range							
LD	22312							
	22390							
	mean	0	10	16	17	38	44	81
	range							
MD	22375							
	mean	8	-9	19	18	33	39	61
	range							
HD	22353							
	22448							
	22409							
	22408							
	22310							
	22429							
	22456							
	mean	8	-28	32	20	34	40	59
	range							

Findings classified by the sponsor as "variations" are summarized in the sponsor's table below. Of note were the following: (a) an increase in unossified sternebra(e) #5 and/or #6 in MD and HD fetuses [no. of affected fetuses and litters] compared to CP, (b) an increase in malaligned sternebra(e) at the HD in terms of affected litters (but not fetuses) compared to CP and CU, (c) an increase in malaligned costal cartilage at the HD [no. of affected fetuses and litters], (d) an increase in reduced ossification of the skull in HD fetuses in terms of no. of affected fetuses, but not litters, [the incidence of this finding was markedly lower in the CU compared to CP and dosed grps], (e) an increase in unossified hyoid at the MD and HD [for no. of affected fetuses] and at the HD [for no. of affected liters] compared to CP [the incidence of this finding was significantly lower in the CU compared to CP], (f) the incidence of bent rib(s) was reduced in CU and at all doses compared to CP [no. of affected fetuses and litters].

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TABLE 10

STUDY NO.: 3477.9 SELEGILINE TRANSDERMAL SYSTEM: A SEGMENT II STUDY IN RATS PAGE 1
CLIENT: SOMERSET PHARMACEUTICALS SUMMARY OF FETAL OBSERVATIONS - VARIATIONS
CLIENT NO.: TOX-541-98B

	FETUSES					LITTERS				
GROUP:	1	2	3	4	5	1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):	0	0	10	30	75	0	0	10	30	75
NUMBER EXAMINED EXTERNALLY	342	333	331	346	362	24	24	23	25	25
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	172	168	166	169	181	24	24	23	25	25
DISTENDED URETER(S)	6	3	1	3	7	4	3	1	2	3
NUMBER EXAMINED SKELETALLY	170	165	165	177	181	24	23	23	25	25
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	17	5	8	31	26	8	5	7	15	14
14TH RUDIMENTARY RIB(S)	12	15	11	7	5	7	9	8	6	4
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	18	27	19	23	28	10	13	16	13	17
COSTAL CARTILAGE MALALIGNED	2	1	0	3	6	2	1	0	3	5
REDUCED OSSIFICATION OF THE SKULL	13	2	5	5	18	9	2*	4	4	10
REDUCED OSSIFICATION OF THE VERTEBRAL ARCHES	2	0	0	1	2	2	0	0	1	2
7TH CERVICAL RIB(S)	11	0	2	4	4	3	0	2	3	3
HYOID UNOSSIFIED	13	2	13	23	29	10	2*	9	11	14
BENT RIB(S)	8	1	3	1	1	6	1	2	1*	1*
COSTAL CARTILAGE VARIATION	2	0	0	0	1	1	0	0	0	1
27 PRESACRAL VERTEBRAE	0	0	1	0	1	0	0	1	0	1
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	0	0	1	1	1	0	0	1	1	1
METACARPAL(S) AND/OR METATARSAL(S) UNOSSIFIED	1	0	0	0	0	1	0	0	0	0
ENTIRE STERNUM UNOSSIFIED	1	0	0	0	0	1	0	0	0	0
PUBIS UNOSSIFIED	1	1	0	0	0	1	1	0	0	0
REDUCED OSSIFICATION OF THE 13TH RIB(S)	0	3	0	2	0	0	1	0	1	0
25 PRESACRAL VERTEBRAE	0	1	0	0	1	0	1	0	0	1

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05
NOTE: GROUP 1 RECEIVED THE PLACEBO, GROUP 2 WAS UNTREATED AND GROUPS 3, 4 AND 5 RECEIVED SELEGILINE.

Toxicokinetics: the data were summarized in the following sponsor's tables:

Table 1. Mean ± SD Levels of selegiline and its metabolites in maternal rat plasma

Analyte	Mean ± SD (N=6) Analyte Concentration (ng/mL) in Maternal Plasma		
	23 hr post dose GD 6	23 hr post dose GD 12	23 hr post dose GD 17
10 mg/kg/day targeted (actual mean dose applied 8.1 – 9.3 mg/kg/day)			
Selegiline	3.28 ± 1.06	4.34 ± 1.45	4.46 ± 2.29
N-Desmethylselegiline	1.02 ± 0.32	1.11 ± 0.35	1.06 ± 0.59
Amphetamine	1.52 ± 0.57	1.51 ± 0.43	1.70 ± 0.70
Methamphetamine	2.02 ± 0.76	2.04 ± 0.58	2.52 ± 1.16
30 mg/kg/day targeted (actual mean dose applied 29.7 – 31.0 mg/kg/day)[a]			
Selegiline	20.2 ± 3.9	17.7 ± 6.1	17.3 ± 3.5
N-Desmethylselegiline	3.97 ± 1.26	3.38 ± 1.35	3.97 ± 1.12
Amphetamine	4.93 ± 1.62	6.65 ± 3.21	7.02 ± 2.30
Methamphetamine	7.73 ± 1.82	8.73 ± 3.04	9.97 ± 2.73
75 mg/kg/day targeted (actual mean dose applied 73.2 – 74.2 mg/kg/day)			
Selegiline	53.9 ± 3.8	49.7 ± 5.4	47.4 ± 8.7
N-Desmethylselegiline	8.41 ± 1.90	10.0 ± 2.6	11.2 ± 3.3
Amphetamine	12.1 ± 7.0	23.7 ± 9.5	23.9 ± 11.3
Methamphetamine	21.6 ± 9.0	32.4 ± 11.2	33.5 ± 14.1

[a] N=5

Table 2. Mean ± SD concentrations of selegiline and its metabolites in maternal rat plasma, fetal plasma, and amniotic fluid.

Analyte	Mean ± SD (N=6) Analyte Concentration (ng/mL) in Matrix 23 hr post dose GD 19		
	Maternal Plasma	Fetal Plasma	Amniotic Fluid
10 mg/kg targeted (actual mean dose applied 7.4 mg/kg)			
Selegiline	4.16 ± 3.51	3.72 ± 2.17	9.03 ± 6.09
N-Desmethylselegiline	0.913 ± 0.682	0.196 ± 0.305	0.461 ± 0.374
Amphetamine	1.58 ± 0.95	1.70 ± 1.38	2.50 ± 1.62
Methamphetamine	2.32 ± 1.14	1.94 ± 1.36	3.14 ± 1.57
30 mg/kg targeted (actual mean dose applied 31.5 mg/kg)			
Selegiline	18.2 ± 10.8[a]	16.4 ± 4.6[b]	50.3 ± 29.0[b]
N-Desmethylselegiline	4.86 ± 2.41[a]	2.72 ± 1.40[b]	2.93 ± 1.57[b]
Amphetamine	8.86 ± 2.41[c]	9.19 ± 2.96[a]	10.8 ± 3.9[a]
Methamphetamine	14.3 ± 2.8[c]	12.0 ± 4.1[a]	15.1 ± 6.4[a]
75 mg/kg targeted (actual mean dose applied 78.6 mg/kg)			
Selegiline	52.8 ± 18.9	51.7 ± 20.0	101 ± 49
N-Desmethylselegiline	11.1 ± 3.4	6.99 ± 1.97	7.56 ± 3.34
Amphetamine	32.0 ± 10.3	31.5 ± 17.7	44.7 ± 33.3
Methamphetamine	47.1 ± 12.5	44.3 ± 23.3	57.4 ± 35.4

[a] N=4
[b] N=5
[c] N=3

Body wt and pregnancy data were collected in satellite animals. Mean body wt was consistently lower in MDF [3-7%] and HDF [6-14%] than in LDF [no C data provided for comparison]. However, the primary effect was a dose-related body wt loss in MDF and HDF on Days 6-9. Catch-up growth was evident on Days 12-15. Overall mean body wt gain was lower in MDF [23%] and HDF [35%] than in LDF. Only one MD animal was not pregnant.

5. Study Type: range-finding Segment II study in rabbit.
Study Title: **Selegiline Transdermal System: a range-finding developmental study (Segment II) in New Zealand White rabbits** (Study No: TOX-542-98B, Vol #1.042, Conducting laboratory and location: [redacted], date of study initiation: 12/15/98, GLP, QA)

Methods
Dosing

species/strain: female New Zealand White rabbits
#/sex/group or time point: 6/grp
age: ≈5 mo on Gestation Day 0
weight: 3.1-4.0 kg on Gestation Day 0
satellite groups used for toxicokinetics or recovery: no
dosage groups in administered units: CP, CU, 2.5, 5, 10, 20, 40 mg/kg
route, form, volume, and infusion rate: transdermal. Patches were applied daily to a clipped area of skin, and were held in place with "...a 2 inch tubular cotton stockinette with four holes cut out for legs and a foam collar for the neck region". Patches were reapplied or replaced when necessary.
duration of dosing: from Gestation Day 6 through 18

Drug, lot#, and % purity: STS, patch lot no. 26E007D, drug substance lot no. 10017, purity of drug substance: [redacted].
Formulation/vehicle: STS [20 mg/20 cm^2].

Observations and times:

Clinical signs: animals were checked twice daily. Dermal irritation was assessed on Gestation Days 6, 9, 12, 15, 19, 24, and 29 using the Macroscopic Dermal Grading System.

Body weights: body wts were recorded on Gestation Days 0, 4, 6, 9, 12, 15, 19, 24, and 29. Corrected maternal body wts were calculated on Gestation Day 29.

Food consumption: n/a

Ophthalmoscopy: n/a

ECG: n/a

Hematology: n/a

Clinical chemistry: n/a

Urinalysis: n/a

Gross pathology: animals that aborted were sacrificed and necropsied. The following parameters were recorded: no. of implantations (uterus), no. of corpora lutea (ovary). Aborted fetuses were examined if possible.

Survivors were sacrificed on Gestation Day 29 and necropsied. Gross lesions were stored (10% neutral buffered formalin). Uteri were weighed and examined. The following parameters were recorded: live/dead fetuses, early/late resorptions, placental abnormalities, no. of corpora lutea. Uteri with no evidence of pregnancy were "...placed in 10% aqueous ammonium sulfide solution for detection of early embryolethality..."

Fetal examination: viable fetuses were examined for the following: external findings, wt, sex, crown-rump length.

Organ weights: uterus only.

Histopathology: n/a

Toxicokinetics: blood samples were collected on Gestation Days 6 [at 2, 6, 12, and 23 hrs postdosing], 12 [23 hrs postdosing], and 18 [23 hrs postdosing]. Three animals per grp were sampled according to different schedules: 6 hrs postdosing on Gestation Day 6 and at 23 hrs postdosing on Gestation Days 6 and 12 in Set 1; 2 and 12 hrs postdosing on Gestation Day 6 and at 23 hrs postdosing on Gestation Day 18. Selegiline and metabolites were quantitated in plasma by [redacted] using GC/MS. The LLOQ for all compounds was [redacted] ng/mL.

Results

Mortality: one 5-mg/kg female aborted on Gestation Day 25, and was sacrificed.

Clinical signs: selected findings are summarized in the following table [data are expressed as no. of observations/no. of affected animals]:

SIGN	DOSE (mg/kg)						
	0 (CP)	0 (CU)	2.5	5	10	20	40
few feces	0/0	0/0	0/0	1/1	1/1	1/1	8/2
no feces	0/0	0/0	0/0	0/0	0/0	0/0	3/1
fecal stain	0/0	0/0	0/0	0/0	1/1	2/2	7/2
emaciation	0/0	0/0	0/0	0/0	0/0	0/0	4/1
decreased food intake	0/0	2/1	4/2	1/1	4/3	1/1	8/2
patches off							
0.25	2/2		0/0	0/0	1/1	0/0	3/2
0.5	1/1	--	0/0	0/0	0/0	0/0	2/2
1.0	6/4		0/0	0/1	1/1	0/0	9/4
patches replaced	2/2	0/0	0/0	1/1	0/0	0/0	8/6

Dermal irritation was observed in all grps, including CP. The incidence and severity of erythema and edema were not affected in a clear dose-related manner. However, several findings were observed only in treated animals, at 20 and 40 mg/kg. These consisted of

the following: (a) exfoliation eschar [1/1 at 20 mg/kg, 2/2 at 40 mg/kg], (b) purple discoloration at the application site [1/1 at 20 mg/kg, 2/2 at 40 mg/kg], (c) whitish creamy substance at the application site [1/1 at 20 mg/kg, 2/2 at 40 mg/kg], (d) "maximized grade 4" [it is not clear what this observation was] [1/1 at 40 mg/kg], and (e) superficial lightening, affecting 10-25% of the application site in 2 HDF [2/2] ["superficial lightening" was defined as "Characterized by pale area(s) (almost a burn-like appearance) in the test site; superficial lightening was not considered a "notable dermal lesion" since it is only a superficial injury (according to the sponsor)]. These data would suggest some drug-related irritation [i.e., independent of patch].

Body weights: mean body wt tended to be lower (compared to CP) at the HD [4-9%] throughout the dosing period. Final mean corrected maternal wt was 6% lower (compared to CP) at 20 and 40 mg/kg. Mean corrected maternal wt was slight reduced (4%) in CP compared to CU. Body wt loss was observed in all treatment grps sometime during the first 3 days of dosing; however the effect was not completely dose-related [loss at 5, 20, and 40 mg/kg, but not 10 mg/kg during Days 6-9].

Gross pathology: 1 CPF and 3 HDF were not pregnant; of those that were pregnant, all had viable fetuses. There were no apparent drug-related gross lesions in dams. There were also no apparent drug-related effects on reproductive parameters, although the no. of corpura lutea, implantation site, and viable fetuses were all 50-55% lower in the HD grp compared to CP. There were dead fetuses in any dam (including both C grps), and no dose-related effect on early/late resorption or post-implantation loss.

Fetal Examination: fetal body wt was not affected by drug, and no external findings were detected in any fetus (including C fetuses).

Organ Weights: mean uterus wt was slightly lower in HDF (22%) compared to CP.

Toxicokinetics: the data were summarized in the following sponsor's table:

Table 1. Mean ± SD concentrations of selegiline and its metabolites in pregnant rabbits.

Analyte	Mean ± SD (N=3) Analyte Concentration (ng/mL) in Maternal Plasma		
	23 hr post dose GD 6	23 hr post dose GD 12	23 hr post dose GD 18
2.5 mg/kg/day targeted			
Selegiline	0.960 ± 0.843	1.83 ± 0.54[a]	1.63 ± 0.38
N-Desmethylselegiline	2.29 ± 0.66	2.80 ± 0.72	2.64 ± 0.33
Amphetamine	0.595 ± 0.127	1.02 ± 0.36	1.08 ± 0.20
Methamphetamine	0.518 ± 0.181	0.837 ± 0.061	0.980 ± 0.136
5 mg/kg/day targeted			
Selegiline	2.05 ± 0.09	4.53 ± 2.36	3.52 ± 0.83
N-Desmethylselegiline	4.16 ± 0.64	6.60 ± 0.91	5.20 ± 0.71
Amphetamine	1.17 ± 0.36	1.89 ± 0.27	1.86 ± 0.62
Methamphetamine	0.952 ± 0.213	1.62 ± 0.22	1.52 ± 0.65
10 mg/kg/day targeted			
Selegiline	3.26 ± 1.10	7.16 ± 0.51	6.19 ± 1.83
N-Desmethylselegiline	7.74 ± 1.44	11.6 ± 1.2	9.82 ± 0.90
Amphetamine	2.26 ± 0.35	4.53 ± 1.67	3.44 ± 0.58
Methamphetamine	2.03 ± 0.42	4.06 ± 1.84	2.88 ± 0.33
20 mg/kg/day targeted			
Selegiline	8.72 ± 0.97	20.5 ± 2.7	12.6 ± 8.0
N-Desmethylselegiline	16.1 ± 3.7	28.1 ± 2.6	22.4 ± 3.2
Amphetamine	4.71 ± 1.52	6.55 ± 1.09	6.31 ± 0.42
Methamphetamine	3.78 ± 1.06	6.72 ± 0.84	5.82 ± 0.91
40 mg/kg/day targeted			
Selegiline	17.8 ± 6.3	56.5 ± 1.3[a]	64.2 ± 10.5
N-Desmethylselegiline	38.8 ± 18.0	52.5 ± 3.5[a]	60.9 ± 10.1
Amphetamine	12.2 ± 6.1	13.4 ± 1.7[a]	12.5 ± 3.4
Methamphetamine	8.06 ± 0.62	14.0 ± 4.0[a]	10.7 ± 3.2

[a] N=2

6. Study Type: embryofetal development (Segment II) study in rabbit.
Study Title: **Selegiline Transdermal System: a developmental toxicity study (Segment II) in New Zealand White Rabbits** (Study No: TOX-543-98B, Vol #1.044, Conducting laboratory and location: [redacted], date of study initiation: 3/30/99, GLP, QA)

Methods
Dosing

species/strain: New Zealand White rabbit [redacted]
#/sex/group or time point: 20/grp
age: ≈5 mo
weight: 3.3-3.8 kg
satellite groups used for toxicokinetics or recovery: no
dosage groups in administered units: 0 (CP), 0 (CU), 2.5, 10, 40 mg/kg
route, form, volume, and infusion rate: transdermal, patches applied daily for 24 hr/day.
duration of dosing: daily from Gestation Days 6 through 18.

Drug, lot#, and % purity: STS, lot no's 26E007N and 26E006L, lot no. 10027 (drug substance), drug substance purity: [redacted].

Formulation/vehicle: STS, 20 mg/20 cm^2. Patches were applied to clipped skin and held in place with a tubular cotton stockinette in which leg holes had been cut and a foam collar around the neck. Patches were checked twice daily and reapplied or replaced as necessary.

Observations and times:

Clinical signs: animals were observed twice daily. Dermal irritation was assessed on Gestation Days 6, 9, 12, 15, 19, 24, and 29 using the Macroscopic Dermal Grading System.

Body weights: body wts were recorded on Gestation Days 0, 4, 6, 9, 12, 15, 29, 24, and 29. Corrected maternal body wts were calculated on Day 29 wts.

Food consumption: food intake was recorded daily starting on Gestation Day 6.

Gross pathology: animals that aborted or were moribund were sacrificed and a complete necropsy was performed. The uterus (and contents) were examined and the no. of implants and the no. of corpora lutea (on each ovary) were recorded. Fetuses were "examined" and discarded.

Surviving females were sacrificed on Day 29 of gestation and necropsied. The uterus and ovary were examined and the following parameters recorded: live/dead fetuses, early/late resorptions, no. of corpora lutea. In addition, the placentas were examined and abnormalities recorded. Uteri with no evidence of pregnancy were placed in 10% aqueous ammonium sulfide for detection of embryolethality.

Organ weights: uterus only.

Fetal Examination: all fetuses were examined for external, visceral [Staples method), and skeletal findings [1-2% aqeous potassium hydroxide solution, Alizarin Red S, glycerin]. In addition, fetuses were weighed and sexed.

Histopathology: n/a

Toxicokinetics: blood samples were collected from the first 6/grp for TK analysis. Samples were collected via the marginal ear vein at ≈23 hrs postdosing on Gestation Days 6, 12, and 18. Samples were frozen and shipped to [redacted] for analysis. In addition, 3 unused patches per grp [LD, MD, HD] and the used patches (removed at 23 hrs postdosing) from animals in the LD, MD, and HD grps were also sent the [redacted] for analysis.

Results

Mortality: there were no drug-related deaths. Two F [1 CP, 1 HD] aborted their litters on Day 20 or 25. One CPF was sacrificed moribund on Day 23.

