Uterine Changes

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
Endometrial epithelial hyperplasia	2/50	2/50	2/49	11/48	12/50
Pyometra	0/50	0/50	0/49	0/48	8/50
Squamous Cell metaplasia	1/50	1/50	0/49	3/48	4/50
Hemometra	0/50	0/50	0/49	0/48	4/50

Vaginal Changes

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
Squamous epithelium with keratin	1/47	1/48	3/47	9/44	27/50
Squamous cell Hyperplasia	0/47	0/48	0/47	0/44	3/50

Neoplastic:

Summary Tumor Incidence

PROJECT ID: 12485 FATES: ALL
DAYS: ALL SEX: MALE
GROUP: Contr. I Contr.II III IV V

	#	%	#	%	#	%	#	%	#	%
Total Animals/Group	50		50		50		50		50	
Total Primary Tumors	75	(150)	77	(154)	57	(114)	63	(126)	71	(142)
Total Animals with Tumors	42	(84)	42	(84)	40	(80)	43	(86)	37	(74)
Total Animals w/ Multiple Tumors	22	(44)	24	(48)	16	(32)	17	(34)	20	(40)
Total Benign ##	58	(77)	58	(75)	46	(80)	52	(82)	58	(81)
Total Malignant ##	17	(22)	19	(24)	11	(19)	11	(17)	13	(18)
Total Malignant with Metastasis #	6	(35)	10	(52)	6	(54)	7	(63)	1	(7)

Percentage value is Total Benign or Malignant Tumors divided by the Total Primary Tumors

Percentage value is Total Metastasized Tumors divided by the Total Malignant Tumors

Comparison of group 2 with group 1 (negative control)
Comparison of groups 3 to 5 with group 2 (vehicle control)

* significantly different from control ($p \leq 0.05$)
** significantly different from control ($p \leq 0.01$)

Figure 80, from page 1169 of Report — 12485/99

Summary Tumor Incidence

PROJECT ID: 12485 FATES: ALL
DAYS: ALL SEX: FEMALE
GROUP: Contr. I Contr.II III IV V

	#	%	#	%	#	%	#	%	#	%
Total Animals/Group	50		50		50		50		50	
Total Primary Tumors	95	(190)	99	(198)	94	(188)	73	(146)	60	(120)
Total Animals with Tumors	48	(96)	49	(98)	45	(90)	44	(88)	31	(62)**
Total Animals w/ Multiple Tumors	29	(58)	29	(58)	28	(56)	19	(38)	17	(34)*
Total Benign ##	79	(83)	75	(75)	76	(80)	51	(69)	39	(65)
Total Malignant ##	16	(16)	24	(24)	18	(19)	22	(30)	21	(35)
Total Malignant with Metastasis #	6	(37)	14	(58)	2	(11)	9	(40)	10	(47)

Percentage value is Total Benign or Malignant Tumors divided by the Total Primary Tumors

Percentage value is Total Metastasized Tumors divided by the Total Malignant Tumors

Comparison of group 2 with group 1 (negative control)
Comparison of groups 3 to 5 with group 2 (vehicle control)

* significantly different from control ($p \leq 0.05$)
** significantly different from control ($p \leq 0.01$)

Figure 81, from page 1173 of Report — 12485/99

Testicular Leydig Cell Hyperplasia

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
Unilateral	2/50	3/50	1/50	9/50	11/50
Bilateral	1/50	2/50	10/50	15/50	25/50
Combined	3/50	5/50	11/50	24/50	36/50

Testicular Leydig Cell Adenomas

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
Unilateral	1/50	2/50	3/50	7/50	7/50
Bilateral	1/50	0/50	6/50	9/50	12/50
Combined	2/50	2/50	9/50	16/50	19/50

NOTE: there is a discrepancy between the incidence of unilateral adenomas in Dr. Kelley's table and the incidence in the histopathology report. This table reflects the incidence from page 1,183 of the report.