Clinical signs: there were no drug-related clinical signs. The incidences of patch off/replaced are summarized in the following table:

SIGN	DOSE (mg/kg)			
	0 (CP)	2.5	10	40
patch off				
0.25	5/4	0/0	5/4	4/4
0.5	4/3	4/4	6/4	1/1
whole	11/7	0/0	5/5	5/5
patch replaced	17/9	6/3	14/7	9/8
patch reapplied	2/2	1/1	2/2	2/2

Clinical signs observed in the HDF that aborted [i.e., scabs, ocular discharge, few feces, reddish discharge] were not different from those observed in the CPFs who aborted or were sacrificed moribund.

Dermal irritation was detected in all grps receiving patches; findings were fairly similar among grps, with no apparent drug-related effect.

Body weights: mean body wt was significantly lower in CPF than in CUF during the dosing period; however, final mean corrected maternal wt was similar among grps [3427, 3531, 3434, 3433, and 3385 gm in CP, CU, LD, MD, and HD grps, respectively]. Mean body wt loss was evident in all treated grps and the CP during the first few days of dosing;

however, only in HDF was the loss greater than that observed in CPF [-30 gm for CFP, -74 gm for HDF]. Body wt loss continued in treated grps [-15, -9, and -30 gm in LDF, MDF, and HDF, respectively] during the next few days of dosing [i.e., Gestation Days 9-12], whereas body wt gain was evident in the C grps. Body wt loss was evident only at the HD [-37 gm] during Gestation Days 12-15 [CPF gained 14 gm during this period]. [It should be noted that body wt loss was also observed prior to start of dosing, i.e., on Gestation Days 0-6, in CPF, LD, and HD (on Days 0-4) and in LDF, MDF, and HDF (on Days 4-6).]

Food consumption: mean food intake was significantly reduced in HDF [29-35%] compared to CP during the dosing period. Overall mean daily intake was reduced by 28% at the HD (compared to CP). [Overall mean daily intake also tended to be lower in LD and MD grps (8 and 11%, respectively); however, these differences were not statistically significant.]

Gross pathology: there were 16, 18, 20, 19, and 16 pregnant females in the CP, CU, LD, MD, and HD grps, respectively; all of these dams had viable fetuses. There were no apparent drug-related gross lesions. In terms of reproductive parameters, the only significant findings were (a) a decrease in implantation sites at the HD (16%) and (b) a decrease in viable fetuses (16%) at the HD; there were no drug-related effects on no. of corpora lutea, dead fetuses, early/late resorptions, post-implantation loss, fetal wt, or fetal sex.

Organ weight: there was a slight (non-significant) decrease in gravid uterus wt at the HD (14%).

Fetal examination: the sponsor noted that 30 fetuses [fetus(litter): 9/2 CP, 4/1 CU, 10/2 LD, 6/3 MD, and 1/1 HD] could not be examined for skeletal findings due to "moderate or severe disarticulation of the ..skeletons..." It was suggested that the disarticulations may have results from placement of fetuses "...in the maceration solution (1.5% aqueous potassium hydroxide) prior to complete fixation in alcohol". One MD fetus was "slightly disarticulated", but was examined for skeletal findings.

Fetal malformations were summarized in the following sponsor's table:

TABLE 9

STUDY NO.: 3477.10 — SELEGILINE TRANSDERMAL SYSTEM: A DEVELOPMENTAL TOXICITY — PAGE 1
CLIENT: SOMERSET PHARMACEUTICALS — (SEGMENT II) STUDY IN NEW ZEALAND WHITE RABBITS
CLIENT NO.: TOX-543-98B — SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

GROUP:	1	2	3	4	5	1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):	0	0	2.5	10.0	40.0	0	0	2.5	10.0	40.0
NUMBER EXAMINED EXTERNALLY	152	155	181	164	127	16	18	20	19	16
DOMED HEAD	4	0	0	1	0	1	0	0	1	0
FLEXED PAW	0	0	1	0	0	0	0	1	0	0
NUMBER EXAMINED VISCERALLY	152	155	181	164	127	16	18	20	19	16
LUNG AGENESIS	4	3	6	5	8	3	3	2	3	5
DIAPHRAGMATIC HERNIA	0	0	0	0	2	0	0	0	0	1
KIDNEY(S) UNASCENDED	0	0	1	0	0	0	0	1	0	0
HYDROCEPHALY	1	0	0	0	0	1	0	0	0	0
TRACHEA ANOMALY	0	0	0	0	1	0	0	0	0	1
HEART AND/OR GREAT VESSEL ANOMALY	0	0	0	1	0	0	0	0	1	0
NUMBER EXAMINED SKELETALLY	152[a]	155[b]	181[c]	164[d]	127[e]	16[a]	18[b]	20[c]	19[d]	16[e]
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	1	1	0	0	0	1	1	0	0	0
EXTRA SITE OF OSSIFICATION ANTERIOR TO STERNEBRA #1	7	2	4	9	6	5	2	3	5	4
STERNEBRA(E) MALALIGNED (SEVERE)	0	0	1	0	0	0	0	1	0	0
RIB ANOMALY	0	0	0	1	0	0	0	0	1	0
CERVICAL VERTEBRAE ANOMALY	0	0	0	0	3	0	0	0	0	1

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL
NOTE: GROUP 1 RECEIVED THE PLACEBO, GROUP 2 WAS UNTREATED AND GROUPS 3, 4 AND 5 RECEIVED SELEGILINE.
[a] INCLUDES NINE DISARTICULATED FETUSES FROM TWO LITTERS.
[b] INCLUDES FOUR DISARTICULATED FETUSES FROM ONE LITTER.
[c] INCLUDES TEN DISARTICULATED FETUSES FROM TWO LITTERS.
[d] INCLUDES SIX DISARTICULATED FETUSES FROM THREE LITTERS.
[e] INCLUDES ONE DISARTICULATED FETUS FROM ONE LITTER.

TABLE 9

STUDY NO.: 3477.10 SELEGILINE TRANSDERMAL SYSTEM: A DEVELOPMENTAL TOXICITY PAGE 2
CLIENT: SOMERSET PHARMACEUTICALS (SEGMENT II) STUDY IN NEW ZEALAND WHITE RABBITS
CLIENT NO.: TOX-543-98B SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

GROUP:	1	2	3	4	5	1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):	0	0	2.5	10.0	40.0	0	0	2.5	10.0	40.0
TOTAL MALFORMATIONS										
NUMBER WITH EXTERNAL MALFORMATIONS	4	0	1	1	0	1	0	1	1	0
NUMBER WITH SOFT TISSUE MALFORMATIONS	5	3	7	6	11	4	3	3	4	7
NUMBER WITH SKELETAL MALFORMATIONS	8	3	5	9	9	6	3	4	5	5
TOTAL NUMBER WITH MALFORMATIONS	16	6	12	14	19	9	5	6	7	9

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL
NOTE: GROUP 1 RECEIVED THE PLACEBO, GROUP 2 WAS UNTREATED AND GROUPS 3, 4 AND 5 RECEIVED SELEGILINE.

The sponsor considered there to be no drug-related findings. Of note, however, was an increase in the incidence (affected fetuses and litters) of lung agenesis at the HD and the occurrence of trachea anomaly and cervical vertebrae anomaly only in HD fetuses. The data for lung agenesis expressed as % affected fetuses (% affected litters) were 2.6% (10%) in CP and 6.3% (31%) in HD fetuses. Also, the total number of visceral malformations was increased in HD fetuses. Individual data on malformations are provided in the table below

GRP	DAM	FETUS	TYPE	FINDING
CP	R0082	2	E, V	domed head[slight], hydrocephaly [internal, moderate]
		5	E, V	domed head [slight], hydrocephaly [internal, moderate]
		7	E, V	domed head [slight], hydrocephaly [internal, moderate]
			S	extra site of ossification anterior to sternebra #1
		9	E, V	domed head [slight], hydrocephaly [internal, moderate]
		10	V	hydrocephaly [internal]
	R0003	8	V	lung agenesis [post-caval lobe]
		9	V	lung agenesis [post-caval lobe]
	R0040	7	V	lung agenesis [post-caval lobe]
	R0061	6	V	lung agenesis [post-caval lobe]
	R0028	6	S	extra site of ossification anterior to sternebra #1, with associated slight malalignment of sternebra #1
		8	S	extra site of ossification anterior to sternebra #1
	R0069	5	S	vertebral anomaly w/wo associated rib anomaly [arches #10 (right), #11 (left) enlarged and misshapen with associated ribs slightly enlarged in comparison with the contralateral rib]
	R0078	1	S	extra site of ossification anterior to sternabra #1 [2]
		7	S	extra site of ossification anterior to sternabra #1
	R0091	4	S	extra site of ossification anterior to sternabra #1
	R0094	8	S	extra site of ossification anterior to sternabra #1
CU	R0005	3	V	lung agenesis [post-caval lobe]
		5	S	extra site of ossification anterior to sternabra #1
	R0037	10	V	lung agenesis [post-caval lobe absent]
	R0105	8	V	lung agenesis [post-caval lobe]
	R0025	11	S	extra site of ossification anterior to sternabra #1 [one, tread-like atachment between extra site and sternebra #1]
	R0043	9	S	vertebral anomaly w/wo associated rib anomaly [absent arch and hemicentra at #9 (right), #7 and #8 vertebrae malaligned, ribs #8 and #9 (right) fused at proximal portions and articulating with arch #8, ribs #7 and #8 (left) fused at proximal portions]

GRP	DAM	FETUS	TYPE	FINDING
LD	R0042	2	V	lung agenesis [post-caval lobe]
		6	E, V	flexed paw [left forepaw]
			V	lung agenesis [post-caval lobe]
		7	V	lung agenesis [post-caval lobe]
		9	V	lung agenesis [post-caval lobe]
	R0021	5	V	kidney unascended [right, also smaller than normal]
		8	S	extra site of ossification anterior to sternabra #1 [2]
	R0029	1	V	lung agenesis [post-caval lobe]
		2	V	lung agenesis [post-caval lobe]
	R0050	4	S	extra site of ossification anterior to sternabra #1 [2]
	R0051	1	S	sternebra(e) severely malaligned [#5 (bipartite) right portion fused to #4, #4 malaligned (moderate) and right portion fused to #3, #3 and #2 malaligned (slight)]
MD	R0085	5	E	domed head [slight]
	R0056	2	V	lung agenesis [post-caval lobe]
			S	extra site of ossification anterior to sternabra #1
		4	V	lung agenesis [post-caval lobe]
	R0058	1	S	extra site of ossification anterior to sternabra #1
		8	V	lung agenesis [post-caval lobe absent]
	R0085	5	V	heart and/or great vessel anomaly [bulbous aortic arch, stenotic pulmonary trunk and ductus arteriosus, left atrium enlarged, hypoplastic right ventricle, small quantity of clear amber fluid in thoracic cavity]
	R0106	5	V	lung agenesis [post-caval lobe]
		6	V	lung agenesis [post-caval lobe]
	R0001	1	S	rib anomaly [#10 and #11 (right) fused at articulating heads and articulate with arch #10 (right)], extra site of ossification anterior to sternabra #1
	R0002	2	S	extra site of ossification anterior to sternabra #1
		3	S	extra site of ossification anterior to sternabra #1 [2]
		4	S	extra site of ossification anterior to sternabra #1
		7	S	extra site of ossification anterior to sternabra #1
		8	S	extra site of ossification anterior to sternabra #1
	R0020	5	S	extra site of ossification anterior to sternabra #1 [sternebra #1 slightly misshapen]
HD	R0011	4	V	lung agenesis [post-caval lobe]
	R0018	3	V	diaphragmatic hernia [muscular portion on right side, portion of medial lobe protrudes through opening into thoracic cavity]
		4	V	diaphragmatic hernia [muscular portion, portion of medial liver lobe protrudes into thoracic cavity]
			S	cervical vertebrae anomaly [arch #2 (right) formed in two pieces with ventral aspect small, with associated malpositioning (right) and malalignment of vertebra #2]
		6	S	cervical vertebrae anomaly [arch #1 (right) small and misshapen, left ventral aspect small]
		12	S	cervical vertebrae anomaly [arch #1 (bilateral) formed in two pieces, ventral aspect of arch #1 (right) small, arch #2 (left) formed in two pieces and misshapen, ventral spect small, cervical centra #3 and #4 fused]
	R0049	6	V	lung agenesis [post-caval lobe]
		7	S	extra site of ossification anterior to sternabra #1
	R0070	6	V	trachea anomaly [cartilaginous rings malformed and nonsymmetrical, entire length]
	R0088	2	V	lung agenesis [post-caval lobe]
		4	V	lung agenesis [post-caval lobe]
		5	V	lung agenesis [post-caval lobe]
	R0096	2	S	extra site of ossification anterior to sternabra #1 [2]

GRP	DAM	FETUS	TYPE	FINDING
HD (con't)	R0096 (con't)	3	V	lung agenesis [post-caval lobe]
		6	V	lung agenesis [post-caval lobe]
		8	S	extra site of ossification anterior to sternabra #1 [2]
	R0097	3	V	lung agenesis [post-caval lobe]
	R0022	5	S	extra site of ossification anterior to sternabra #1
	R0026	2	S	extra site of ossification anterior to sternabra #1
		4	S	extra site of ossification anterior to sternabra #1

Data on variations were summarized in the following sponsor's table:

TABLE 10

STUDY NO.: 3477.10 — SELEGILINE TRANSDERMAL SYSTEM: A DEVELOPMENTAL TOXICITY — PAGE 1
CLIENT: SOMERSET PHARMACEUTICALS — (SEGMENT II) STUDY IN NEW ZEALAND WHITE RABBITS
CLIENT NO.: TOX-543-98B — SUMMARY OF FETAL OBSERVATIONS - VARIATIONS

GROUP:	1	2	3	4	5	1	2	3	4	5
TARGET LEVEL (MG/KG/DAY):	0	0	2.5	10.0	40.0	0	0	2.5	10.0	40.0
NUMBER EXAMINED EXTERNALLY	152	155	181	164	127	16	18	20	19	16
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	152	155	181	164	127	16	18	20	19	16
RETROCAVAL URETER	1	8	3	8	3	1	6	3	4	3
MAJOR BLOOD VESSEL VARIATION	1	5	2	4	4	1	3	2	4	2
GALLBLADDER ABSENT OR SMALL	1	0	0	0	1	1	0	0	0	1
HEMORRHAGIC RING AROUND THE IRIS	0	0	0	1	1	0	0	0	1	1
SPLEEN PALE IN COLOR	0	0	0	0	3	0	0	0	0	1
NUMBER EXAMINED SKELETALLY	152[a]	155[b]	181[c]	164[d]	127[e]	16[a]	18[b]	20[c]	19[d]	16[e]
13TH FULL RIB(S)	53	52	63	47	47	12	14	15	14	14
27 PRESACRAL VERTEBRAE	14	21	19	15	22	7	10	11	7	9
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	6	18	9	6	12	6	9	7	5	9
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	7	9	10	5	4	5	4	6	4	3
13TH RUDIMENTARY RIB(S)	35	33	33	32	26	14	15	14	15	12
STERNEBRAE WITH THREAD-LIKE ATTACHMENT	0	3	6	0	5	0	3	2	0	2
ACCESSORY SKULL BONE(S)	1	1	2	2	4	1	1	1	2	4
HYOID ARCH(ES) BENT	1	3	11	7	5	1	2	6	5	5
ZYGOMATIC ARCH FUSION	0	3	1	3	2	0	2	1	3	1
7TH STERNEBRA	0	0	1	1	2	0	0	1	1	2
7TH CERVICAL RIB(S)	10	6	3	3	3	4	4	2	2	2
COSTAL CARTILAGE MALALIGNED	0	0	0	0	2	0	0	0	0	2

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL
NOTE: GROUP 1 RECEIVED THE PLACEBO, GROUP 2 WAS UNTREATED AND GROUPS 3, 4 AND 5 RECEIVED SELEGILINE.
[a] INCLUDES NINE DISARTICULATED FETUSES FROM TWO LITTERS.
[b] INCLUDES FOUR DISARTICULATED FETUSES FROM ONE LITTER.
[c] INCLUDES TEN DISARTICULATED FETUSES FROM TWO LITTERS.
[d] INCLUDES SIX DISARTICULATED FETUSES FROM THREE LITTERS.
[e] INCLUDES ONE DISARTICULATED FETUS FROM ONE LITTER.

Although the sponsor did not consider any findings drug-related, two findings were notable, i.e., accessory skull bone(s) and costal cartilage malaligned. These incidence of these findings [% affected fetuses (% affected litters)] were dose-related, as shown below:

FINDING	DOSE (mg/kg)				
	0 (CP)	0 (CU)	2.5	10	40
accessory skull bone(s)	0.7 (6.2)	0.6 (5.6)	1.1 (5.0)	1.2 (10.5)	3.2 (25)
costal cartilage malaligned	0 (0)	0 (0)	0 (0)	0 (0)	1.6 (12.5)

The incidence of accessory skull bone(s) at the HD are within sponsor's HC range [affected fetuses: mean: 1.08% (0-4.58%); affected litters: mean: 6.84% [range: 0-31.58%]. However, the HC represented the results of 11 studies; the incidence of accessory skull bone(s) was markedly higher in 1 of the 11 studies. With this study

removed [i.e., 4.58% affected fetuses, 31.58% affected litters], the mean (range) for affected fetuses is 0.73% (range: 0-1.83%) and the mean (range) for affected litters is 4.37% (range: 0-10%). The incidence of costal cartilage malalignment at the HD exceeds the sponsor's HC range [affected fetuses: mean: 0.2% (range: 0-1.52%); affected litters: mean: 1.4% (0-9.52%].

Toxicokinetics: the data were summarized in the following sponsor's table:

Table 1. Mean ± SD concentrations of selegiline and its metabolites in maternal rabbit plasma.

Analyte	Mean ± SD (N=6) Analyte Concentration (ng/mL) in Maternal Plasma		
	23 hr post dose GD 6	23 hr post dose GD 12	23 hr post dose GD 18
2.5 mg/kg/day targeted			
Selegiline	0.790 ± 0.981	1.23 ± 1.01	1.18 ± 1.08
N-Desmethylselegiline	2.55 ± 0.90	3.66 ± 1.14	2.79 ± 0.93
Amphetamine	0.786 ± 0.310	1.10 ± 0.30	0.948 ± 0.177
Methamphetamine	0.753 ± 0.362	1.03 ± 0.26	0.812 ± 0.194
10 mg/kg/day targeted			
Selegiline	5.31 ± 1.40	6.61 ± 0.82	4.84 ± 0.99
N-Desmethylselegiline	12.8 ± 1.9	14.2 ± 3.9	8.99 ± 1.47
Amphetamine	4.34 ± 1.71	4.31 ± 1.64	2.98 ± 1.05
Methamphetamine	4.19 ± 1.96	4.13 ± 1.75	2.79 ± 1.36
40 mg/kg/day targeted			
Selegiline	29.2 ± 4.0	57.8 ± 21.5	42.4 ± 11.1
N-Desmethylselegiline	49.1 ± 11.6	65.5 ± 21.1	47.7 ± 8.1
Amphetamine	11.1 ± 2.7	14.2 ± 2.6	11.1 ± 1.6
Methamphetamine	12.2 ± 3.2	15.6 ± 4.8	10.6 ± 2.3

7. Study Type: peri-postnatal development (Segment III) study in rat.
Study Title: **Selegiline Transdermal System: a prenatal and postnatal (Segment III) study in Sprague-Dawley rats** (Study No: TOX-544-98B, Vol #1.038, Conducting laboratory and location: [redacted] date of study initiation: 1/22/99, GLP, QA)

Methods

Dosing

species/strain: Sprague-Dawley rat [redacted]
#/sex/group or time point: 25/grp
age: ≈88 days of age
weight: 200-269 gm
satellite groups used for toxicokinetics or recovery: 6/grp for CU and 75 mg/kg (TK analysis)
dosage groups in administered units: 0 (CP), 0 (CU), 10, 30, 75 mg/kg. [The doses were based on the results of a dose-range finding Segment II study in rat.]
route, form, volume, and infusion rate: transdermal. Patches were applied to clipped skin and held in place with binding material, a shoulder strap, and athletic tape.

Duration of dosing: F_0 dams were dosed from Day 6 through Day 21 of gestation, and on Days 1-21 of lactation. Dosing was withheld from Gestation Day 22 through parturition [i.e., Lactation Day 0] in order not to unnecessarily disrupt delivery.

Drug, lot#, and % purity: STS, patch lot no. 26E006L, purity of drug substance:

Formulation/vehicle: STS, 20 mg/20 cm^2. Patches were not analyzed by [redacted] however, the Lactation Day 20 patches for the dosed grps [main-study; 6/grp] were stored and shipped to [redacted] for analysis.

Observations and times:

Clinical signs: animals were observed twice daily. Dermal irritation was assessed on Gestation Days 6, 9, 12, 15, 18, and 20, and on Days 1, 4, 7, 10, 14, 17, and 21 of lactation [parturition was designated Day 0 of lactation] using the Macroscopic Dermal Grading System.

Body weights: body wts were recorded on Gestation Days 0, 6, 9, 12, 15, 18, and 20 and on Days 1, 4, 7, 10, 14, 17, and 21 of lactation.

Food consumption: food intake was recorded on Gestation Days 0-6, 6-9, 9-12, 12-15, 15-18, and 18-20. Food consumption was not recorded during the lactation period.

Ophthalmoscopy: n/a

ECG: n/a

Hematology: n/a

Clinical chemistry: n/a

Urinalysis: n/a

Reproduction parameters: females were transferred to breeding cages on Day 18 of gestation. Animals were check twice daily for signs of parturition; "Signs of difficult or prolonged delivery were recorded..." Pups remained with dams throughout the 21-day lactation period. "Abnormal nursing and nesting behaviors were recorded..." On Day 6 postpartum, dams were tested for pup retrieval. Pups were placed on the opposite area of the cage from the dam, and the number of pups retrieved by the dam within 5 min was recorded.

Gross pathology: a complete necropsy was performed on all dams. All F_0 dams with a litter were sacrificed on Day 21 of lactation. All F_0 dams losing an entire litter were sacrificed following the death of all pups. All F_0 dams that did not deliver were sacrificed 25 days following evidence of mating; uteri with no evidence of pregnancy were placed in a10% aqueous ammonium sulfide solution and examined for early embryofetal deaths.

Organ weights: n/a

Histopathology: n/a

Toxicokinetics: blood and milk samples were collected from satellite animals on Day 10 of lactation [23 hrs following patch application on Day 9] for quantitation of selegiline and metabolites. Blood samples were also collected from main-study animals [6/grp] on Day 21 of lactation [the same animals for which patches were saved for analysis]; the schedule for blood collection was the same as that for the satellite animals. The analyses were conducted by .

Pup (F_1) parameters: following pup examination and weighing on Day 4 of lactation, each litter was culled to 8 pups [4 M, 4 F] where possible. Culled pups were sacrificed with no further examination. Pups were examined on Days 0, 4, 7, 14, and 21 of lactation. Sex was recorded on Day 0 and confirmed on Days 4 and 21 of lactation. Pup body wts were recorded on Days 1, 4, 7, 14, and 21 of lactation. Dead pups (if intact) were necropsied. Cannibalized pups were discarded without examination.

Developmental and functional parameters were as follows: pinnae detachment [from Day 4 until complete detachment of both pinnae observed], surface righting response [from Day 5 until a positive response was observed (i.e., righting within 15 sec)], cliff aversion [from Day 9 until a positive response (i.e., backing away from cliff edge within 30 sec) was observed], eye opening [from Day 14 until both eyes completely open], startle [from Day 14 until a positive response (i.e., jump or flinch) was observed] and auditory [on Day 21 using a Galton whistle with both ears equidistant from the whistle; movement of the ears (i.e., Preyer's reflex) was considered a positive response] responses.

F_1 reproductive parameters: following weaning, pups [20/sex/grp] were selected for evaluation of

vaginal opening [from postpartum Day 33 until vaginal opening occurred] or preputial separation [from postpartum Day 40 until complete separation was observed], behavioral testing [open-field evaluation (between Days 35-45 postpartum; no. of squares entered was recorded), Biel (multiple-T) water maze (between Days 45-55 postpartum; swimming ability, T-maze performance and recall)], and reproductive performance. Selection of pups for F_1 reproductive performance evaluation occurred on Days 9 and 28 postpartum. When possible, 1/sex/litter was selected. According to the report, "Each pup was examined externally prior to selection. Only animals of suitable health were included in the selection process." F_1 pups were observed twice daily for mortality; body wt was recorded twice a week in males, and twice a week in females until evidence of mating was detected. F_1 pups not selected for assessment of reproductive performance were sacrificed, and examined for external and visceral findings. At ≈10 wks of age, F_1 animals were mated [sibling matings were avoided] for up to 20 days. The following parameters were recorded in F_1 females: clinical signs [daily], body wt [Gestation Days 0, 4, 7, 10, 14, 17, and 20 and Days 1, 4, 7, 10, 14, 17, and 21 of lactation], delivery, lactation behavior [F_2 pups remained with F_1 dams to Day 21 of lactation]. F_1 dams were sacrificed according to the same criteria and schedule as for F_0 dams. F_2 pups were culled (to 4/sex/litter) on Day 4 of lactation.

F_2 parameters: the following parameters were recorded in F_2 pups: viability [daily], sex, body wt [Lactation Days 1, 4, 7, 14, and 21], gross pathology [Day 21 of lactation]; pups found dead, but not cannibalized were necropsied, whereas those that were cannibalized were discarded without examination.

Experimental design: summarized in the following sponsor's figure:

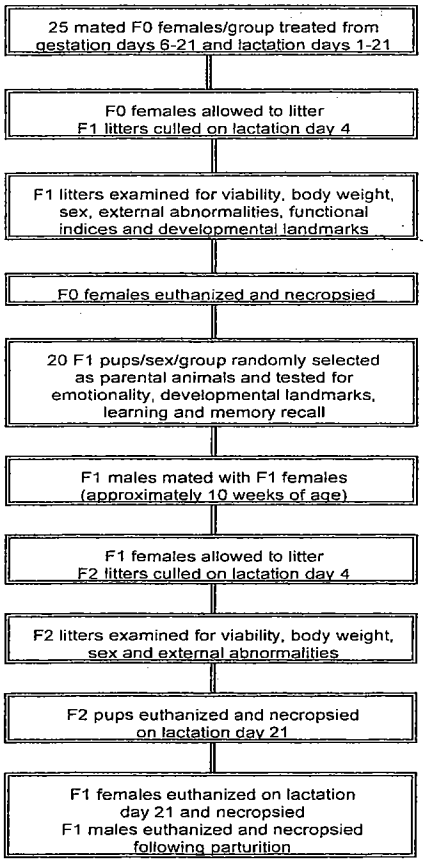

Figure 1. Experimental Design

Results

Mortality: no unscheduled deaths occurred in F_0 dams.

Pregnancy: 4 females were not pregnant [1 CP, 1 CU, 1 LD, 1 HD]. Therefore, reproductive data were available for 24/grp in CP, CU, LD, and HD, and for 25 F in the MD grp.

Clinical signs: selected clinical signs [no. of occurrences/no. of affected animals] are summarized in the following table:

SIGN	DOSE (mg/kg)				
	0 (CP)	0 (CU)	10	30	75
total litter loss	0/0	0/0	0/0	0/0	8/8
cool to touch	0/0	0/0	0/0	0/0	2/2
feces small in size	0/0	0/0	2/2	3/2	10/6
few feces	0/0	0/0	0/0	0/0	15/8
brownish mucoid vaginal discharge	1/1	0/0	0/0	0/0	4/4
reddish colored vaginal discharge	1/1	0/0	0/0	4/3	13/12
urine stain	1/1	0/0	0/0	2/2	39/17
scab-nose area	0/0	0/0	4/2	122/11	149/17
swelling-nose area	0/0	0/0	0/0	3/2	4/3
dk material around nose	32/10	0/0	20/12	47/9	170/23
dk material around mouth	0/0	0/0	1/1	3/2	9/6
patch off					
0.125	1/1		4/3	2/2	1/1
0.25	0/0	--	0/0	2/2	1/1
0.5	0/0		0/0	4/2	0/0
whole	34/11		0/0	0/0	3/2

Dermal irritation was observed in all grps receiving patches. The only possible drug-related finding was an increase in severity of erythema: Grade 2 erythema was observed in 12/7 CPF, 12/8 LDF, 15/10 MDF, and 34/19 HDF [data expressed as "no. of occurrences/no. of animals affected"].

Body weights: during the gestation period, mean body wt was 9-11% lower in CP as compared to CU. Compared to CP, mean body wt was significantly lower at the MD and HD on Day 9 [6 and 12%, respectively], and on Days 12 and 15 at the HD [6-7%]. The primary effect on body wt gain was noted during the first 3 days of dosing, i.e., body wt loss was noted at the MD [-6 gm] and HD [-27 gm], compared to gains of 7-13 gm/day in the other grps. During Days 9-12, mean body wt gain was highest in HD animals [29 gm/day vs 15-19 gm/day in other grps], indicative of some catch-up growth. At other times, mean body wt gain was fairly similar among grps, except for an increase in body wt gain in the CU [Day 0-6, Day 15-18].

During the lactation period, mean body wt was lower [2-9%] in CP as compared to CU. The mean body wt gain in HDF was only slightly lower than in CP [2-4%]; this difference was not statistically significant. Mean body wt gain was similar among grps, with the exception of a lower wt gain during Lactation Days 10-14 at the HD [53%, i.e., 9 gm/day vs 19 gm/day].

Food consumption: food intake was significantly reduced in MDF (23%) and HDF (45%) during Gestation Days 6-9, compared to CP. In addition, food intake in CP was 15-20% lower compared to CU during Gestation Days 0-6 and 6-9.

Reproductive parameters: pup retrieval was reduced at all doses compared to CP, and in CP compared to CU. [The sponsor summarized the data only in terms of % of total fetuses.] The total number of pup deaths at parturition was significantly elevated in HDF. These data are summarized in the following table:

FINDING	DOSE (mg/kg)				
	0 (CP)	0 (CU)	10	30	75
litter retrieval					
% of total pups	95.8	100*	87.8**	82.4**	83.9**
pups retrieved/total pups	183/191	180/180	159/181	155/188	94/112
dead pups (total number), D0	13	15	11	14	30*

*p< 0.05, **p<0.01

There were no apparent drug-related effects on gestation length, post implantation loss, no. of live pups (Day 0 of lactation), litter size (Day 0 of lactation), or sex ratio. However, the sponsor did note that post-implantation loss tended to be higher in the MD and HD grps [1.7 ± 1.57, 1.8 ± 1.81, 1.5 ± 0.89, 2.4 ± 1.71, and 2.3 ± 2.07 in CP, CU, LD, MD, and HD grps, respectively].

Gross pathology: there were no apparent drug-related gross findings in F_0 dams, including those that lost their entire litter, except for implantation scars [uterine horn] which were observed in all F_0 dams that lost their entire litter.

Toxicokinetics: the data were summarized in the following sponsor's tables:

Table 1. Mean concentrations of selegiline and its metabolites in maternal rat plasma and milk

Analyte	Mean ± SD (N=5) Analyte Concentration (ng/mL) 23 hr post dose Lactation Day 9		Mean Milk to Plasma Ratio
	Plasma	Milk	
75 mg/kg targeted (actual mean dose applied 77.5 mg/kg)			
Selegiline	39.2 ± 8.7	580 ± 203	14.8
N-Desmethylselegiline	9.86 ± 1.58	47.3 ± 18.5	4.80
Amphetamine	21.0 ± 7.3	102 ± 53	4.84
Methamphetamine	32.5 ± 7.6	160 ± 60	4.91

Table 2. Mean ± SD concentrations of selegiline and its metabolites in maternal rat plasma 23 hr post dose Lactation Day 20

Analyte	Mean ± SD (N=6) Analyte Concentration (ng/mL) in Maternal Plasma 23 hr post dose Lactation Day 20		
	10 mg/kg targeted (actual 7.3 mg/kg)	30 mg/kg targeted (actual 29.9 mg/kg)	75 mg/kg targeted (actual 76.6 mg/kg)
Selegiline	3.34 ± 0.89	18.1 ± 3.5	42.5 ± 11.7
N-Desmethylselegiline	1.02 ± 0.32	4.62 ± 1.49	12.6 ± 4.4
Amphetamine	1.90 ± 0.67	8.06 ± 2.32	18.5 ± 6.6
Methamphetamine	2.30 ± 0.52	11.2 ± 3.0	32.0 ± 11.9

F_1 pup parameters: pup survival during the lactation period was reduced in MD and HD grps. Pup survival was reduced in CP as compared to CU during the first 4 days of lactation. The data were summarized in the following sponsor's table:

TABLE 13

STUDY NO.: 3477.11 SELEGILINE TRANSDERMAL SYSTEM: A SEGMENT III STUDY IN RATS PAGE 2
CLIENT: SOMERSET PHARMACEUTICALS SUMMARY OF F1 PUP VIABILITY
CLIENT NO.: TOX-544-98B

DURING LACTATION

	GROUP:	1	2	3	4	5
	TARGET LEVEL (MG/KG/DAY):	0	0	10	30	75
	TEST ARTICLE:	PLACEBO	UNTREATED	SELEGILINE	SELEGILINE	SELEGILINE
DAY 1	NO. ALIVE/NO. PUPS	314/319	314/318	311/323	290/332	204/308
	PERCENT	98.4	98.7	96.3	87.3**	66.2**
DAY 4 BEFORE SELECTION	NO. ALIVE/NO. PUPS	300/319	311/318	293/323	259/332	158/308
	PERCENT	94.0	97.8*	90.7	78.0**	51.3**
DAY 4 AFTER SELECTION	NO. ALIVE/NO. PUPS	192/192	180/180	183/183	194/194	129/129
	PERCENT	100.0	100.0	100.0	100.0	100.0
DAY 7	NO. ALIVE/NO. PUPS	191/192	178/180	181/183	187/194	108/129
	PERCENT	99.5	98.9	98.9	96.4	83.7**
DAY 14	NO. ALIVE/NO. PUPS	191/192	177/180	180/183	184/194	99/129
	PERCENT	99.5	98.3	98.4	94.8*	76.7**
DAY 21	NO. ALIVE/NO. PUPS	191/192	176/180	180/183	182/194	99/129
	PERCENT	99.5	97.8	98.4	93.8**	76.7**

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01

There were a number of dose-related clinical findings observed in treated pups during the lactation period. Selected findings are summarized in the following table. [Data are expressed as "no. of occurrences/no. of affected animals"(% affected animals).]

FINDING	DOSE (mg/kg)				
	0 (CP)	0 (CU)	10	30	75
dead					
found dead	25/25 (8)	20/20 (6)	32/32 (10)	57/57 (18)	89/89 (35)
missing	7/7 (2)	5/5 (2)	10/10 (3)	33/33 (11)	72/72 (28)
constriction(s)	4/2 (0.6)	9/5 (2)	13/9 (3)	3/2 (0.6)	15/10 (4)
mid/distail tail necrosis	1/1 (0.3)	10/5 (2)	8/6 (2)	7/6 (2)	23/14 (5)
open lesion(s)	1/1 (0.3)	2/2 (0.6)	3/3 (1)	22/20 (6)	25/25 (10)
cool to touch	6/6 (2)	0/0 (0)	1/1 (0.3)	18/18 (6)	20/20 (8)
pale in color	0/0 (0)	0/0 (0)	6/6 (2)	3/3 (1)	14/12 (5)
scabs	29/20 (6)	22/17 (5)	26/20 (6)	74/54 (17)	60/45 (18)
small in size	1/1 (0.3)	2/2 (0.6)	2/2 (0.6)	4/4 (1)	4/3 (1)
subcutaneous hemorrhage(s)	6/6 (2)	7/7 (2)	12/10 (3)	27/27 (9)	28/25 (10)
no apparent eyeball	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	1/1 (0.3)
bent toe(s)	1/1 (0.3)	2/1 (0.3)	3/2 (0.6)	4/4 (1)	14/13 (5)
missing digit(s)	11/5 (2)	18/11 (3)	15/8 (2)	5/4 (1)	18/13 (5)
no observable milk in stomach	0/0 (0)	0/0 (0)	0/0 (0)	1/1 (0.3)	3/3 (1)
portion of tail absent	0/0 (0)	8/4 (1)	14/10 (3)	8/3 (1)	14/6 (2)
short tail	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	3/1 (0.4)
tail tip absent	0/0 (0)	3/2 (0.6)	0/0 (0)	23/10 (3)	10/7 (3)

Pup body wt was reduced in CP compared to CU throughout the lactation period [10-24%]. Pup wt was reduced at the MD [9-18%] and HD [17-33%] compared to CP.

Selected gross findings in pups found dead or sacrificed moribund during the lactation period are summarized in the following table [data are expressed as no. of pups affected/total no. of pups examined (%)]:

FINDING	DOSE (mg/kg)				
	0 (CP)	0 (CU)	10	30	75
tail absent	0/26 (0)	0/20 (0)	0/31 (0)	0/57 (0)	4/93 (4)
tail necrotic	0/26 (0)	0/20 (0)	0/31 (0)	1/57 (2)	6/93 (6)
abdominal body fat depletion	0/26 (0)	0/20 (0)	0/31 (0)	0/57 (0)	4/93 (4)
bone fracture	0/26 (0)	0/20 (0)	0/31 (0)	0/57 (0)	1/93 (1)
microphthalmia	0/26 (0)	0/20 (0)	0/31 (0)	0/57 (0)	1/93 (1)
pale liver	1/26 (4)	1/20 (5)	1/31 (3)	5/57 (9)	5/93 (5)
open skin lesion	0/26 (0)	0/20 (0)	0/31 (0)	3/57 (5)	10/93 (11)
scabbing	0/26 (0)	0/20 (0)	5/31 (16)	1/57 (2)	13/93 (14)
subcutaneous hemorrhaging	0/26 (0)	0/20 (0)	0/31 (0)	0/57 (0)	2/93 (2)
milk not present in stomach	17/26 (65)	15/20 (60)	15/31 (48)	30/57 (53)	37/93 (40)

The sponsor indicated that "There was a slight increase in the incidence of pale liver and milk not present in the stomach of F_1 pups in the 30 and 75 mg/kg/day groups..." However, based on total number of pups per grp [not per sex/grp], the % of affected animals was not increased in a dose-related manner. There were no apparent drug-related gross findings in pups sacrificed on schedule.

Drug-related effects were observed on most of the developmental parameters tested. The data [%] are summarized in the attached table [data have been pooled across sex, unless sex-specific]. [Total n = 191, 179, 181, 189, and 112 CP, CU, LD, MD, and HD pups, respectively.]

There were no apparent drug-related effects on exploratory behavior in the open-field test or on T-maze performance in either males or females.

F_1 generation selected for assessment of reproductive parameters (postweaning phase):

Mortality: one F_1 LDF died on Day 23 post-weaning. [This animal did not appear to be among those selected for assessment of reproductive performance.] No additional unscheduled deaths occurred postweaning.

Clinical signs: no additional clinical signs [other than those apparent during lactation (i.e., bent toes, tail tip absent)] were observed.

Body wt: in males, mean body wt was reduced in CP (12%) compared to CU throughout the 98-day postweaning period. Compared to the CP, mean body wt was reduced at the MD [15-16% through Day 10] and HD [10-29% through Day 63]. Mean body wt on Day 98 was similar in CP and treated grps; however, body wt at the HD tended to be lower (but not significantly; 7%). Body wt gain was primarily affected in MD during the first 7 days postweaning (16-24%) and in HD pups during the first 10 days postweaning (25-28%). Body wt gain was not consistent greater in CU as compared to CP [significant only on Days 10-14 and 17-21 postweaning].