Uterine Neoplasms

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
Adenocarcinoma	0/50	0/50	2/49	4/48	2/50
Squamous Cell Carcinoma	0/50	0/50	0/49	1/48	3/50

The following table is taken form Dr. Roswitha Kelly's statistical review (pages 9 to 11)

Table 3: Tumor Trends in Female Rats*

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	HIGH	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic Method)	Pair-wise P-Value (Exact)	Pair-wise P-Value (Asymptotic)
1	Abdomen	33	FIBROADENOMA	0	1	0	0	0	1.0000	0.9185	1.0000	0.9205
1	Abdomen	34	FIBROADENOMA MAMMA	0	1	0	0	0	1.0000	0.8923	1.0000	0.8855
1	Abdomen	36	FIBROSARCOMA	0	0	1	0	0	0.7419	0.8328	n/a	n/a
1	Abdomen	49	LIPOMA	0	1	0	0	0	1.0000	0.9127	1.0000	0.8203
1	Abdomen	75	SQUAMOUS CELL CARCINOMA	0	0	0	1	0	0.4286	0.5806	n/a	n/a
10	Ear	58	MALIGNANT SCHWANNOMA	0	1	0	0	0	1.0000	0.9185	1.0000	0.9205
12	Eyes	2	ADENOCARCINOMA HARDERIAN GLAND	0	0	0	0	1	0.1935	0.0671	0.4286	0.2067
13	Genital region	34	FIBROADENOMA MAMMA	1	0	0	0	0	n/a	n/a	n/a	n/a
14	Haematopoietic tissue	47	LARGE GRANULAR LYMPH LEUCAEMIA	0	0	0	0	1	0.3200	0.1565	0.5333	0.2738
14	Haematopoietic tissue	54	LYMPHOMA LYMPHOCYTIC TYPE	0	0	0	0	1	0.6667	0.3918	0.8000	0.4668
18	Hindleg foreleg	46	KERATOACANTHOMA	0	1	0	0	0	1.0000	0.8923	1.0000	0.8855
18	Hindleg foreleg	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	0	0	1	0	0.5391	0.7054	n/a	n/a
19	Ileum	48	LEIOMYOSARCOMA	0	1	0	0	0	1.0000	0.9185	1.0000	0.9205
2	Adrenals	28	CORTICAL ADENOMA bilat	1	0	0	0	0	n/a	n/a	n/a	n/a
2	Adrenals	29	CORTICAL ADENOMA unilat	6	2	1	0	5	0.0303	0.0301	0.2500	0.1734
2	Adrenal	37	GRANULAR CELL TUMOR	1	0	0	0	0	n/a	n/a	n/a	n/a
2	Adrenals	66	PHAEOCHROMOCYTOMA	3	1	1	0	0	0.9504	0.9326	1.0000	0.9205
20	Kidneys	70	RENAL ADENOCARCINOMA	0	0	1	0	0	0.6190	0.8441	n/a	n/a
20	Kidneys	71	RENAL LIPOMA	0	0	0	1	0	0.5391	0.7054	n/a	n/a
21	Lacrimal glands	1	ADENOCARCINOMA	0	0	0	1	0	0.5391	0.7054	n/a	n/a
22	Liver	42	HAEMANGIOSARCOMA	0	1	0	0	0	1.0000	0.8923	1.0000	0.8855
22	Liver	43	HEPATOCELLULAR ADENOMA	0	0	1	1	0	0.6557	0.8157	n/a	n/a
23	Lower jaw	75	SQUAMOUS CELL CARCINOMA	0	0	0	1	0	0.4286	0.5806	n/a	n/a
24	Lungs with bronchi	76	SQUAMOUS CELL CARCINOMA (kera)	0	0	0	1	0	0.3548	0.6280	n/a	n/a
26	Mammary gland	1	ADENOCARCINOMA	5	6	4	3	3	0.8820	0.8980	0.9612	0.9346
26	Mammary gland	21	CARCINOMA ARISING IN FIBROADE	0	1	2	0	0	0.9539	0.9496	1.0000	0.9205

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	HIGH	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic Method)	Pair-wise P-Value (Exact)	Pair-wise P-Value (Asymptotic)
26	Mammary gland	33	FIBROADENOMA	8	6	15	7	2	0.9931	0.9923	0.9585	0.9239
26	Mammary gland	35	FIBROMA	1	0	1	0	0	0.7419	0.8328	n/a	n/a
26	Mammary gland	36	FIBROSARCOMA	0	1	0	0	0	1.0000	0.9185	1.0000	0.9205
26	Mammary gland	46	KERATOACANTHOMA	0	1	0	0	0	1.0000	0.9185	1.0000	0.9205
26	Mammary gland	50	LIPOSARCOMA	1	0	0	0	1	0.2609	0.1209	0.5357	0.2753
26	Mammary gland	51	LIPOSARCOMA MYXIOD TYPE	1	0	0	0	0	n/a	n/a	n/a	n/a
26	Mammary gland	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	0	1	0	0	0.7739	0.8725	n/a	n/a
26	Mammary gland	6	ADENOMA	2	1	1	0	0	0.9504	0.9326	1.0000	0.9205
27	Mononuclear phagocytic tissue	45	HISTIOCYTIC SARCOMA	2	3	1	0	0	0.9970	0.9756	1.0000	0.9784
28	Nasal cavity I (incl nasoph)	1	ADENOCARCINOMA	0	0	0	1	0	0.5200	0.7148	n/a	n/a
29	Neck Flank	3	ADENOCARCINOMA MAMMA	3	0	0	0	0	n/a	n/a	n/a	n/a
29	Neck Flank	34	FIBROADENOMA MAMMA	0	2	4	0	1	0.8495	0.8800	0.8312	0.7068
29	Neck Flank	36	FIBROSARCOMA	0	1	0	0	0	1.0000	0.9185	1.0000	0.9205
29	Neck Flank	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	1	0	0	0	1.0000	0.9185	1.000	0.9205
3	Anus	36	FIBROSARCOMA	0	0	0	0	1	0.2609	0.1209	0.5357	0.2753
30	Ovaries	39	GRANULOSA CELL TUMOR unilat	0	3	0	0	0	1.0000	0.9664	1.0000	0.9742
30	Ovaries	52	LUTEOMA unilat	0	0	1	0	0	0.7739	0.8725	n/a	n/a
30	Ovaries	77	THECOMA unilat	0	0	0	0	1	0.1935	0.0671	0.4286	0.2067
30	Ovaries	80	YORK SAC CARCINOMA	0	0	0	0	1	0.2609	0.1209	0.5357	0.2753
30	Ovaries	81	SERTOLI CELL TUMOR bilat	1	3	0	0	0	1.0000	0.9738	1.0000	0.9818
31	Pancreas	12	ADENOMA ISLET CELL	1	2	0	1	1	0.5542	0.6288	0.8794	0.7819
32	Parathyroids	6	ADENOMA	0	2	1	0	1	0.6849	0.7372		
33	Pituitary	15	ADENOMA PARS DISTALIS	38	36	35	33	17	1.0000	1.0000	0.9999	0.9997
33	Pituitary	4	ADENOCARCINOMA PARS DISTALIS	2	5	1	2	4	0.2288	0.2729	0.7080	0.6226
37	Shoulder	21	CARCINOMA ARISING IN FIBROADE	0	0	1	0	0	0.7739	0.8725	n/a	n/a
38	Skin (left flank)	76	SQUAMOUS CELL CARCINOMA (kera)	0	0	1	0	0	0.7739	0.8725	n/a	n/a
4	Axilla	3	ADENOCARCINOMA MAMMA	1	2	0	0	0	1.0000	0.9533	1.0000	0.9618
4	Axilla	33	FIBROADENOMA	0	1	0	0	0	1.0000	0.8923	1.0000	0.8855
4	Axilla	5	ADENOLIPOMA MAMMA	1	0	0	0	0	n/a	n/a	n/a	n/a
4	Axilla	34	FIBROADENOMA MAMMA	2	2	3	1	1	0.8279	0.8598	0.8794	0.7819
4	Axilla	67	PLEOMORPH LIPOSARCOMA	0	0	0	0	1	0.2609	0.1209	0.5357	0.2753
41	Spleen	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	0	0	0	1	0.2609	0.1209	0.5357	0.2753
42	Stomach (fundus r pars prove)	75	SQUAMOUS CELL CARCINOMA	0	0	0	1	0	0.4286	0.5806	n/a	n/a