In females, mean body wt was significantly higher in CU as compared to CP only on during the first 7 days postweaning [14-18%]. Compared to CP, mean body wt was reduced in HD pups [10-25%] through Day 28 postweaning. Body wt in HD pups was only slightly lower (8%) than in CP on Day 98 postweaning. Body wt gain was reduced only in HD pups, and only sporadically [Days 3-7, Days 52-56]. During the gestation period, mean body wt was significantly reduced only at the HD (11%) and only on Day 20; however, body wt tended to be lower in HDF (7-8%) compared to CP throughout the gestation period. Body wt gain was reduced at the HD (37-29%) during Days 14-20 of gestation. During lactation, mean body wt was consistently lower in HDF (6-10%) compared to CP; however, the effect was statistically significant only on Days 4 and 17

TEST	DAY	DOSE (mg/kg)				
		0 (CP)	0 (CU)	10	30	75
pinnae detachment	4	100	99.4	97.3	94.3	88.6
	5		100	100	100	96.7
	6					100
surface righting	5	82.7	82.7	76.2	64.6	59.8
	6	95.8	97.8	94.5	89.9	92.9
	7	98.4	100	97.8	96.8	93.8
	8	100		98.3	98.9	97.3
	9			99.4	99.5	100
	10			100	100	
cliff aversion	9	95.8	92.1	91.7	88.2	84.3
	10	100	98.9	99.4	96.8	97.1
	11		100	100	99.5	99.0
	12				100	100
eye opening	14	67.5	65.0	73.9	47.8	17.2
	15	93.7	97.2	98.9	85.3	68.7
	16	100	99.4	100	98.9	91.9
	17		100		98.9	99.0
	18				100	100
startle response	14	100	99.4	87.2	79.3	63.6
	15		100	98.9	95.1	81.8
	16			100	98.9	93.9
	17				100	99.0
	18					100
auditory response	21	100	100	100	100	100
vaginal opening	33	80.0	95.0	85.0	60.0	40.0
	34	95.0	100	95.0	90.0	70.0
	35	100		95.0	90.0	75.0
	36			95.0	95.0	85.0
	37			100	95.0	90.0
	38				95.0	90.0
	39				95.0	90.0
	40				95.0	95.0
	41				100	95.0
	42					95.0
	43					95.0
	44					95.0
	45					95.0
	46					100
preputial separation	40	0.0	10.0	5.0	5.0	0.0
	41	5.0	40.0	20.0	10.0	0.0
	42	40.0	60.0	35.0	10.0	0.0
	43	65.0	90.0	55.0	15.0	5.0
	44	90.0	95.0	75.0	35.0	15.0
	45	95.0	95.0	80.0	65.0	25.0
	46	100	100	90.0	65.0	40.0
	47			95.0	70.0	60.0
	48			100	75.0	70.0
	49				85.0	70.0
	50				95.0	80.0
	51				95.0	90.0
	52				95.0	90.0
	53				95.0	100
	54				95.0	
	55				100	

of lactation (10-8%). Body wt gain was increase in HFD_1 (compared to CP) during Days 4-7 of lactation (10 gm vs 0 gm).

Reproductive parameters: there were no clear drug-related effects on copulation index, fertility index, precoital interval, gestation length]. However, 1 HDF_1 was the only female to have lost the entire litter. One LDF_1 was found dead prior to mating and 1 CP, 5 CU, 2 MDF_1, and 5 HDF_1 were not gravid at necropsy. Therefore, the total number of females with live pups was 19/20, 15/20, 19/20, 18/20, and 14/20 in CP, CU, LD, MD, and HD grps, respectively. The mean no. of implantation scars was reduced (21%) in HDF; however, post-implantation loss [i.e., implantation scar - no. of live pups (Day 0)] was not affected. No data were provided on the no. of corpora lutea or pre-implantation loss. On Lactation Day 0, mean litter size was significantly reduced [13.7, 14.0, 14.5, 12.8, and 10.8 in CP, CU, LD, MD, and HD, respectively] and M:F ratio was significantly increased [127:134, 104:106, 126:149, 121:109, and 90:61 in CP, CU, LD, MD, and HD, respectively] at the HD.

Gross pathology: no gross lesions were evident in the 1 LDF_1 found dead.

In males (sacrificed on Day 97/98), findings consisted of the following: (a) bent toes [0/20, 0/20, 0/20, 1/20, and 3/20 in CP, CU, LD, MD, and HD, respectively], (b) tail tip absent [0/20, 0/20, 0/20, 2/20, and 4/20 in CP, CU, LD, MD, and HD, respectively], (c) small epididymes and testes were noted in 3/20 HDM_1 [#533-02, #533-09, #466-04]. In addition, soft testes were noted in HDM_1 #466-04 and #533-02. The sponsor noted that #533-02 and #533-09 were littermates, and that the females mated with these males "...failed to deliver offspring". Small epididymides and discolored (purplish-tan) testes were detected in 1 MDM_1 [#574-07].

There were no apparent gross findings in females sacrificed on Lactation Day 21. Of the females that did not deliver and were sacrificed on Gestation Day 25, only 1 HDF_1 was ammonium sulfide positive [i.e., gravid] upon examination. The sponsor noted that this female [#533-14] was from the same litter as the 2 HDM_1 that exhibited epididymal and testes lesions. Of the females with no evidence of mating (that were sacrificed 25 days after the mating period, a bent tail (medial portion) and tail tip absent were detected in 1 HDF_1. In the 1 HDF_1 with total litter loss, gross findings consisted of the following: bent tail, liver lesion [i.e., "medial lobe misshapen with additional portion of liver within bifurcation"], reddened thymus, and presence of implantation scars and abnormal contents in uterine horns.

F_2 generation

Pup viability: similar among grps during the lactation period [i.e., D1-21].

Clinical signs: the total no. of pups evaluated were 261, 209, 275, 228, and 150 in CP, CU, LD, MD, and HD, respectively.

Body wt: there were no drug-related effects.

Gross pathology: there were no apparent drug-related findings in pups found dead during lactation [M: 10, 2, 4, 1, and 4 in CP, CU, LD, MD, and HD grps, respectively; F: 4, 2, 8, 6, and 3 in CP, CU, LD, MD, and HD grps, respectively] or in survivors.

Summary of individual study findings:

Mating and fertility: in the Segment I study conducted in Sprague-Dawley rat, both males and females were treated with selegiline STS. Males were treated for 4 wks prior to mating, throughout the ≤14-day mating period, and to sacrifice. Females were treated for 2 wks prior to mating, throughout the ≤14-day mating period, and until Gestation Day 6, and sacrificed on Gestation Day 15. Doses were 0 [placebo control, CP], 0 [untreated control, CU], 10, 30, and 75 mg/kg. [No data were provided on the actual

doses delivered.] Observations consisted of the following: clinical signs, body wt, food consumption, gross pathology [male and female reproductive organs], sperm analysis, organ wts [selected, in males only]. There were no drug-related deaths. Clinical signs consisted of scabs and dark material around the nose and/or mouth [primarily HD]. As in the chronic toxicity studies, there were numerous instances in which patches were found off [whole or in part]. To what extent this compromised dosing is unknown; however, individual females were not notably affected and the percentage of dosing days on which at least 50% of the patch was found to be off or damaged was ≥10% only in 3/25 HDM. Therefore, the problems with patch adherence probably did not significantly compromise the study. TK analysis was not conducted during this study. Dermal irritation was evident in C and treated grps, with no obvious differences in incidence or severity among grps. Mean body wt was reduced in CP as compared to CU in both males [15-27%] and female [6-10%] throughout the pre-mating and mating periods. No drug-related effects were noted on mean body wt in males. In females, mean body wt [compared to CP] and body wt gain was reduced at the MD and HD during the pre-mating period. During gestation, body wt was reduced at the MD only on Day 0 of gestation, but was reduced through most of the gestation period at the HD [6-8% compared to CP; 11-13% compared to CU]. Body wt gain was consistently affected during gestation, being increased in MDF and HDF during the first 4 days of gestation and decreased at the HD during Gestation Days 8-15. Body wt gain was also increased in all treated grps during Gestation Days 12-15. Also, body wt gain was not consistently higher in CU compared to other grps during the gestation period. Food consumption was reduced in CP when compared to CU; however, no drug-related effects were observed in males. In females, food intake was reduced in CP (compared to CU) only during the gestation period. Food intake was further reduced in MDF (10%) and HDF (20%) during the premating, and in HDF during the gestation period. Taken together, the body wt and food consumption data suggest some drug effect on body wt in females at the MD and HD during the premating period and at the HD during the gestation; also, the patch/wrapping procedure had a consistent adverse effect on body wt and food consumption throughout the premating and gestation periods.

At necropsy, the only drug-related effect on male reproductive parameters was a decrease in prostate wt in HDM compared to both C grps. [There was also a small decrease in body-wt corrected epididymis wt at the HD.] There were no gross lesions apparent in reproductive organs. Sperm concentration and total sperm count were reduced in HDM; sperm motility and morphology were not affected. Sperm parameters were not affected by the presence of the patch/wrappings alone. There were no clear drug-relate effects on reproductive parameters, including copulation index and fertility index. The fertility index was lower in all treated grps compared to C grps [due to an increase in nongravid females]; however, the effect was not dose-related.

Therefore, in this study the only evidence of reproductive toxicity was observed in males [i.e., sperm, prostate wt]. The effects on sperm were not sufficient to interfere with fertility; however, successful impregnation is a very insensitive measure of male fertility. The NOEL for these findings was the MD. Since TK data were not collected, actual exposures achieved in this study were not documented. However, the same doses (and route) were used in the embryofetal development and the peri/postnatal development studies in rat. Based on the TK data from those studies, the AUC at the HD (a NOEL for female fertility) is estimated to be ≈15-19, 8-9, 7-13, and 5-8 times the AUCs for selegiline, N-desmethylselegiline, amphetamine, and methamphetamine, respectively, in humans at the proposed clinical dose. Plasma exposure (AUC) at the NOEL for male effects, using TK data [23-hr postdosing values x 24 hrs] collected in the 6-mo transdermal toxicity study, is estimated to be ≈4, 2, 1, and 0.5 times the AUCs for selegiline, N-desmethylselegiline, amphetamine, and methamphetamine, respectively, in humans at the proposed clinical dose.

Embryofetal development: studies were conducted in Sprague-Dawley rat and New Zealand White rabbit.

In the rat study, selegiline STS was administered on Gestation Day 6-17 at doses of 0 [placebo control, CP], 0 [untreated control, CU], 10, 30, and 75 mg/kg. [The actual delivered doses were not estimated.] Doses were based on the results of a dose-range finding study conducted (in 7/grp) using doses of 12.5-75 mg/kg. In that study, the primary finding was a transient body wt loss at all doses, although final corrected maternal body wts were fairly similar among grps. Malformations [micrognathia (maxillary or mandibular) and microstomia] were detected only in 1 HD fetus. Observations in the definitive study consisted of the following: clinical signs, body wt, food consumption, gross pathology, uterus wt, fetal examinations [Gestation Day 20], and TK (satellite animals). There were no unscheduled deaths. Clinical signs [limping/swelling of hindlimbs, urine stains, dark material around the nose and/or mouth] were evident primarily in HD animals, although there was a dose-related increase in the incidence of dark material around the nose. In contrast to the chronic toxicity studies and the mating and fertility study, there were only 2 instances [1 each at the LD and HD] in which patch integrity or adherence was impaired. Local irritation was detected in all grps, with no clear drug-related effects. This is in contrast to the dose-range finding study in which severity of erythema was increased at doses of 50 and 75 mg/kg. Mean body wt was reduced in CP (11%) compared to CU, and in HDF compared to CP. Mean body wt loss was observed during the first few days of dosing at the MD and HD; overall body wt gain was reduced at these doses (25-27%). Effects on food consumption were consistent with those observed on body wt. There were no apparent drug-related effects on the number of corpora lutea, implantation sites, pre-implantation loss or live/dead fetuses. There were increases in early and late resorptions, post-implantation loss, and a decrease (6%) in fetal wt at the HD; only the effect on fetal wt was statistically significant. There was also an increase in the number of visceral and total malformations at the HD. The sponsor argued that these findings were not indicative of a teratogenic effect since there was no apparent pattern of malformations and were accompanied by evidence of maternal toxicity [resulting from drug "...coupled with the stress of 24-hour transdermal exposure"]. However, maternal toxicity was not particularly severe and an examination of individual data indicated that dams with the greatest body wt effect were not necessarily those in which fetuses with malformations were observed. [Also, the presence of the CP grp controlled for the effect of patch/wrap placement alone on fetal effects.] It should also be noted that the only incidence of malformations observed in the dose-range finding study occurred at the HD (75 mg/kg). The sponsor is correct in that there was no clear pattern of malformations. The incidences of several skeletal variations [unossified #5 and/or 6 sternebra, malaligned sternebra, malaligned costal cartilage, unossified hyoid] were slightly increased in HD litters. The NOEL for maternal effects was the LD and the NOAEL for developmental toxicity was the MD. Steady-state trough plasma exposure [23 hrs postdosing; units = ng/mL], and estimated AUCs [based on 23-hr data] at these doses are provided below:

DOSE (mg/kg)	selegiline		N-desmethylselegiline		amphetamine		methamphetamine	
	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$
10	4.46 ± 2.29	107	1.06 ± 0.59	25.4	1.70 ± 0.70	41	2.52 ± 1.16	60.5
30	17.3 ± 3.5	415.2	3.97 ± 1.12	95.3	7.02 ± 2.30	168	9.97 ± 2.73	239
human [20 mg]		66.1		35.3		60.9		147

The sponsor also quantitated selegiline and metabolites in fetal blood and amniotic fluid. Fetal plasma levels at 23 hrs postdose were similar to maternal plasma levels, except for N-desmethylselegiline which was lower in fetal plasma particularly at the LD [20, 56, and 63% of maternal levels at LD, MD, and HD, respectively]. Amniotic fluid contained much higher levels of selegiline than maternal plasma [2-3 fold] at all doses, whereas levels of amphetamine and methamphetamine were slightly higher and levels of N-desmethylselegiline were slight lower compared to maternal plasma.

In the rabbit study, selegiline STS was administered [to 20/grp] at daily doses of 0 [placebo control, CP], 0 [untreated control, CU], 2.5, 10, and 40 mg/kg on Gestation Days 6 through 18. [Actual delivered doses were not estimated.] Doses were based on the results of a dose-range finding study conducted using

doses of 2.5-40 mg/kg t.d. In that study, transient body wt loss was observed in all treatment grps; final corrected maternal body wt was slightly reduced (6%) at 20 and 40 mg/kg. No reproductive effects were observed. In the definitive study, observations consisted of clinical signs, body wt, food consumption, gross pathology, fetal examination, and TK [in a subset of animals]. There were no apparent drug-related deaths or clinical signs. Patches were found off at some time during the dosing period in a number of animals (all grps affected), with the greatest incidence in the CP grp. Mean body wt was reduced in CPF (as compared to UCF) during the dosing period. Body wt loss was evident in all treated grps and in CPF during the first few days of dosing, with the loss being greatest in HDF. Body wt loss continued longer in treated grps (particularly in HDF) than in C grps. However, maternal body wt was not markedly affected since mean corrected wt was similar among grps. Food consumption was significantly reduced only in HDF. No gross lesions were detected in dams. A sufficient number of dams produced viable fetuses to allow for an adequate assessment [i.e., ≥16/grp]. Reductions in implantation sites and in the number of viable fetuses were observed at the HD; there were no drug-related effects on the number of corpora lutea, dead fetuses, early/late resorptions, post-implantation loss, fetal body wt or fetal sex ratio. The total number of fetuses with malformations was similar among grps; however, the number of fetuses with visceral malformations was increased at the HD, both in terms of affected fetuses [5/152 (3%), 3/155 (2%), 7/181 (4%), 6/164 (4%), and 11/127 (9%) in CPF, UCF, LDF, MDF, and HDF, respectively] and affected litters [4/16 (25%), 3/18 (17%), 3/20 (15%), 4/19 (21%), and 7/1644%) in CPF, UCF, LDF, MDF, and HDF, respectively]. This increase was due to the following: (a) an increase in lung agenesis at the HD, (b) diaphragmatic hernia and trachea anomaly at the HD only. The only skeletal finding of note (in terms of malformations) was an increase in cervical vertebrae anomaly, detected in 3 fetuses from 1 HD litter. An examination of individual data indicated that 1 HD litter was particularly affected; one HDF produced 2 fetuses with diaphragmatic hernia and 3 fetuses with cervical vertebrae anomaly. There were no differences in malformations between the 2 C grps. The incidences of 2 skeletal variations [accessory skull bone(s), costal cartilage malaligned] were increased in HDF, and the incidence of one [accessory skull bone(s)] was slightly increased at the MD. [The sponsor did not consider these drug-related. The sponsor also noted that a number of fetuses could not be examined for skeletal finding due to methodological problems; the CP and LD grps were particularly affected.] Based on these data, the LD could be considered a NOEL for maternal effects, although maternal toxicity was not particularly notable at higher doses. The MD could be considered a NOEL for fetal toxicity. The sponsor considered the HD a NOEL for developmental toxicity; however, the increases in certain visceral malformations and skeletal variations at the HD preclude designation of the HD as a NOEL. "Trough" [i.e., 23 hr postdosing] plasma levels at these doses are provided in the following table, along with estimated AUCs [calculated from the 23-hr data] and comparisons to the human AUC (at the proposed clinical dose):

DOSE (mg/kg)	selegiline		N-desmethylselegiline		amphetamine		methamphetamine	
	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$
2.5	1.18 ± 1.08	28.3	2.79 ± 0.93	67	0.948 ± 0.177	22.8	0.812 ± 0.194	19.5
10	4.84 ± 0.099	116	8.99 ± 1.47	216	2.98 ± 1.05	71.5	2.79 ± 1.36	67
human [20 mg]		66.1		35.3		60.9		147

Peri/postnatal development: the effect of selegiline STS on peri/postnatal development was assessed in Sprague-Dawley rats [25/grp] at doses of 0 [placebo control, CP], 0 [untreated control, CU], 10, 30, and 75 mg/kg. [Actual deliver doses were not estimated.] Additional CP and HD animals [6/grp] were dosed in order to collect TK data. Dose selection was based on the results of an embryofetal development dose-range-finding study. Observation in the definitive study consisted of the following: F_0 [clinical signs, body wt, food consumption, reproduction parameters, gross pathology, and TK (satellite animals)], F_1 (culled to 4/sex/litter on Day 4) [viability, body wt, physical/behavioral development, reproductive performance (≈1/sex/litter)], F_2 [viability, body wt, gross pathology].

There were no unscheduled deaths during the study. The total number of gravid females and live pups per grp was sufficient to allow for adequate assessment of peri/postnatal development, although 8/25 HDF lost their entire litter. Drug-related clinical signs included reduced feces, cool to the touch, vaginal discharge (brown, red), urine stain, scabbing, and dark material around the nose and/or mouth. One or more clinical signs were evident at all doses; however, incidences were greatest at the HD. Patch retention was, overall, not a problem in this study. Dermal irritation was observed in all grps receiving patches; however, there was an increase in severity of erythema at the HD. Mean body wt was slightly reduced (9-11%) in CP as compared to CU. Transient decreases in body wt occurred at the MD and HD. On Gestation Day 20, mean body wts were similar among grps, except that body wts in CPF were still lower than those in CUF. During the lactation period, body wt remained reduced in CP compared to CU; however, body wt and body wt gain were fairly similar among the other grps except for a transient decrease in body wt gain in HDF. Food consumption was transiently reduced in MDF and HDF, and in CPF when compared to CUF.

As noted, 8/25 HDF lost their entire litter at some point during the lactation period. There was an increase in dead pups in HDF on Day 0 of lactation. Pup deaths continued to be higher in HDF by Day 4 (prior to culling), and on Days 7 and 14 of lactation (post-culling), resulting in a significantly reduced number of live pups at the HD. The rate of pup deaths was also increased in MD during the lactation period. Although the number of pup deaths at the MD was similar to the CPF on Day 0 of lactation, an increase in pup deaths occurred on Lactation Days 1 and 4 (prior to culling), and on Days 7, 14, and 21, resulting in a significant decrease in the % of live pups at the MD during the lactation period. Pup retrieval was significantly reduced at all doses compared to CP, and in CPF as compared to CUF when tested on Day 6 of lactation. Whether reduced pup retrieval was due to an effect on maternal behavior or on pup behavior is unclear, as is the extent to which impaired maternal behavior may have adversely impacted pup survival. [It should be noted that pup survival was not affected at the LD, whereas pup retrieval was significantly reduced.] Drug-related clinical signs were evident at all doses in F_1 pups. Pale in color was the primary sign at the LD. Open lesion(s), cool to the touch, pale in color, scabs, subcutaneous hemorrhage(s), and tail tip absent were noted at the MD and HD. Constriction(s), mid/distal tail necrosis, bent toe(s), and missing digits were observed primarily at the HD. No observable milk in the stomach was reported in only 1 MD and 3 HD fetuses. Similar findings were observed upon necropsy of pups that died or were sacrificed moribund. Pup body wt was reduced in CP as compared to CU pups, and at the MD and HD compared to the CP. Delays were noted in most of the developmental parameters assessed. At the LD, delays were observed in surface righting, startle response, vaginal opening, and preputial separation. These were delayed at the higher doses, along with cliff aversion and eye opening. Pinnae detachment was delayed only at the HD. In contrast to these delays, no effects were observed on performance in behavioral tests of learning and memory. Behavioral testing was performed on Days 35-45 postpartum; by this time, measured developmental parameters had been obtained in 100% of fetuses/grp, except for delays in vaginal opening and preputial separation.

In animals selected for assessment of F_1 reproductive performance, there were no drug-related deaths. In F_1 males, mean body wt was reduced in CPM-F_1 compared to CUM-F_1, and in MDM-F_1 and HDM-F_1 compared to CPM-F_1; however, by the end of the postweaning period, body wts were fairly similar among grps. In F_1 females, mean body wt was reduced in CPF-F_1 compared to CUF-F_1; body wt in HDF-F_1 was only slightly lower than body wt in CUF-F_1. Mean body wt was not consistently affected during the gestation and lactation periods; however, body wt tended to be lower at the HD. There were no clear drug-related effects on reproductive parameters, i.e., copulation index, fertility index, precoital interval, and gestation length. However, the litter size on Day 0 was reduced at the HD. [Post-implantation loss was not affected; pre-implantation loss did not appear to have been assessed.] At necropsy, bent toes and absent tail tip were observed in MDM_1 and HDM_1. In addition, small epididymes and testes were noted in 3 HDM_1, and soft testes were noted in an additional HDM_1. [Small epididymides and discolored testes were detected in 1 MDM_1.] Two of the HDM_1 with small epididymes and testes were littermates, and

both failed to produce offspring; one HDF_1 that had total litter resorption was also from the same litter. No notable gross lesions were observed in treated F_1 females. There were no effects on F_2 pup viability during the lactation period, no effect on pup body wts, and no apparent drug-related gross findings in F_2 pups at necropsy.

The sponsor considered the LD to be the NOEL for both maternal and developmental toxicity. Drug-related effects [clinical signs, body wt] were noted in F_0 dams primarily at the MD and HD; however, maternal effects did not appear severe enough to adversely impact on peri/postnatal development even at the HD. The LD cannot be considered a NOEL for developmental toxicity since delays in certain developmental parameters were noted at that dose; therefore, no NOEL was established for developmental toxicity. TK data were collected in satellite and main-study F_0 animals. [Satellite animals were dosed only at the HD.] Plasma levels [at 23 hrs postdosing] were similar in HD satellite and main-study animals. Analysis of milk samples indicated that selegiline and all metabolites were secreted into milk, with milk levels of all compounds greater than plasma levels; the greatest milk:plasma ratio was obtained for selegiline. Plasma AUCs [mean ± SD; ng/mL or ng•hr/mL] were estimated from 23-hr values; these data are summarized in the following table, along with human data.

DOSE (mg/kg)	selegiline		N-desmethylselegiline		amphetamine		methamphetamine	
	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$	$C_{23\ hr}$	$AUC_{(0\text{-}24\ hr)}$
10	3.34 ± 0.89	80	1.02 ± 0.32	24	1.90 ± 0.67	46	2.30 ± 0.52	55
30	18.1 ± 3.5	434	4.62 ± 1.49	111	8.06 ± 2.32	193	11.2 ± 3.0	269
75	42.5 ± 11.7	1020	12.6 ± 4.4	302	18.5 ± 6.6	444	32.0 ± 11.9	768
human [20 mg]		66.1		35.3		60.9		147

Reproductive and developmental toxicology summary and conclusions: the effects of selegiline STS on mating and fertility were assessed in Sprague-Dawley rats. No clear dose-limiting effects were observed; however, effects on body wt probably would have precluded using significantly higher doses. There were numerous cases in which patch adherence was not maintained throughout a 24-hr period; however, from an examination of individual data, it did not appear that dosing was particularly compromised in the majority of animals. No adverse effect on mating and fertility were observed at intended doses of 0, 0, 10, 30, and 75 mg/kg. [Actual delivered doses were not estimated, but were in all probability much lower than the intended doses.] Adverse effects on sperm [i.e., reduced concentration and total count] were, however, observed at the HD. Although not affected, fertility in rats is recognized as an insensitive measure of adverse effects on spermatogenesis. Prostate wt was reduced, but microscopic analyses were not performed. No clear effects on male reproductive organs were observed in the 6-mo STS study [marked tubular atrophy and aspermia were detected in testis in 1/20 HDM] or in the carcinogenicity studies. No adequate oral mating and fertility study has been submitted.

The effects of selegiline STS on embryofetal development were assessed in Sprague-Dawley rat [0, 0, 10, 30, 75 mg/kg] and New Zealand White rabbit [0, 0, 2.5, 10, 40 mg/kg]. In the rat study, maternal effects consisted of nonspecific clinical signs and transient body wt loss [resulting in a decrease in overall body wt gain] at the MD and HD. Increases in early/late resorptions and post-implantation loss, and a decrease in fetal wt were observed at the HD. There were also increases in total malformations at the HD; this increase was due to an increase in visceral malformation. [One HD (75 mg/kg) fetus with malformations was detected in a dose-range finding study.] No pattern(s) of malformations was observed. There was also an increase in certain skeletal variations at the HD. The sponsor attributed the increase in malformations to maternal toxicity; however, it did not appear that maternal effects were severe enough to be a cause. Fetal exposure to selegiline and metabolites, N-desmethylselegiline, amphetamine, and methamphetamine were documented. In the rabbit study, the only maternal effect was a transient body wt loss at the HD. Although all grps exhibited body wt loss, body wt loss continued longer at the HD. However, mean corrected maternal wt was similar among grps. Decreases in implantation site and in the

number of viable fetuses were observed at the HD. The total number of malformations was similar among grps; however, the number of visceral malformations [primarily due to an increase in lung agenesis] was increased at the HD. In addition, the incidences of certain skeletal variations were increased at the HD. Although body wt effects were observed at the HD in both the rat and rabbit studies, higher doses could probably have been used in both studies and particularly in the rabbit study. The data indicate adverse effects on embryofetal development in both rat and rabbit following STS dosing. No teratogenic effects were observed in oral embryofetal development studies in Sprague-Dawley rat and New Zealand White rabbit. [In the rabbit study, there was an inadequate number of evaluable fetuses at all but the LD (5 mg/kg).] There were, however, adverse effects on fetal body wt in the oral rat study, and increases in total resorptions and post-implantation loss and decreases in the number of viable fetuses at the HD [50 mg/kg] in the rabbit study. When comparing the results of the STS and oral studies, it must be noted that circulating levels of selegiline are much lower (and those of metabolites relatively higher) following oral dosing as compared to STS dosing.

The effects of selegiline STS on peri/postnatal development were assessed in Sprague-Dawley rats [0, 0, 10, 30, 75 mg/kg]. The actual delivered doses were not estimated; however, patch adherence did not appear to be a particular problem in this study and TK data were collected. Nonspecific clinical signs were evident at all doses, but were minimal at the LD. Transient decreases in body wt were noted during gestation and lactation at the MD and HD. Pup deaths were increased on Day 0 at the HD, and % survival was reduced throughout the lactation period at the MD and HD. Eight of 25 HDF_1 lost their entire litter at some point during the lactation period. Pup retrieval was reduced at all doses when assessed on Day 6 of lactation. Whether this was due to impaired maternal behavior or reflected direct drug-related effects on the pups is unknown. It is notable that pup retrieval was reduced at the LD, but pup survival was unaffected. Nonspecific clinical signs [reflecting general poor condition or rough handling by dams] and reduced body wt were observed in MD and HD F_1 pups. Attainment of developmental milestones was delayed at all doses, but primarily at the MD and HD. Performance on behavioral tasks [assessed postweaning] was unaffected. Reproductive performance of the F_1 generation, as reflected by copulation index, fertility index, precoital interval, and gestation length, was not affected; however, litter size on Day 0 was reduced at the HD. Pup viability was not affected during the lactation period, and there were no apparent drug-related findings in F_2 pups. Necropsy findings in F_1 males indicated adverse effects on male reproductive organs in 4 HDM_1 and in 1 MDM_1. Therefore, it would appear that selegiline STS had adverse effects on the survival and development of the F_1 generation and viability of the F_2 generation (observed on the day of delivery). TK data documented transfer of selegiline and metabolites into milk and, therefore, "direct" dosing of F_1 pups. The study did not establish a NOEL for developmental toxicity; maternal effects were detected at the MD and HD.

In a peri/postnatal development study of oral selegiline conducted in Sprague-Dawley rat, increases in still births, decreases litter size, pup survival, and pup body wt were observed at doses of 16 and 64 mg/kg (MD, HD); no HD pups survived to Day 4 postpartum. Minimal effects were observed on developmental parameters; however, no HD pups were available for testing. The reproductive performance of the F_1 generation was not assessed. According to the abstract of a published article (the full article was not available), Whitaker-Azmitia *et al.* [Whitaker-Azmitia PM, Zhang X, Clarke C. *Neuropsychopharmacology* 11(2):125-132, 1994] reported adverse effects in rat pups exposed to selegiline (3 mg/kg) and clorgyline (3 mg/kg) [route not specified] throughout gestation or throughout gestation "...and to sacrifice..." The following findings were reported: (a) no effect on dopamine receptor terminal density, (b) severe effects on serotonergic receptor terminal density "...particularly in the cortex that showed a significant reduction of innervation at 30 days postnatal", (c) normal achievement of developmental milestones and "..no changes in measure of anxiety (% light/dark)..", (d) effects on possible measures of "impulsively", i.e., increased open field activity and impaired passive-avoidance performance. In addition, the authors noted that "The MAO-I-sac animals were severely impaired, showing stereotypic behavior, seizures, and eventually visual impairments". The authors

concluded that "...our results should be used to caution against the use of MAO-Is in women of child-bearing age".

The results of the battery of reproductive toxicity studies conducted using selegiline STS (and oral selegiline) indicate possible adverse effects on male fertility, and adverse effects on the offspring of treated dams. The latter includes increased mortality, possible teratogenic effects, and impairment of postnatal development. No-effect levels were established for all but adverse effects on postnatal development.

Labeling recommendations: none.

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VII. GENETIC TOXICOLOGY

The following genotoxicity studies were submitted to NDA 20-647: Ames test [Somerset Study No. 97090], *in vitro* mouse lymphoma assay [Somerset Study No. 97092], *in vivo* cytogenetics assay [Somerset Study No. 97096]. Reviews of these are included here, in addition to reviews of the new study reports: *in vitro* mouse lymphoma, *in vitro* chromosomal aberration assay in human lymphocytes (2 studies).

1. **Microbial mutagenesis testing of selegiline HCl using the *Salmonella Typhimurium/Escherichia Coli* plate incorporation test with and without metabolic activation (The Ames test)** (Study No. 97090, conducting laboratory and location: [redacted], study initiation date: 9/18/97, GLP, Vol 8.1)

Methods: selegiline (lot no. 901121RS) was tested using *Salmonella* tester strains, TA1535, TA1537, TA98, and TA100, and *E. coli* tester strain, WP2*uvr*A with and without metabolic activation (Aroclor 1254-induced Sprague-Dawley rat liver S9) using the plate incorporation assay. Postive controls were as follows: sodium azide (TA1535, TA100), 9-aminoacridine (TA1537), 4-nitro-*o*-phenylenediamine (TA98), and N-ethyl-N'-nitro-N-nitrosoguanidine (WP2*uvr*A) in the absence of S9, and 2-anthramine (=2-aminoanthracene) for all tester strains in the presence of S9. Two separate definitive assays were performed. In the first, selegiline was tested at concentrations of 0.00005, 0.000158, 0.0005, 0.00158, and 0.005 µg/plate in the absence of metabolic activation, and at 0.5, 1.58, 5.0, 15.8, and 50 µg/plate in the presence of S9. In the repeat assay, concentrations were 0.5, 1.58, 5.0, 15.8, and 50 µg/plate in the absence of S9, and 5.0, 15.8, 50, 158, and 500 µg/plate in the presence of S9. In each assay, selegiline was tested in 3 plates/concentration, as were positive controls.

Concentrations for the first assay were based on the results of a preliminary study in which selegiline was tested at concentrations of 0.000005-5000.0 µg/plate (± S9). Cytotoxicity was defined by the sponsor as "a decrease in the number of colonies, or a clearing of the density of the background lawn, or both". In the absence of S9, the number of revertants was reduced at concentrations ≥50 µg/plate and ppt was noted at concentrations ≥0.5 µg/plate. In the presence of S9, a decrease in revertants was observed at concentrations of 500 and 5000 µg/plate.

A positive response was defined by the sponsor as "..a dose-related increase in the number of revertants observed over three concentrations for at least one strain, with the highest increase equal to at least two times the solvent control value..."

Results: in the initial assay, there were no increases in revertants with any tester strain, with or without S9. However, there was also no evidence of cytotoxicity. Slight ppt was reported at the highest concentration (+S9) for tester strains TA100 and WP2*uvr*A. Positive controls produced 3-300 fold increases in revertants.

In the repeat assay, there were also no increases in revertants with any tester strain, with or without S9. However, as with the initial assay, there was a lack of demonstrated cytotoxicity with most of the tester strains. In the absence of S9, no cytotoxicity was demonstrated with strains, TA1535, TA1537, TA98, or TA100; with *E coli* WP2*uvr*A, the number of revertants was reduced in 1 of 3 replicates, suggesting possible cytotoxicity at the HC. In the presence of S9, cytotoxicity was demonstrated only for tester strains, TA1535 (decrease in revertants, ppt) and TA100 (decrease in revertants), at the HCs. Positive controls produced 3.4-600 fold increases in revertants.

2. **Mammalian cell mutagenesis testing of selegiline HCl using the L5178Y/*tk*+/- mouse lymphoma cell assay with colony sizing, with and without metabolic activation** (Study No. 97092, conducting laboratory and location: [redacted] study initiation date: 8/9/97, GLP, QA: Y, Vol 8.1).

Methods: selegiline (lot no. 901121RS) was tested two separate assays. In the initial test, selegiline was tested at concentrations of 50, 100, 200, 300, 350, 400, and 450 µg/mL in the absence of metabolic activation and at 2.5, 5, 7.5, 10, 12.5, and 15.0 µg/mL in the presence of metabolic activation (Aroclor-1254-induced rat liver S9). In the repeat assay, concentrations of 50, 100, 200, 250, 300, 325, 350, and 375 µg/mL in the absence of S9 and 2.5, 5, 7.5, and 12.5 µg/mL in the presence of S9 were tested. Concentrations for the definitive studies were based on the results of a preliminary assay, in which selegiline was tested at concentrations of 0.5-5000 µg/mL (±S9). In both assays, the duration of incubation with drug was 4 hrs at 37° C.

Colony sizing was performed in both definitive assays at selected concentrations. Hycanthone and cyclophosphamide were used as positive controls in the absence and presence of S9, respectively. The criteria for a positive response were as follows: (1) a "definitive" positive (i.e., ++) required a dose-reponse relationship "...and an induced mutation frequency of at least 100 x 10^{-6} at a relative total growth of ≥20%, (2) a "limited" positive (i.e., +) response required a dose-response effect and "...an induced mutation frequency of at least 70 x 10^{-6} at a RTG ≥ 10%". Cytotoxicity was considered to negate an otherwise positive response if the RTG was 10-20%. A response was considered equivocal if a positive response was not reproducible in two separate, adequate assays. No statistical analysis was performed.

Results: in the preliminary study, excessive cytotoxicity (RSG <10%) at concentrations ≥500 µg/mL and ≥50 µg/mL without and with S9, respectively. At the next lower concentrations, i.e., 158 (-S9) and 15.8 (+S9) µg/mL, RSG was 42.3 and 77.7%, respectively.

In the initial assay, RSG was >10% at all concentrations tested in the absence of S9. At 400 and 450 µg/mL, RSG was 12.2 and 16.0%, respectively, indicating that sufficiently high concentrations were tested (-S9). At concentrations ≥500 µg/mL, RSG was <1%. In the presence of S9, the RSG was >20% at all concentrations tested. At higher concentrations (i.e., ≥17.5 µg/mL), RSG was <10%. Mutation frequency was increased in a dose-related manner both in the absence and presence of S9. [The data were summarized in the attached sponsor's Tables 2-3. It was noted that "IMF" or Induced MF was calculated by subtracting the mean control MF from the MF of each test culture. This was consistent with the data except at 2.5 µg/mL (+S9) in Table 3.] The positive control in the absence of S9 could not be adequately evaluated due to methodological problems; however, it was the opinion of the Study Director that hycanthone (the PC) was positive in this assay due to "...a high number of mutant colonies..." and "...an appropriately high ratio of small colony mutants..." It was the sponsor's opinion that selegiline was negative in the absence of S9; this position was based on the fact that the increase in MF did not meet the criteria for a positive response, i.e., (a) IMF > 100 x 10^6 and (b) the RTG was <10%. However, the MF at 300 µg/mL was 96 x 10^6 [i.e., not notably different than 100 x 10^6 and almost 3-fold higher than the negative control mean] and the RTG was 18%.

In the repeat assay (data summarized in the attached sponsor's Tables 4), RSG was >20% at all concentrations tested in the absence of metabolic activation, and at concentrations of 2.5-7.5 µg/mL in the presence of S9. Mutation frequency was increased at all concentrations tested; however, the response was not dose-related at concentrations <350 µg/mL. In the presence of S9, the RSG was ≈10% at 12.5 µg/mL but <10% at concentrations of 10 and ≥15.0 µg/mL. Mutation frequency was increased at all concentrations tested in the absence of S9, and at 2.5, 7.5, and 12.5 µg/mL in the presence of S9. The

sponsor, however, considered only the increase at the HC in the absence of S9, i.e., 375 µg/mL, to be positive.

Colony sizing indicated increases in both small and large colonies (data summarized in the attached sponsor's Table 5) under the conditions (and concentrations) selected for the initial and repeat assays.

Table 2. Initial L5178Y/*tk*$^{+/-}$ Mouse Lymphoma Mutagenesis Assay of Selegiline: Results in the Absence of Metabolic Activation

Chemical	+/- S9	Conc./ml	RSG(%)	RCE(%)	RTG (%)	MF x 10^{-6}	IMF x 10^{-6}	Notes
PBS	-	10 µl	100.0	104.9	104.9	28		
PBS	-	10 µl	100.0	95.1	95.1	42		
Selegiline	-	5 µg*	84.3					
Selegiline	-	25 µg*	72.1					
Selegiline	-	50 µg	83.8	69.0	57.8	42	7	
Selegiline	-	100 µg	62.1	76.1	47.3	42	7	
Selegiline	-	150 µg*	45.4					
Selegiline	-	200 µg	42.9	64.7	27.7	57	21	
Selegiline	-	250 µg*	46.3					
Selegiline	-	300 µg	37.3	48.5	18.1	96	61	
Selegiline	-	350 µg	26.8	34.8	9.3	186	151	
Selegiline	-	400 µg	12.2	27.5	3.3	280	245	
Selegiline	-	450 µg	16.0	48.2	7.7	210	175	
Selegiline	-	500 µg*	0.7					
Selegiline	-	550 µg*	0.0					
Selegiline	-	600 µg*	0.0					
Selegiline	-	650 µg*	0.0					
Selegiline	-	700 µg*	0.0					
Selegiline	-	750 µg*	0.0					
Selegiline	-	800 µg*	0.0					
Hycanthone	-	5 µg	41.0	1.6	—**	—**	—**	Positive**
Hycanthone	-	7.5 µg	35.1	1.9	—**	—**	—**	Positive**

See text for definitions: RSG = Relative suspension growth; RCE = Relative cloning efficiency; RTG = Relative total growth; MF = Mutant frequency; IMF = Induced mutant frequency. PBS = Phosphate buffered saline.