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	HIGH	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic Method)	Pair-wise P-Value (Exact)	Pair-wise P-Value (Asymptotic)
43	Tail	36	FIBROSARCOMA	1	0	1	0	0	0.7419	0.8328	n/a	n/a
45	Thorax groin	35	FIBROMA	0	0	1	0	0	0.7419	0.8328	n/a	n/a
45	Thorax groin	59	MALIGNANT SCHWANNOMA TYPE B	0	0	0	1	0	0.5200	0.7148	n/a	n/a
46	Thymus	78	THYMOMA	0	0	1	0	0	0.7739	0.8725	n/a	n/a
47	Thyroids	10	ADENOMA C CELL unilat	4	1	1	0	1	0.5449	0.5794	0.7347	0.5739
47	Thyroids	11	ADENOMA FOLLICUL CELL unilat	0	2	1	1	1	0.6600	0.7152	0.9062	0.8193
47	Thyroids	22	CARCINOMA FOLLICUL CELL unilat	0	0	1	1	0	0.6629	0.8128	n/a	n/a
48	Tongue (incl base)	37	GRANULAR CELL TUMOR	0	1	0	0	0	1.0000	0.9127	1.0000	0.8203
49	Uterus (incl cervix)	1	ADENOCARCINOMA	0	0	2	4	2	0.2473	0.2850	0.1071	0.0430
49	Uterus (incl cervix)	17	ADENOSQUAMOUS CELL CARCINOMA	0	0	0	1	0	0.4286	0.5806	n/a	n/a
49	Uterus (incl cervix)	24	CARCINOSARCOMA	0	0	0	1	0	0.5500	0.7142	n/a	n/a
49	Uterus (incl cervix)	39	GRANULOSA CELL TUMOR unilat	0	0	0	1	0	0.5391	0.7054	n/a	n/a
49	Uterus (incl cervix)	65	PAPILLOMA CERVIX	0	0	0	0	1	0.2609	0.1209	0.5357	0.2753
49	Uterus (incl cervix)	68	POLYP ENDOMETRIAL STROMAL	4	1	3	3	5	0.0851	0.0948	0.1192	0.0736
49	Uterus (incl cervix)	69	POLYP GLANDULAR	3	2	3	1	1	0.8279	0.8598	0.8794	0.7819
49	Uterus (incl cervix)	75	SQUAMOUS CELL CARCINOMA	0	0	0	1	3	0.0228	0.0156	0.1465	0.0741
50	Vagina	37	GRANULAR CELL TUMOR	0	0	1	1	0	0.6629	0.8128	n/a	n/a
50	Vagina	62	MYOMA	0	0	0	0	1	0.2609	0.1209	0.5357	0.2753
6	Brain (brain stem)	18	ASTROCYTOMA	0	0	1	0	0	0.7419	0.8328	n/a	n/a
6	Brain (brain stem)	63	OLIGODENDROGLIOMA	0	0	1	0	0	0.7419	0.8328	n/a	n/a
8	Brain (cerebrum)	18	ASTROCYTOMA	1	0	0	1	0	0.4286	0.5806	n/a	n/a
8	Brain (cerebrum)	61	MIXED GLIOMA	0	1	0	0	1	0.4554	0.4427	0.7890	0.6333

*Control Group 1 given for information only; trend test with control group 2 (vehicle), low, medium, and high dose groups; Pair-wise comparison between control group 2 and high dose.

The following table is taken form Dr. Roswitha Kelly's statistical review (pages 14 to 16)

Table 4: Tumor Trends for Male Rats*

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	HIGH	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic)	Pair-wise P-Value (Exact)	Pair-wise P-Value (Asymptotic)
11	Epididymides	49	LIPOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
14	Haematopoietic tissue	47	LARGE GRANULAR LYMPH LEUCAEMIA	0	0	1	0	1	0.3189	0.3594	0.5000	0.2525
14	Haematopoietic tissue	54	LYMPHOMA LYMPHOCYTIC TYPE	0	1	0	2	0	0.6950	0.8018	1.0000	0.9088

Organ Code	Organ Name	Tumor Code	Tumor Name	CTRL	CTR2	LOW	MED	HIGH	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic)	Pairwise P-Value (Exact)	Pairwise P-Value (Asymptotic)
14	Haematopoietic tissue	55	LYMPHOMA PLEOMORPHIC TYPE	1	1	0	0	0	1.0000	0.9098	1.0000	0.9088
15	Harderian glands	8	ADENOMA bilat	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
16	Head	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	1	0	0	0	1.0000	0.9123	1.0000	0.9288
17	Heart (left and right ventricl	19	ATRIOCAVAL MESOTHELIOMA	0	0	1	0	2	0.0829	0.0635	0.2143	0.1015
17	Heart (left and right ventricl	31	ENDOCARDIAL SCHWANNOMA	0	0	0	0	1	0.1818	0.0653	0.5000	0.2525
17	Heart (left and right ventricl	36	FIBROSARCOMA	0	0	0	0	1	0.1818	0.0653	0.5000	0.2525
17	Heart (left and right ventricl	72	SCHWANNOMA	0	0	1	0	0	0.7368	0.8617	n/a	n/a
2	Adrenals	27	CORTICAL ADENOCARCINOMA	0	1	0	0	0	1.0000	0.9396	1.0000	0.8556
2	Adrenals	29	CORTICAL ADENOMA unilat	2	1	1	2	4	0.0585	0.0617	0.1851	0.1147
2	Adrenals	40	HAEMANGIOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
2	Adrenals	60	MIMICKS TUMOR PARS INTERMEDIA	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
2	Adrenals	66	PHAEOCHROMOCYTOMA	7	1	4	2	4	0.2099	0.2415	0.1827	0.1129
20	Kidneys	30	CYSTADENOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
22	Liver	43	HEPATOCELLULAR ADENOMA	2	1	0	1	1	0.4041	0.4597	0.7532	0.5938
22	Liver	44	HEPATOCELLULAR CARCINOMA	0	1	0	0	1	0.4583	0.4280	0.7532	0.5938
23	Lower jaw	46	KERATOACANTHOMA	0	1	0	0	0	1.0000	0.9123	1.0000	0.9088
25	Lymph node (mesenteric)	40	HAEMANGIOMA	1	0	1	0	0	0.8182	0.8608	n/a	n/a
25	Lymph node (mesenteric)	42	HAEMANGIOSARCOMA	0	1	0	0	1	0.4583	0.4280	0.7532	0.5938
25	Lymph node (mesenteric)	53	LYMPHANGIOMA	0	0	0	0	1	0.2632	0.1198	0.5000	0.2525
26	Mammary gland	1	ADENOCARCINOMA	0	1	0	0	1	0.3864	0.3752	0.7500	0.5932
26	Mammary gland	20	BASAL CELL CARCINOMA	0	0	0	1	0	0.5000	0.6918	n/a	n/a
26	Mammary gland	33	FIBROADENOMA	0	0	1	0	0	0.7368	0.8617	n/a	n/a
26	Mammary gland	35	FIBROMA	0	0	2	0	0	0.8092	0.8927	n/a	n/a
26	Mammary gland	36	FIBROSARCOMA	0	0	0	0	1	0.1818	0.0653	0.5000	0.2525
26	Mammary gland	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	0	0	1	1	0.1908	0.2133	0.5000	0.2525
27	Mononuclear phagocytic tissue	45	HISTIOCYTIC SARCOMA	3	3	1	2	0	0.9345	0.9478	1.0000	0.9729
29	Neck Flank	20	BASAL CELL CARCINOMA	0	1	0	0	1	0.4583	0.4280	0.7532	0.5932
29	Neck Flank	36	FIBROSARCOMA	0	0	0	0	1	0.3077	0.1500	0.5000	0.2525
29	Neck Flank	42	HAEMANGIOSARCOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
29	Neck Flank	46	KERATOACANTHOMA	0	2	0	0	0	1.0000	0.9455	1.0000	0.9516
29	Neck Flank	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	0	0	2	0	0.4617	0.6299	n/a	n/a
29	Neck Flank	79	TRICHOFOLLICULOMA	0	0	0	2	0	0.5132	0.6603	n/a	n/a
31	Pancreas	12	ADENOMA ISLET CELL	2	5	1	1	4	0.3485	0.4011	0.7183	0.6363
31	Pancreas	23	CARCINOMA ISLET CELL	1	0	0	0	0	n/a	n/a	n/a	n/a
31	Pancreas	7	ADENOMA ACINAR CELL	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
32	Parathyroids	1	ADENOCARCINOMA	0	0	0	0	1	0.2632	0.1198	0.5000	0.2525
32	Parathyroids	6	ADENOMA	1	2	0	0	0	1.0000	0.9455	1.0000	0.9516
33	Pituitary	15	ADENOMA PARS DISTALIS	26	27	18	16	11	0.9987	0.9987	0.9998	0.9996