*Not cloned.

**The agar in the plates used to assess cloning efficiency for the cultures exposed to Hycanthone did not gel properly, and, as a result, the cloning efficiency for these samples was too low and valid calculations could not be made for relative total growth, mutant frequency or induced mutant frequency. Hycanthone was evaluated as positive based on the mutant colony plates, as discussed in the text.

Table 3. Initial L5178Y/*tk*$^{+/-}$ Mouse Lymphoma Mutagenesis Assay of Selegiline: Results in the Presence of Metabolic Activation

Chemical	+/- S9	Conc./ml	RSG(%)	RCE(%)	RTG (%)	MF x 10^{-6}	IMF x 10^{-6}	Notes
PBS	+	10 µl	93.3	108.2	101.0	19		
PBS	+	10 µl	106.7	91.8	97.9	23		
Selegiline	+	2.5 µg	100.8	91.6	92.3	17	10	
Selegiline	+	5.0 µg	79.2	84.9	67.2	16	0	
Selegiline	+	7.5 µg	73.6	74.9	55.1	26	5	
Selegiline	+	10.0 µg	36.0	51.8	18.6	49	28	
Selegiline	+	12.5 µg	33.0	76.0	25.0	107	86	Positive(+)
Selegiline	+	15.0 µg	23.2	86.7	20.1	157	136	Positive(++)
Selegiline	+	17.5 µg*	5.0					
Selegiline	+	20.0 µg*	7.6					
Selegiline	+	22.5 µg*	1.5					
Selegiline	+	25.0 µg*	0.2					
Selegiline	+	27.5 µg*	0.1					
Selegiline	+	30.0 µg*	0.0					
Selegiline	+	32.5 µg*	0.0					
Selegiline	+	35.0 µg*	0.0					
Selegiline	+	37.5 µg*	0.0					
Selegiline	+	40.0 µg*	0.0					
Selegiline	+	42.5 µg*	0.0					
Selegiline	+	45.0 µg*	0.0					
Selegiline	+	47.5 µg*	0.0					
Selegiline	+	50.0 µg*	0.0					
Cyclophosphamide	+	1.5 µg*	108.2					
Cyclophosphamide	+	2.0 µg	74.5	53.8	40.1	341	324	Positive(++)

See text for definitions: RSG = Relative suspension growth; RCE = Relative cloning efficiency; RTG = Relative total growth; MF = Mutant frequency; IMF = Induced mutant frequency; Positive(+) = Limited positive; Positive(++) = Definitive positive. PBS = Phosphate buffered saline.

*Not cloned.

Table 4. Repeat L5178Y/*tk*$^{+/-}$ Mouse Lymphoma Mutagenesis Assay of Selegiline

Chemical	+/- S9	Conc./ml	RSG(%)	RCE(%)	RTG (%)	MF x 10^{-6}	IMF x 10^{-6}	Notes
PBS	-	10 µl	99.6	96.6	96.3	20		
PBS	-	10 µl	100.4	103.4	103.7	23		
Selegiline	-	25 µg*	76.0					
Selegiline	-	50 µg	62.4	120.1	74.9	33	11	
Selegiline	-	100 µg	54.1	99.5	53.8	63	41	
Selegiline	-	200 µg	51.4	65.5	33.7	47	25	
Selegiline	-	225 µg*	40.5					
Selegiline	-	250 µg	38.4	86.7	33.3	55	33	
Selegiline	-	275 µg*	32.4					
Selegiline	-	300 µg	38.0	74.8	28.4	64	42	
Selegiline	-	325 µg	31.9	77.5	24.8	58	36	
Selegiline	-	350 µg	25.4	57.5	14.6	83	61	
Selegiline	-	375 µg	21.9	62.4	13.7	110	88	Positive(+)
Hycanthone	-	5 µg	35.8	58.8	21.1	1,050	1,028	Positive(++)
Hycanthone	-	7.5 µg	23.8	66.8	15.9	1,600	1,578	Positive(++)
PBS	+	10 µl	91.3	112.9	103.1	14		
PBS	+	10 µl	108.7	87.1	94.7	18		
Selegiline	+	2.5 µg	79.9	112.7	90.1	26	10	
Selegiline	+	5.0 µg	56.3	84.9	47.8	16	0	
Selegiline	+	7.5 µg	25.9	108.2	28.0	52	36	
Selegiline	+	10.0 µg*	6.7					
Selegiline	+	12.5 µg	9.5	85.7	8.2	175	159	
Selegiline	+	15.0 µg*	3.5					
Selegiline	+	17.5 µg*	3.8					
Selegiline	+	20.0 µg*	0.2					
Selegiline	+	22.5 µg*	0.0					
Selegiline	+	25.0 µg*	0.0					
Selegiline	+	27.5 µg*	0.0					
Selegiline	+	30.0 µg*	0.0					
Cyclophosphamide	+	2.0 µg*	68.6					
Cyclophosphamide	+	3.0 µg	58.8	76.2	44.8	7,442	7,426	Positive(++)

See text for definitions: RSG = Relative suspension growth; RCE = Relative cloning efficiency; RTG = Relative total growth; MF = Mutant frequency; IMF = Induced mutant frequency; Positive(+) = Limited positive; Positive(++) = Definitive positive. PBS = Phosphate buffered saline.

*Not cloned.

Table 5. Small Colony (σ), Large Colony (λ) and Total Mutant Frequencies of the Solvent and Positive Control Cultures and Relevant Treated Cultures in the Initial and Repeat L5178Y/*tk*$^{+/-}$ Mouse Lymphoma Mammalian Cell Mutagenesis Assays of Selegiline

Chemical	+/- S9	Concentration /ml	σ MF x 10^{-6}	λ MF x 10^{-6}	Total MF x 10^{-6}
Initial Mouse Lymphoma Assay					
PBS	-	10.0 µl	18	10	28
PBS	-	10.0 µl	31	11	42
Hycanthone*	-	5.0 µg			
Hycanthone*	-	7.5 µg			
PBS	+	10.0 µl	1	14	19
PBS	+	10.0 µl	1	18	23
Selegiline	+	12.5 µg	69	38	107
Selegiline	+	15.0 µg	113	44	157
Cyclophosphamide	+	2.0 µg	262	79	341
Repeat Mouse Lymphoma Assay					
PBS	-	10.0 µl	13	7	20
PBS	-	10.0 µl	16	7	23
Selegiline	-	350 µg	43	20	83
Selegiline	-	375 µg	59	29	88
Hycanthone	-	5.0 µg	936	114	1,050
Hycanthone	-	7.5 µg	1,468	110	1,578
PBS	+	10.0 µl	7	7	14
PBS	+	10.0 µl	11	7	18
Cyclophosphamide	+	3.0 µg	7,441	1	7,442

See text for definitions: σ = Small colony; λ = Large colony; MF = Mutant frequency. PBS = Phosphate buffered saline.

*As described in the text and in the footnote to Table 2, the agar in the plates used to assess cloning efficiency for the cultures exposed to Hycanthone in the initial mouse lymphoma assay did not gel properly, and, as a result, the cloning efficiency for these samples was too low, and valid calculations could not be made for σ, λ, or total mutant frequencies. However, in this assay 5.0 µg Hycanthone/ml yielded 78.0% σ mutants, and 7.5 µg Hycanthone/ml yielded 85.2% σ mutants.

3. ***In vivo* cytogenetics testing of selegiline HCl using the mouse bone marrow micronucelus test preceded by dose range-finding** (Study No. 97096, conducting laboratory and location: [redacted], study initiation date: 8/26/97, GLP, QA, Vol 8.1)

Methods: selegiline (lot no. 901121RS; vehicle: sterile water) was administered to Swiss-Webster mice (20-30 gm) at oral (gavage) doses of 0, 37.5, 75, 300, and 600 mg/kg (15/sex/grp). [1200 and 1800 mg/kg dose grps were originally included; however, a number of deaths occurred at these doses (1200 mg/kg: 4M, 1F; 1800 mg/kg 8M, 7F). Animals that died at 300 and 600 mg/kg were replaced. An additional 5/sex received the positive control (triethylenemelanmine) i.p. Animals receiving selegiline were sacrificed at ≈27, 36, and 45 hrs postdosing (5/sex/time point); those receiving positive control were sacrificed at ≈27 hrs postdosing. Animals were observed twice daily for clinical signs. Slides were prepared of bone marrow cells from femora. The following were scored for micronuclei: 150, 300, and 600 mg/kg at 27 and 36 hrs. At 45 hrs, there were insufficient slides available for males at 600 mg/kg. Therefore, slides from males at the 75 mg/kg dose were scored; since slides from only 4 males were available, twice as many cells were scored for 1 of the 4 males (to make up for the missing male tissue).

2000 PCEs per slide were scored for micronuclei and PCE:NCE ratios were calculated based on 1000 erythrocytes.

The criteria for a positive response were as follows: (1) dose-related response in males or females at one time point or (2) statistically significant increase in micronuclei at one or more doses in males or females at one sampling time.

Doses were selected on the basis of the results of a preliminary dose-range finding study, in which mice (3/sex/grp) received selegiline at oral doses of 20, 63.2, 200, 632, and 2000 mg/kg.

Results: in the preliminary study, all HD animals died; prior to death clinical signs included tremors, labored breathing, convulsions, humched posture, and lethargy. No signs of toxicity were observed at the lower doses.

In the definitive study, all animals (5/sex/grp) died at 1800 mg/kg (clinical signs: convulsions, labored breathing, tremors), 4/5 M and 1/5 F died at 1200 mg/kg (clinical signs: convulsions, labored breathing, ataxia, tremors, lethargy), 15/23 M and 3/18 F died at 600 mg/kg (clinical signs: similar to those at the higher doses and salivation), and 1/15 M died at 300 mg/kg (clinical signs in survivors: hyperactivity, aggression). Hyperactivity was observed at 150 mg/kg, whereas no clinical signs were observed at the two lowest doses.

There were no increases in micronuclei in either males or females at any of the time points sampled. There was also no evidence of bone marrow toxicity. The positive control produced statistically significant decreases in the PCE ratio and increases in micronuclei.

4. Study Title: ***In vitro*** **mammalian cell gene mutation test (L5178Y/TK+/-) mouse lymphoma assay** [Somerset Study No: AA13MM.704.BTL, Vol #1.046, Conducting laboratory and location: [redacted], date of study initiation: 3/1/99, GLP, QA].

Methods: selegiline HCl [lot no. 9803007; vehicle: sterile water; purity stated tc [redacted] is [redacted] expiration date: 6/99] was tested at concentrations of 0, 50, 100, 150, 200, 300, and 400 µg/mL (4-hr) and 10-150 µg/mL (24 hr) in the absence of metabolic activation and at concentrations of 0, 20, 30, 40, 50, and 60 µg/mL in the presence of metabolic activation [male Sprague-Dawley rat liver S9, Aroclor-1254 induced]. [Stability tests were conducted on the drug in vehicle.] The drug concentrations to be tested were selected based on data from a preliminary study. Methyl methanesulfonate (MMS; -S9) and 7,12-dimethyl-benz(a)anthracene (7,12-DMBA; +S9) were used as positive controls.

Drug concentrations were tested in duplicate. Treatment periods were 4 (±S9) and 24 (-S9) hrs. Following treatment, plates were incubated at 37° C for 10-14 days. At the end of the incubation period, vehicle control plates were evaluated [total number of colonies per plate, total relative growth (TRG)]. "The TFT-resistant colonies were then counted for each culture with ≥10% total relative growth. TFT colonies from culture with 9% total growth were counted in the independent repeat assay". [This was a deviation from protocol.] Colony sizing was performed on negative and positive controls, and on any treated cultures with an increase in MF.

The criteria for a positive response were as follows: (a) concentration-related increase in MF and "...one or more dose levels with 10% or greater total growth exhibited mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level". (b) an equivocal result was described as a MF between "...55 and 99 mutants per 10^6 clonable cells over the background level". An MF <55 mutants per 10^6 clonable cells over background was considered a negative result.

Results: in the preliminary study, RSG was "0" at concentrations ≥500 [-S9, 4 hr], ≥150 [+S9, 4 hr], and ≥150 µg/mL [-S9, 24-hr]. The first two "definitive" assays in the presence of S9 failed, the first due to the lack of a positive response in the positive controls and the second "...due to complete lack of growth in the cloning plates". The 3rd assay produced useable results. In the independent repeat using a 24-hr treatment period (-S9 only), the first assay failed due to low MF in the negative control. The 2nd independent repeat was useable.

With 4-hr treatment in the absence of S9, R167154 produced an increase in MF at 200 and 300 µg/mL; total growth at the HD was <10% and was not scored. The data were summarized in the sponsor's Table 2 (provided below; VC = viable counts). The sponsor considered the one value, MF = 142, at the HC to be notable. Colony sizing on the positive control indicated an increase in colonies of 0.3-0.9 mm in diameter [range of all -S9 assays].

TABLE 2

CLONING DATA FOR L5178Y/TK+/- MOUSE LYMPHOMA CELLS TREATED WITH Selegiline HCl IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION Initial Assay

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	54	67	71	64 ±7	194	189	181	188	±5	68		
Solvent	2	61	53	52	55 ±4	176	171	183	177	±5	63		
Mean Solvent Mutant Frequency= 65													
50	A	61	53	61	58 ±4	191	166	168	175	±11	67	1	62
50	B	70	76	50	65 ±11	153	163	152	156	±5	84	18	70
100	A	67	59	57	61 ±4	188	170	173	177	±8	69	4	52
100	B	77	70	81	76 ±5	177	181	169	176	±5	87	21	51
150	A	46	58	61	55 ±6	207	185	192	195	±9	57	-9	60
150	B	70	67	67	68 ±1	117	148	146	137	±14	99	34	42
200	A	79	84	64	76 ±8	157	168	138	154	±12	98	33	32
200	B	66	71	50	62 ±9	136	118	137	130	±9	96	30	28
300	A	57	63	65	62 ±3	110	104	117	110	±5	112	46	18
300	B	65	74	87	75 ±9	118	100	101	106	±8	142	76	14
400	A				++				++				
400	B				+++	65	70	54	63	±7			4
Positive Control - Methyl Methanesulfonate (µg/mL)													
10		130	162	113	135 ±20	127	112	124	121	±6	223	158	50
20		81	107	84	91 ±12	32	41	38	37	±4	490	425	10

Solvent = water

A and B or 1 and 2 are duplicate cultures

++ - Too toxic to clone

+++ - Too toxic to count, total growth <10%

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

In the presence of S9, R167154 also produced an increase in MF at the HC tested. The data were summarized in the sponsor's Table 4 (below). The sponsor considered one value at 50 µg/mL [MF = 89]

and both values at the HC [MF = 148 and 136] to be notable. Colony sizing on the positive drug-treated cultures, i.e., at 60 µg/mL, indicated an increase in colonies with diameters of 0.2-1.1 mm, ie., small, medium, and large colonies. Colony sizing performed on the positive control (7,12-DMBA) indicated an increase in colonies of 0.4-0.9 mm in diameter.

TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH Selegiline HCl IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	17	27	25	23 ±4	176	175	136	162	±19	28		
Solvent	2	18	30	64	37 ±19	164	136	158	153	±12	49		
Mean Solvent Mutant Frequency= 39													
20	A	33	38	28	33 ±4	165	176	189	177	±10	37	-1	96
20	B	24	22	35	27 ±6	168	182	189	180	±9	30	-9	104
30	A	27	33	20	27 ±5	172	148	151	157	±11	34	-5	75
30	B	31	35	38	35 ±3	153	165	175	164	±9	42	4	81
40	A	39	45	48	44 ±4	149	156	164	156	±6	56	18	60
40	B	72	+	+	72 ±0	151	148	149	149	±1	96	58	54
50	A	39	33	44	39 ±4	126	157	142	142	±13	55	16	30
50	B	71	59	55	62 ±7	143	123	151	139	±12	89	50	29
60	A	80	86	76	81 ±4	119	99	109	109	±8	148	109	12
60	B	85	84	79	83 ±3	136	125	105	122	±13	136	97	14
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)													
2.5		101	113	125	113 ±10	160	199	177	179	±16	126	88	85
4		138	146	152	145 ±6	140	130	117	129	±9	225	187	60

Solvent = water

A and B or 1 and 2 are duplicate cultures

\+ - Culture lost to contamination

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

The sponsor considered these data to indicate an "equivocal" response in the absence of S9 and a "positive" response in the presence of S9.

With the 24-hr treatment in the absence of S9, there was an increase in MF at the highest concentration scored (60 µg/mL); however, it did not meet the sponsor's ≥55 mutants above background criterion for a positive response. The data from this assay are summarized in the sponsor's Table 6 (below):

TABLE 6

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH Selegiline HCl IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Independent Repeat Assay (24-hour exposure)

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts			Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	15	25	14	18 ±5	203	196	186	195 ±7	18		
Solvent	2	20	24	31	25 ±5	172	198	195	188 ±12	27		
Mean Solvent Mutant Frequency= 23												
30	A	31	28	39	33 ±5	144	137	137	139 ±3	47	24	26
30	B	40	24	25	30 ±7	149	109	148	135 ±19	44	21	24
40	A	25	27	47	33 ±10	176	123	140	146 ±22	45	23	18
40	B	27	32	40	33 ±5	113	101	159	124 ±25	53	31	15
50	A	31	26	41	33 ±6	145	142	136	141 ±4	46	24	14
50	B	31	20	27	26 ±5	142	138	133	138 ±4	38	15	12
60	A	35	33	34	34 ±1	122	112	94	109 ±12	62	40	9
60	B	44	37	33	38 ±5	100	137	113	117 ±15	65	43	9
75	A			++		96	93	93	94 ±1			5
75	B			++		78	104	+	91 ±11			6
Positive Control - Methyl Methanesulfonate (µg/mL)												
2.5		66	61	71	66 ±4	137	124	169	143 ±19	92	70	73
5		79	85	85	83 ±3	86	90	91	89 ±2	187	164	34

Solvent = water

A and B or 1 and 2 are duplicate cultures

\+ - Culture lost to contamination

++ - Too toxic to count, total growth <10%

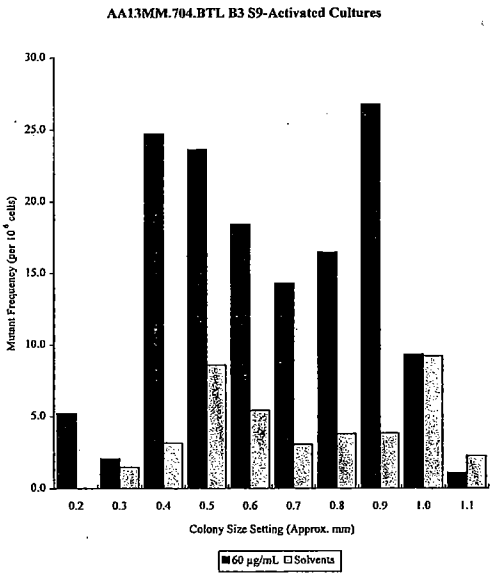
[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

The sponsor considered selegiline to be equivocally positive in the absence of S9 (4-hr exposure) and positive in the presence of S9 (4-hr exposure); selegiline was considered negative in the absence of S9 (24-hr treatment). Colony sizing in the presence of S9 demonstrated an increase in small, medium, and large colonies with selegiline [sponsor's Table 3 follows]:

Colony Size Distribution in the Presence of Metabolic Activation
(Test Article-Treated Cultures Compared with Solvent Control)
Repeat of Initial Assay with S9 Activation



5. Study Title: ***In vitro* chromosomal aberration assay of selegiline HCl in human lymphocytes with and without metabolic activation** [Somerset Study No: 97094, Vol #1.046, Conducting laboratory and location: [redacted], date of study initiation: 1/98, (protocol signed: 7/7/97), GLP, QA].

In a "REVIEW SUMMARY", the sponsor noted that this study conducted by [redacted] was subjected to an independent review resulting from questions raised during Somerset's review of the draft study report. The independent peer review was conducted by [redacted]. Dr. [redacted] questioned the scientific validity of the study results and recommended that the study be repeated. A repeat study was conducted by [redacted] [Study AA13MM.341.BTL]. The sponsor noted that

The sponsor provided the study report prepared by [redacted], but not the letter from [redacted] Ph.D. of [redacted] documenting his expert opinion [stated to be provided as Appendix B; however, Appendix B was not included].

Methods: human lymphocytes were obtained from one healthy human volunteer per assay. The Study Director donated blood for the concentration range-finding study. Blood samples were obtained from a 21-yr old female to provide lymphocytes for the initial and a repeat *in vitro* chromosomal aberration assays. Blood samples from a 29-yr old and a 32-yr old male provided lymphocytes for additional *in vitro* chromosomal aberration assays. All human volunteers appeared to be employees of [redacted]

In the initial assay, selegiline HCl [lot no. 901121RS] was tested [in duplicate cultures] at concentrations of 50, 100, 150, 300, 400, and 500 µg/mL in the absence and presence of S9 [Aroclor-1254 induced male Sprague-Dawley rat liver microsomes]; however, the study did not provide useful data because of excessive cytotoxicity at all concentrations. Doses had been based on preliminary data collected at

concentrations of 0.158-5000 µg/mL. In the preliminary study, MI was reduced by 74 and 87% at 500 µg/mL in the absence and presence of metabolic activation, respectively. In the initial assay, cells were treated for 22 hrs [-S9]

In a repeat assay, concentrations of 0 [PBS], 8.9, 15.8, 28.1, 50, 89, and 158 µg/mL were tested [in duplicate cultures] in the absence and presence of S9 [Aroclor-1254 induced male Sprague-Dawley rat liver microsomes]. MI was assessed based on 1000 cells per concentration; 100 cells per slide [200 cells per concentration] were scored for chromosomal aberrations. Positive controls were Mitomycin C (-S9; only 60 cells were available for scoring) and cyclophosphamide (+S9). No useable data were available for the cyclophosphamide-treated culture; therefore a repeat study was conducted.

In a 2nd repeat assay, selegiline HCl was tested [in duplicate cultures] at concentrations of 0, 10, 20, 40, 80, and 160 µg/mL in the presence of S9. Lower doses of cyclophosphamide were used. MI was calculated based on 1000 cells per concentration; 100 cells per slide [200 cells per concentration] were analyzed for chromosomal aberrations.

In a 3rd repeat assay, selegiline HCl was tested [in single cultures] at concentrations of 0, 10, 15, 20, 25, and 30 µg/mL in the absence and presence of S9. Mitomycin C and cyclophosphamide were used as positive controls. Two cultures were prepared per concentration, one harvested at 72 hrs and the other harvested at 96 hrs. One hundred cells per slide [200 cells per concentration, but from only 1 culture for each harvest time] were scored for chromosomal aberrations. Differences in methodology from the initial assays were as follows: (a) lower concentrations were used, both without and with metabolic activation, (b) treatment with selegiline was longer in the absence of S9, (c) harvesting of cells following treatment was later, both without and with S9, and (d) use of only single cultures per concentration per harvest time. [The exact number of cells per concentration was not clearly stated; from the descriptions is would appear that 200 cells were scored per concentration; however, only single cultures were prepared. Therefore, either only 100 cells were scored for each harvest time, or 200 cells were scored from each slide.]

Apparently, in the "initial" assay [or 1st and 2nd repeats], cultures were treated with selegiline for 22 hrs (-S9) or 4 hrs (+S9), washed, then sampled 2 (-S9) or 20 (+S9) hrs later. In the repeat assay [or 3rd repeat], cultures were treated with selegiline for 22 or 46 hrs in the absence of S9, and for 4 hrs in the presence of S9. Sampling time was 2 hrs following the end of treatment in the absence of S9 and 20 or 44 hrs following the end of treatment in the presence of S9. The sponsor summarized the treatment schedules in the following table:

Treatment Schedules for the Preliminary Assay and the Chromosomal Aberration Assays

	PHA-M	Test Article	Wash	Colcemid	Harvest
Preliminary Assay and Initial Chromosomal Aberration Assay					
-S9	0 hr	48 hr	70 hr	70.5 hr	72 hr
+S9	0 hr	48 hr	52 hr	70.5 hr	72 hr
Repeat Chromosomal Aberration Assay					
-S9	0 hr	48 hr	70 hr	70.5 hr	72 hr
-S9	0 hr	48 hr	94 hr	94.5 hr	96 hr
+S9	0 hr	48 hr	52 hr	70.5 hr	72 hr
+S9	0 hr	48 hr	52 hr	94.5 hr	96 hr

The sponsor's criteria for a "biologically significant" response were as follows: (a) concentration-related increases in aberrations, (b) increases in aberrations should be higher than the historical control range.

Results: in the "initial" chromosomal aberration assay, concentrations of selegiline scored in the absence and presence of S9 were as follows: 8.9, 15.8, 28.1, and 50 µg/mL (-S9), 10, 20, 40 µg/mL (+S9). [Higher concentrations were not scored due to >50% inhibition of MI.] In the absence of S9, selegiline produced increases in cells with structural aberrations at all but the LC. In the presence of S9, cells with structural aberrations were increased at the HC of selegiline. Cytotoxicity was evident at all concentrations tested [MI = 25-4% of C]. The data are summarized in the following sponsor's Table 2:

Table 2

INITIAL CHROMOSOME ABERRATION ASSAYS OF SELEGILINE
72 Hour Harvest Time

Chemical	Conc/ml	±S9	Mitotic Index (‰)[1]	No. of Cells[2]	Chromatid Breaks	Chromatid Xchgs	Chromosome Breaks	Chromosome Xchgs	Other Abs.	% Aberrant Cells[3]	Aberrations per 100 Cells[3]
PBS	10 µl	-	22	200	5	1	0	0	0	2.0 ± 2.8	3.0 ± 4.2
Selegiline	8.9 µg	-	20	200	9	1	0	0	0	4.0 ± 1.4	4.5 ± 2.1
Selegiline	15.8 µg	-	16	200	32	2	0	0	0	9.0 ± 2.8♦	17.0 ± 12.7♦
Selegiline	28.1 µg	-	11	200	34	6	0	0	0	10.0 ± 2.8♦	20.0 ± 9.9♦
Selegiline	50 µg	-	4	200	54	6	0	0	0	15.5 ± 0.7♦	30.0 ± 2.8♦
Mitomycin C	0.5 µg	-	2	60*	159	80	0	0	2	66.3 ± 5.3♦	371.3 ± 129.0♦
PBS	10 µl	+	25	200	5	0	0	0	0	2.0 ± 2.8	2.5 ± 3.5
Selegiline	10 µg	+	24	200	2	4	0	0	0	2.5 ± 0.7	3.0 ± 1.4
Selegiline	20 µg	+	24	200	0	7	0	0	0	2.5 ± 0.7	3.5 ± 2.1
Selegiline	40 µg	+	14	200	23	10	0	0	0	8.5 ± 2.1♦	16.5 ± 0.7♦
Cyclophosphamide	5 µg	+	5	200	13	14	0	0	0	9.5 ± 2.1♦	13.5 ± 2.1♦

1. Mitotic indices are based on 1,000 cells.
2. Number of cells evaluated for aberrations.
3. Mean ± SD.
PBS = Phosphate buffered saline.
* 40 Metaphases were evaluated from one culture and 20 metaphases from the other.
♦ Biologically relevant positive response based on the magnitude of the response, which exceeded the range of historical negative controls; statistically, all treatments were significantly positive ($p \leq 0.001$).

In the repeat assay, selegiline was tested at concentrations of 20, 25, and 30 µg/mL in the absence of S9 and at 15, 20, and 25 µg/mL in the presence of S9 with a harvest time of 72 hrs. [The MIs were markedly higher in the repeat assay. According to the report, this was due to the fact that the human donor for the repeat assay was "...a long-distance runner (which would have elevated the white blood cell count) who consumes peanut butter daily (which is known to enhance mitogenic responses)..."] Increases in cells with structural chromosomal aberrations were noted at all but the LC in the absence and presence of S9. Cytotoxicity [i.e., a decrease in MI of >50%] was noted only at the HC in the presence of S9 [MI was 29% of C]. The data are summarized in the following sponsor's Table 3:

Table 3

REPEAT CHROMOSOME ABERRATION ASSAY OF SELEGILINE
72 Hour Harvest Time

Chemical	Conc/ml	±S9	Mitotic Index (‰)[1]	No. of Cells[2]	Chromatid Breaks	Chromatid Xchgs	Chromosome Breaks	Chromosome Xchgs	Other Abs.	% Aberrant Cells[3]	Aberrations per 100 Cells[3]
PBS	10 µl	-	155	200	1	3	0	0	0	1.0 ± 0.0	2.0 ± 1.4
Selegiline	20 µg	-	136	200	5	7	0	0	0	4.5 ± 0.7	6.0 ± 2.8
Selegiline	25 µg	-	125	200	8	20	0	0	0	7.5 ± 0.7*	14.0 ± 4.2*
Selegiline	30 µg	-	96	200	12	14	0	0	0	9.5 ± 2.1*	13.0 ± 1.4*
Mitomycin C	0.3 µg	-	78	200	32	33	0	0	0	13.5 ± 2.1*	32.5 ± 6.4*
PBS	10 µl	+	113	200	0	2	0	0	0	1.0 ± 0.0	1.0 ± 0.0
Selegiline	15 µg	+	91	200	2	4	0	0	0	2.0 ± 1.4	3.0 ± 1.4
Selegiline	20 µg	+	69	200	3	10	0	0	0	4.5 ± 2.1*	6.5 ± 2.1*
Selegiline	25 µg	+	33	200	7	9	0	0	0	6.0 ± 1.4*	8.0 ± 2.8*
Cyclophosphamide	2.5 µg	+	51	200	6	14	0	1	0	7.5 ± 0.7*	10.5 ± 3.5*

1. Mitotic Indices are based on 1,000 cells.
2. Number of cells evaluated for aberrations.
3. Mean ± SD.

PBS = Phosphate buffered saline.

* Biologically relevant positive response based on the magnitude of the response, which exceeded the range of historical negative controls; statistically, all treatments were significantly positive ($p \leq 0.001$).

Using a harvest time of 96 hrs, selegiline was tested at concentrations of 20, 25, and 30 µg/mL in the absence of S9 and at 20, 25, and 30 µg/mL in the presence of S9. The number of cells with structural aberrations was increased at all but the LC in the absence and presence of S9. Cytotoxicity was notable (MI decrease by >50%) only at the HC in the presence of S9. The data are provided in the following sponsor's Table 3 (con't):

Table 3, concluded

REPEAT CHROMOSOME ABERRATION ASSAY OF SELEGILINE
96 Hour Harvest Time

Chemical	Conc/ml	±S9	Mitotic Index (‰)[1]	No. of Cells[2]	Chromatid Breaks	Chromatid Xchgs	Chromosome Breaks	Chromosome Xchgs	Other Abs.	% Aberrant Cells[3]	Aberrations per 100 Cells[3]
PBS	10 µl	-	76	200	4	0	2	0	0	2.5 ± 0.7	3.0 ± 1.4
Selegiline	20 µg	-	67	200	3	1	1	2	0	3.0 ± 1.4	3.5 ± 2.1
Selegiline	25 µg	-	56	200	6	7	2	2	0	5.5 ± 0.7*	8.5 ± 2.1*
Selegiline	30 µg	-	53	200	6	17	1	4	0	7.5 ± 0.7*	14.0 ± 1.4*
Mitomycin C	0.05 µg	-	56	200	3	12	4	13	11	9.0 ± 2.8*	21.5 ± 2.1*
PBS	10 µl	+	130	200	3	1	0	0	0	2.0 ± 0.0	2.0 ± 0.0
Selegiline	20 µg	+	107	200	5	5	1	3	0	3.0 ± 0.0	7.0 ± 2.8*
Selegiline	25 µg	+	68	200	1	8	0	5	0	5.0 ± 1.4*	7.0 ± 4.2*
Selegiline	30 µg	+	58	200	4	4	8	3	0	8.0 ± 1.4*	9.5 ± 2.1*
Cyclophosphamide	1.25 µg	+	70	200	6	4	1	8	0	6.5 ± 5.0*	9.5 ± 9.2*

1. Mitotic Indices are based on 1,000 cells.
2. Number of cells evaluated for aberrations.
3. Mean ± SD.

PBS = Phosphate buffered saline.

* Biologically relevant positive response based on the magnitude of the response, which exceeded the range of historical negative controls; statistically, all treatments were significantly positive ($p \leq 0.001$).

Appendix B: the sponsor provided a copy of Appendix B [fax'd 12/10/01] in response to a request by the Division. Appendix B was a letter from [redacted], to Melissa Goodhead, B.Sc., RAC, Director of Operations, Somerset Pharmaceuticals Inc. Dr. [redacted] reported problems with the "...suitability for scoring of the metaphase spreads from Selegiline treated cultures prepared by [redacted], in both trials and in the absence and presence of metabolic activation. Dr. [redacted] concluded that:

> "Because of the extremely poor quality of the slides, they should never have been scored and no conclusions should have been drawn from data generated from scoring the above slides [Trial 1 and 2 slides]. Our opinion is that the data reported in the [redacted] report cannot be considered a reliable evaluation of the clastogenicity of Selegiline HCl. Our recommendation is that the study be repeated and properly evaluated"

6. Study Title: ***In vitro* mammalian chromosome aberration test** (Somerset Study No: AA13MM.341.BTL, Vol #1.046, Conducting laboratory and location: [redacted] date of study initiation: 2/99, GLP, QA:Y).

Methods: human peripheral lymphocytes were obtained from a healthy female volunteer (42 yr). Selegiline [lot no. 9803007; vehicle: purified water] was tested in the absence and presence of metabolic activation [Arochlor 1254-induced male Sprague-Dawley rat liver S9]. [The S9 preparation was characterized using 2-aminoanthracene and 7,12-dimethyl-benz(α)anthracene in the Ames test (tester strain, TA100)]. Drug concentrations for the definitive study were determined based on the results of a preliminary toxicity study at concentrations up to 5000 µg/mL in the absence and presence of metabolic activation.

Mitomycin C and cyclophosphamide were used as positive controls in the absence and presence of metabolic activation, respectively.

Treatment was for 4 hrs (±S9) and for 20 hrs (-S9). Cells were harvested at ≈20 hrs after initiation of treatment. Cells were treated with Colcemid® 2 hrs prior to harvesting. When possible, 200 metaphases [100 per slide] per concentration were scored for chromosomal aberrations. The MI was calculated on the basis of 500 cells in the first definitive assay and on 1000 cells in the repeat study. Polyploidy/endoreduplication was scored in 100 cells per concentration. The sponsor's criteria for a positive response were as follows: (a) a dose-related increase in the percentage of cells with aberrations, (b) a statistically significant increase in chromosomal aberrations at one or more concentrations, or (c) "A reproducible significant increase at one dose level other than the high dose with no dose response.

[Deviations from GLP: (a) no analysis of test or control articles was conducted, (b) no analyses of the test or control mixtures [i.e., homogeneity, concentration] were performed, (c) stability testing was not performed on either test or control article.]

Results: in the preliminary assay, the MI was not affected at concentrations of 0.5 to 500 µg/mL in the absence of S9 (4-hr treatment), but was completely reduced (i.e., 100%) at concentrations of 1500 and 5000 µg/mL. With the 20-hr treatment, the MI was reduced 49% at 500 µg/mL, but completely reduced at concentrations of 1500 and 500 µg/mL. In the presence of S9, the MI was quite variable, but was reduced by 59% at 500 µg/mL and completely reduced at concentrations of 1500 and 5000 µg/mL. Based on these results, the concentrations selected for the definitive assay were 0, 50, 100, 200, 400, 500, 600, 700, and 800 µg/mL.

In the initial definitive assay, insufficient cytotoxicity (i.e., >50% decrease in MI) was obtained in the absence of S9 and with 4-hr treatment. Therefore, this part of the assay was repeated using doses of 400, 500, 600, 650, 700, 750, 800, and 1000 µg/mL. [It is unclear whether or not cells treated at the original concentrations were scored for chromosomal aberrations; no data were provided.] In the 1st repeat, fixative was inadvertently added to cells prior to harvest; therefore, this portion of the assay had to be repeated a 2nd time. In the 2nd repeat assay, concentrations of 650, 700, and 750 µg/mL were scored for aberrations. The MI was reduced by 54% at the HC. With the 20-hr treatment, concentrations of 200, 400, and 500 µg/mL were scored. The MI was reduced by 68% at the HC. In the presence of S9, concentrations of 600, 700, and 800 µg/mL were scored. The MI was reduced by 56% at the HC. The data are summarized in the following sponsor's Table 7:

TABLE 7
SUMMARY

Treatment	S9 Activation	Treatment[1] Time (Hours)	Mitotic Index	Cells Scored	Aberrations Per Cell[2] (Mean ± SD)	Cells With Aberrations[3] (%) Numerical	Structural
Water	-	4	7.8	200	0.015 ± 0.158	0.0	1.0
Selegiline HCl							
650 µg/mL	-	4	4.5	200	0.020 ± 0.140	1.0	2.0
700 µg/mL	-	4	4.0	200	0.000 ± 0.000	0.0	0.0
750 µg/mL	-	4	3.5	200	0.000 ± 0.000	1.0	0.0
MMC, 0.13 µg/mL	-	4	4.8	200	0.105 ± 0.307	1.0	10.5**
Water	+	4	3.9	200	0.000 ± 0.000	0.0	0.0
Selegiline HCl							
600 µg/mL	+	4	2.4	200	0.015 ± 0.122	0.0	1.5
700 µg/mL	+	4	3.1	200	0.030 ± 0.171	0.0	3.0*
800 µg/mL	+	4	1.7	200	0.005 ± 0.071	0.0	0.5
CP, 25 µg/mL	+	4	1.1	200	0.140 ± 0.402	0.0	12.0**
Water	-	20	4.1	200	0.020 ± 0.140	0.0	2.0
Selegiline HCl							
200 µg/mL	-	20	2.5	200	0.020 ± 0.140	0.0	2.0
400 µg/mL	-	20	2.2	200	0.030 ± 0.198	0.0	2.5
500 µg/mL	-	20	1.3	200	0.000 ± 0.000	0.0	0.0
MMC, 0.13 µg/mL	-	20	2.7	200	0.095 ± 0.370	0.0	7.5**

[1] Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
[2] Severely damaged cells were counted as 10 aberrations.
[3] *, p≤0.05; **, p≤0.01; Fisher's exact test.

The statistically significant increase in % of cells with structural aberrations [chromatid breaks; replicates were 2 and 4] was not considered biologically significant since the value fell within the HC range of 0-3.5% [mean ± SD = 0.3 ± 0.6%] for S9-activated solvent controls [data from studies conducted 1995-1997]. These data included data on the following solvents: "... water, saline, DMSO, ethanol, acetone, and other non-standard and Sponsor-supplied vehicles". Positive controls produced increases in structural aberrations consistent with a positive response, but produced no increases in numerical aberrations.

7. ***In vivo* cytogenetics testing of selegiline HCl using the mouse bone marrow micronucelus test preceded by dose range-finding** [Study No. 97096, conducting laboratory and location: [redacted] study initiation date: 8/26/97, GLP, QA, Vol 8.1].

Methods: selegiline (lot no. 901121RS; vehicle: sterile water) was administered to Swiss-Webster mice (20-30 gm) at oral (gavage) doses of 0, 37.5, 75, 300, and 600 mg/kg (15/sex/grp). [1200 and 1800 mg/kg dose grps were originally included; however, a number of deaths occurred at these doses (1200 mg/kg: 4M, 1F; 1800 mg/kg: 8M, 7F). Animals that died at 300 and 600 mg/kg were replaced.] An additional 5/sex received the positive control (triethylenemelamine) i.p. Animals receiving selegiline were sacrificed at ≈27, 36, and 45 hrs postdosing (5/sex/time point); those receiving positive control were sacrificed at ≈27 hrs postdosing. Animals were observed twice daily for clinical signs. Slides were prepared of bone marrow cells from femora. Bone marrow was "...pulled gently out of the canal into the syringe...The syringe was flicked with a finger to break up any cell clumps..." The cells were further processed, placed on slides, and stained with Giemsa stain. "At least two slides were prepared per mouse." [It was noted that "The procedure to remove the bone marrow cells from the femora was a protocol deviation; it was used because it permitted the cells to be obtained more rapidly after the mice had been sacrificed. This deviation did not affect the integrity of the study or interpretation of the results." The protocol called for "...removing each femur and pushing the bone marrow cells through the femur into a centrifuge tube containing serum..."] The following were scored for micronuclei: 150, 300, and 600 mg/kg at 27 and 36 hrs. At 45 hrs, there were insufficient slides available for males at 600 mg/kg. Therefore, slides from males at the 75 mg/kg dose (and sacrificed at 45 hrs postdosing) were scored. In addition, since slides from only 4 males treated at 300 mg/kg were available, twice as many cells were scored for 1 of the 4 males (to make up for the missing male tissue). 2000 PCEs per slide were scored for micronuclei and PCE:NCE ratios were calculated based on 1000 erythrocytes.

The criteria for a positive response were as follows: (1) dose-related response in males or females at one time point or (2) statistically significant increase in micronuclei at one or more doses in males or females at one sampling time.

Doses were selected on the basis of the results of a preliminary dose-range finding study, in which mice (3/sex/grp) received selegiline at oral doses of 20, 63.2, 200, 632, and 2000 mg/kg.

Results: in the preliminary study, all HD animals died; prior to death clinical signs included tremors, labored breathing, convulsions, hunched posture, and lethargy. No signs of toxicity were observed at the lower doses.

In the definitive study, 8/8 M and 7/8 F died at 1800 mg/kg (clinical signs: convulsions, labored breathing, tremors), 4/5 M and 1/5 F died at 1200 mg/kg (clinical signs: convulsions, labored breathing, ataxia, tremors, lethargy), 15/23 M and 3/18 F died at 600 mg/kg (clinical signs: similar to those at the higher doses and salivation), and 1/15 M died at 300 mg/kg (clinical signs in survivors: hyperactivity, aggression). Hyperactivity was observed at 150 mg/kg, whereas no clinical signs were observed at the two lowest doses.

There were no increases in micronuclei in either males or females at any of the doses or time points sampled [150, 300, 600 mg/kg at 27 and 36 hrs; 75, 150, 300 mg/kg (M), 150, 300, 600 mg/kg (F) at 45 hrs]. There was also no evidence of bone marrow toxicity. The positive control produced statistically significant decreases in the PCE ratio and increases in micronuclei at the 27-hr sampling time.

Genetic toxicology summary and conclusions: seven genotoxicity studies were submitted in NDA20-647 for Eldepryl® capsules [2 Ames tests, *in vivo* chromosomal aberration in rat, *in vivo* micronucleus test (rat, mouse), SCE (human lymphocytes), one study used 5 different methods (Ames, 3 gene conversion tests using *Saccharomyces cerevisiae* D_4, UCS)]. Of these, only 2 (Ames, *in vivo* chromosomal aberration assay in rat) were conducted under GLP. The other 5 studies were inadequate for a variety of reasons. The 2 GLP studies were considered inadequate due to the lack of cytotoxicity (Ames), or the lack of an MTD and of sufficient cells examined per animal (*in vivo* chromosomal

aberration). Therefore, the sponsor was asked to complete a full genotoxicity battery [as described in ICH guidance] as a Phase 4 commitment. In a supplement to NDA 20-647, the sponsor provided study reports for 3 genotoxicity studies: Ames test, *in vitro* mouse lymphoma, and an *in vivo* micronucleus assay in mouse. The following studies were submitted only to this NDA [21-336]: *in vitro* mouse lymphoma assay [Somerset Study No. AA13MM.704.BTL, *in vitro* chromosomal aberration assay in human lymphocytes [Somerset Study No. 97094], *in vitro* chromosomal aberration assay in human lymphocytes [Somerset Study No. AA13MM.341.BTL]. Only the studies submitted in the supplement to NDA 20-647 and to NDA 21-336 are discussed below.

The Ames test was conducted by [redacted]. In this study, the mutagenic potential of selegiline HCl was tested [with and without metabolic activation] using *S. Typhimurium* strains, TA1535, TA1537, TA98, and TA100, and *E. coli* strain, WP2*uvr*A. [This comprises an acceptable battery of tester strains.] Two definitive assays were conducted. In the first assay, selegiline was tested at concentrations up to 0.005 µg/plate in the absence of S9 and concentrations up to 50 µg/plate were used in the presence of S9. No increases in revertants were observed; however, no cytotoxicity was detected. A slight ppt was detected at the HC. In the 2nd assay, concentrations up to 50 and 500 µg/plate were used in the absence and presence of S9, respectively. No increases in revertants were observed either without or with S9; however, there was no evidence of cytotoxicity except in tester strains, TA1535 and TA100, in the presence of S9 at the HCs. Therefore, these assays are inadequate to assess the mutagenic potential of selegiline. [It is unclear why such low concentrations were used since in the original Ames test (considered inadequate due to the lack of cytotoxicity) concentrations up to 500 µg/plate were used (±S9).]

The sponsor submitted 2 *in vitro* mouse lymphoma assays, one conducted in 1997 by [redacted]) and a second conducted in 1999 by [redacted]. In the [redacted] study, selegiline was tested in two separate assays. In the initial assay, concentrations of 50-450 µg/mL (-S9) and 2.5-15 µg/mL (+S9) were tested. Sufficient cytotoxicity [i.e., RTG <10%] was observed at concentrations of 350-450 µg/mL (-S9). At the HC tested in the presence of S9, RTG was ≈20%. At the next higher concentration, RTG was <10%. Cultures at this concentration should have been scored; however, the lack of these data is not too problematic since selegiline increased MF at concentrations of 10-15 µg/mL. In the absence of S9, selegiline produced concentration-related increases in MF. The sponsor did not consider these increases to constitute a positive response since the three highest concentrations were associated with an RTG <10%. However, at the next lower concentration, there was a 3-fold increase in MF with an RTG of 18%. In the presence of S9, excessive cytotoxicity was not observed even at the HC; the RTG at the HC was 20%. Selegiline produced dose-related increases in MF. The sponsor considered only the increase at the HC to be positive; however, the MF at concentrations of 10-15 µg/mL were 2-7.5 fold higher than the mean MF for the negative control. Colony sizing indicated that the increase in MF in the presence of S9 [colony sizing was not conducted in the absence of S9] was due primarily to an increase in small colonies. In the repeat assay, concentrations of 50-375 µg/mL (-S9) and 2.5-12.5 µg/mL (+S9) were tested. Sufficient cytotoxicity was observed at the HCs both with and without S9; the RTG at the HCs were 14 and 8% without and with metabolic activation, respectively. In the absence of S9, selegiline produced increases in MF at all but the LC; the increases at the 2 highest concentrations were ≈4 to 5-fold higher than the mean MF for the negative control. The sponsor considered the increase at the HC to be positive; this concentration was associated with an RTG of 14%. In the presence of S9, selegiline produced concentration-related increases in MF at the two highest concentrations scored. The sponsor did not considered these indicative of a positive response for the following reasons: (a) at the HC, the RTG was <10% and (b) at the next lower concentration, the IMF < 100 x 10^6. However, the MF was 3-fold higher than the mean MF for the negative control and the RTG at that concentration was 28%. Colony sizing indicated that the increases in MF in the absence of S9 [colony sizing was not conducted in the presence of S9] were due to increases

in both small and large colonies. Therefore, in these two assays, selegiline was reproducibly positive both in the absence and presence of S9. Colony sizing indicated that the effect was both clastogenic and mutagenic. [The conclusions stated in the study report agreed with this interpretation of the data.] [In the Toxicology summary provided by the sponsor, it was noted that an expert peer review of the draft report of this study was conducted by Ph.D. Dr. ...led to questions regarding the validity of the results presented in the final study report. The summary referred to "Appendix B"; however, there was no Appendix B in the final study report submitted.]

In the study, selegiline was tested at concentrations of 50-400 µg/mL (4-hr incubation) and 10-150 µg/mL (24-hr incubation) in the absence of S9 and at concentrations of 20-60 µg/mL in the presence of S9. With the 4-hr incubation, selegiline produced concentration-related increases in MF both without and with metabolic activation. The sponsor considered the response in the absence of S9 to be equivocal since the IMFs were $<100 \times 10^6$ [RTG at the HC was 14%]. In the repeat (-S9) assay [24-hr incubation], selegiline increased MF at the HC; however, since the IMF was $<55 \times 10^6$ and the RTG was M10%, the sponsor did not consider this a positive response. This negative response with the 24-hr incubation should not be considered a failure to replicate the positive response with the 4-hr incubation. Therefore, in this study, selegiline was positive both in the absence and presence of S9, and colony sizing in the presence of S9 indicated both clastogenic and mutagenic effects.

The sponsor submitted two *in vitro* chromosomal aberration assays in human lymphocytes, one was conducted by) in 1998 and the 2nd study was conducted by in 1999. The sponsor also provided [fax'd upon request by the Division] a copy of a letter from , Ph.D. This letter documented Dr. opinion that the study conducted by was inadequate due to poor quality of the metaphase spreads prepared by Dr. recommended that the study be repeated. [The sponsor also noted that "

In the study, selegiline was tested in two assays considered definitive [additional assays were considered inadequate due to methodological problems]. In the "initial" assay, selegiline was tested (in duplicate cultures) at concentrations of 8.9-50 µg/mL and 10-40 µg/mL in the absence and presence of S9, respectively. [Higher concentrations were tested, but not scored since inhibition of MI exceeded 50% at those concentrations.] Selegiline produced significant increases in the % cells with structural aberrations at concentrations of 15.8-50 µg/mL (-S9) and at 40 µg/mL (+S9). In the repeat assay, selegiline was tested (only in single cultures) at two different harvest times. With a harvest time of 72 hrs, selegiline was tested at concentrations of 20-30 µg/mL in the absence of S9 and at concentrations of 15-25 µg/mL in the presence of S9. Significant increases in % cells with structural aberrations were observed at 25-30 µg/mL (-S9) and at 20-25 µg/mL (+S9). With a 96-hr harvest time, selegiline produced significant increases in the % cells with structural aberrations at concentrations of 25-30 µg/mL (-S9) and 25-30 µg/mL (+S9).

In the study, selegiline was tested in at concentrations of 650-750 µg/mL (-S9) and 600-800 µg/mL (+S9) with a 4-hr treatment time, and at concentrations of 200-500 µg/mL (-S9) with a 20-hr treatment time. Selegiline did not produce concentration-related increases in structural or numerical aberrations under any of the conditions used.

The sponsor submitted an *in vivo* micronucleus assay in mouse performed by Selegiline was tested at doses of 150-600 mg/kg p.o. and at 27, 36, and 45 hrs postdosing, the one exception being that doses of 75-300 mg/kg were tested in male mice at 45 hr postdosing. [The method used to extract bone marrow cells differed from that specified in the protocol; however, it was the

opinion of the Study Director that this deviation did not affect the integrity of the study.] Although no bone marrow cytotoxicity was observed, the HDs used exceeded the MTD based on deaths (300-1800 mg/kg). No increases in micronuclei were observed at any dose or sampling time.

A search of the literature using PubMed and search terms, "selegiline" and "genotoxicity" revealed only one published article. Subramanya *et al.* [Subramanya KS *et al. Toxicol Lett* 66(3):221-230, 1993] reported that selegiline hydrochloride was negative in the *in vivo* micronucleus assay in mouse at doses "...as high as 16-times the clinical dose used in humans". [Only the abstract was available at the time of this review.]

Conclusions: in the original NDA submission for selegiline capsules (NDA 20-647), none of the 7 genotoxicity studies submitted was considered to have adequately assessed the genotoxic potential of selegiline. [Selegiline was negative in the two (of 7) studies conducted under GLP.] The sponsor committed to conducting a full battery of genotoxicity studies postmarketing. Three genotoxicity studies were submitted in a supplement to NDA 20-647: Ames test, *in vitro* mouse lymphoma, and an *in vivo* micronucleus assay in mouse. Three additional genotoxicity assays were submitted to the NDA currently under review: *in vitro* mouse lymphoma assay, 2 *in vitro* chromosomal aberration assays in human lymphocytes. In the genotoxicity studies conducted postmarketing (cf. NDA 20-647), selegiline was positive in the *in vitro* mouse lymphoma assays (conducted by), positive in the *in vitro* chromosomal aberration assay in human lymphocytes conducted by , and negative in the Ames test (conducted by) and the *in vivo* micronucleus assay in mouse (conducted by . In the *in vitro* mouse lymphoma assays, selegiline produced increases in both small and large colonies, consistent with both clastogenic and mutagenic effects.

The original Ames test (submitted to NDA 20-647) was inadequate due, in part, to the lack of cytotoxicity. The repeat Ames test is also inadequate due to the lack of cytotoxicity for 4 of 5 tester strains in the absence of S9 and for 3 of 5 tester strains in the presence of S9. In the toxicology summary report, the sponsor stated that the mouse lymphoma assay conducted by was not a valid study. [No discussion of this was included in the study report and no documentation was provided.] However, since selegiline was also positive in the *in vitro* mouse lymphoma assay conducted by , the problems with the study do not impact on the overall evaluation of the genotoxic potential of selegiline.

The sponsor also stated that the *in vitro* chromosomal aberration assay in human lymphocytes conducted by . was not a valid study, and provided (upon request) a letter from a consultant to document methodological problems with the assay. Selegiline was positive in this assay, but was negative in the *in vitro* chromosomal aberration assay in human lymphocytes conducted by The consultant's letter stating problems with the study is not definitive documentation that the study is invalid; however, the need to resolve the issue is lessened by the positive findings in the *in vitro* mouse lymphoma assay.

The questionable validity of two studies conducted by . does, however, raise questions as to the validity of other studies (i.e., Ames test, *in vivo* micronucleus assay in mouse) also conducted by . The validity of the Ames test and the *in vivo* micronucleus assay was not questioned by the sponsor. [Selegiline was negative in both of these studies, whereas selegiline was positive in the two studies considered invalid by the sponsor.] However, considering the apparent poor quality of the two positive studies conducted by , it would not seem unreasonable to require additional documentation that the *in vivo* micronucleus assay is a valid study. [The Ames test is inadequate due to the lack of cytotoxicity; therefore, there is no need to document the validity of that study.] The sponsor should be asked to provide such documentation. If, however, such documentation is not possible (since the contract laboratory was reported by the sponsor to have closed),

the sponsor should conduct a repeat assay. In addition, the sponsor should justify the use of oral dosing in the *in vivo* micronucleus assay to support a transdermal formulation; no appropriate toxicokinetic data are available by which to determine relative exposure to selegiline using gavage dosing. The sponsor has determined that, in humans, plasma exposure to selegiline is markedly higher with transdermal application as compared to oral dosing. If such justification cannot be provided, then the study should be repeated [and no methods validation is necessary].

Labeling recommendations: none.

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VIII. DETAILED CONCLUSIONS AND RECOMMENDATIONS

Conclusions: conclusions regarding the various nonclinical areas were discussed in the summary/conclusion subsection within each section.

General Toxicology Issues: issues were discussed in the summary/conclusion subsection of the General Toxicology section.

Recommendations: from a pharmacology/toxicology standpoint, it is recommended that this NDA not be approved due to the lack of adequate assessment of carcinogenic potential. Specifically, the sponsor has not provided the data for either the mouse or rat carcinogenicity studies in a format that will allow for independent review of the results. The sponsor has committed to providing electronic datasets for the studies; however, they have not been received.

[In a meeting held on 1/30/02, the Division decided, and the sponsor was informed, that the lack of the datasets for the carcinogenicity studies would not be a reason for a non-approvable decision. However, the Division stated that the datasets must be submitted and the sponsor committed to doing so.]

Although not a basis for the nonapprovable recommendation, the sponsor needs to provide additional information regarding the genotoxic potential of selegiline STS.

The following information should be relayed to the sponsor:

Regarding the genotoxicity studies, you should conduct a repeat Ames test using concentrations of selegiline sufficient to produce cytotoxicity in each tester strain, with and without metabolic activation. You should also conduct a repeat *in vivo* cytogenetics assay unless you can provide (a) justification for the use of the oral route to support your transdermal formulation and (b) documentation that the study was conducted adequately. You have stated that the mouse lymphoma assay and the *in vitro* chromosomal aberration assay conducted by [redacted] were invalid due to serious methodological problems. The *in vivo* cytogenetics assay was also conducted by [redacted] therefore, we need additional assurance that this study was adequately conducted. If you cannot provide justification that the oral route is adequate to support the transdermal route, then a repeat assay should be conducted (using an appropriate route) and no additional validation of the *in vivo* cytogenetics assays would be necessary.

Labeling with basis for findings: labeling recommendations were not made since it has been determined that this NDA is not approvable.

IX. APPENDIX/ATTACHMENTS

Executive CAC
Date of Meeting
Mouse/Rat Carcinogenicity Study

Committee: Joseph Contrera, Ph.D., HFD-900, Chair
Robert Osterberg, HFD-520, Member
John Leighton, HFD-150, Alternate Member
Barry N. Rosloff, Ph.D., HFD-120, Supervisor
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/NDA #21-336
Drug Name: selegiline transdermal system
Sponsor: Somerset Pharmaceuticals, Inc.

Mouse Carcinogenicity Study: the committee concurred with the reviewer that the 78-wk dietary study was inadequate based on the short duration and the lack of a complete battery of tissues examined for histopathology. The committee also agreed that assay sensitivity may have been reduced at the HD due to the excessive body wt effect (the only dose-limiting effect) observed.

Rat Carcinogenicity Study: the committee concurred with the reviewer that: (a) the 104-wk dietary carcinogenicity study in rat was deficient in that a complete battery of tissues was not examined for histopathology. (b) assay sensitivity may have been reduced at the HD due to the excessive body wt effect observed; however, adequate assay sensitivity was achieved by examination of the lower doses.

Executive CAC Recommendations and Conclusions: the committee concluded that the carcinogenic potential of selegiline had not been adequately assessed. Adequate assessment of the carcinogenic potential is of particular concern because of selegiline's positive genotoxicity findings. It was recommended that either a 2-yr or an alternative carcinogenicity study (e.g., TG.AC, p53) be conducted in mouse. The sponsor should provide justification for the assay selected.

Joseph Contrera, Ph.D.
Chair, Executive CAC

cc:\
/Division File, HFD-120
/BNRosloff, HFD-120
/LMFreed, HFD-120
/DBates, HFD-120
/ASeifried, HFD-024

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Lois Freed
3/25/02 01:31:04 PM
PHARMACOLOGIST

Barry Rosloff
3/25/02 01:56:22 PM
PHARMACOLOGIST
I concur with Dr. Freed's conclusion that the carcinogenicity datasets should be submitted. See my memo of 3/25/02 for additional comments concerning the adequacy of the carcinogenicity studies.