Organ Code	Organ Name	Tumor Code	Tumor Name	CTRL	CTR2	LOW	MED	HIGH	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic)	Pair-wise P-Value (Exact)	Pair-wise P-Value (Asymptotic)
33	Pituitary	16	ADENOMA PARS INTERMEDIA	1	0	1	0	0	0.7368	0.8617	n/a	n/a
33	Pituitary	4	ADENOCARCINOMA PARS DISTALIS	1	0	0	0	0	n/a	n/a	n/a	n/a
34	Preputial gland	6	ADENOMA	0	2	0	0	1	0.6152	0.6503	0.8797	0.7822
35	Prostate	1	ADENOCARCINOMA	0	1	0	0	0	1.0000	0.9123	1.0000	0.9088
35	Prostate	6	ADENOMA	0	0	0	0	1	0.2632	0.1198	0.5000	0.2525
36	Salivary glands (2 sections)	72	SCHWANNOMA	0	0	0	1	0	0.5556	0.6545	n/a	n/a
37	Shoulder	35	FIBROMA	0	0	0	2	0	0.5132	0.6603	n/a	n/a
37	Shoulder	36	FIBROSARCOMA	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
37	Shoulder	49	LIPOMA	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
37	Shoulder	74	SOLID BASAL CELL CARCINOMA	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
38	Skin (left flank)	35	FIBROMA	1	0	0	0	0	n/a	n/a	n/a	n/a
38	Skin (left flank)	79	TRICHOFOLLICULOMA	1	1	0	0	0	1.0000	0.9098	1.0000	0.9088
39	Snout	49	LIPOMA	0	0	0	1	0	0.4545	0.6594	n/a	n/a
39	Snout	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	0	0	1	0	0.4545	0.6594	n/a	n/a
4	Axilla	20	BASAL CELL CARCINOMA	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
4	Axilla	35	FIBROMA	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
4	Axilla	36	FIBROSARCOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
4	Axilla	79	TRICHOFOLLICULOMA	0	2	0	0	0	1.0000	0.9455	1.0000	0.9516
40	Spinal cord (3 sections)	18	ASTROCYTOMA	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
40	Spinal cord (3 sections)	26	CHOROID PLEXUS PAPILLOMA	0	1	0	0	0	1.0000	0.9123	1.0000	0.9088
41	Spleen	40	HAEMANGIOMA	0	0	1	0	0	0.8182	0.8608	n/a	n/a
41	Spleen	42	HAEMANGIOSARCOMA	1	1	1	0	0	0.9320	0.9229	1.0000	0.9088
41	Spleen	56	MALIG FIBRO HISTIOCYTOMA (MFH)	0	0	0	1	0	0.5556	0.6545	n/a	n/a
42	Stomach (fundus r pars prove)	36	FIBROSARCOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
44	Testes	10	ADENOMA C CELL unilat	0	0	0	1	0	0.4545	0.6594	n/a	n/a
44	Testes	13	ADENOMA LEYDIG CELL bilat	1	0	6	9	12	0.0015 (!)	0.0013	0.0001 (!)	0.0002
44	Testes	14	ADENOMA LEYDIG CELL unilat	1	2	2	6	7	0.0361	0.0379	0.0800	0.0525
46	Thymus	42	HAEMANGIOSARCOMA	0	1	0	0	0	1.0000	0.9150	1.0000	0.9088
46	Thymus	78	THYMOMA	0	0	0	1	0	0.5000	0.6918	n/a	n/a
47	Thyroids	10	ADENOMA C CELL unilat	4	1	3	1	4	0.1262	0.1425	0.1827	0.1129
47	Thyroids	11	ADENOMA FOLLICUL CELL unilat	2	2	4	5	4	0.3502	0.3891	0.3376	0.2410
47	Thyroids	22	CARCINOMA FOLLICUL CELL unilat	2	0	3	0	0	0.8899	0.9206	n/a	n/a
47	Thyroids	25	C CELL CARCINOMA unilat	1	0	1	0	0	0.7778	0.8859	n/a	n/a
47	Thyroids	9	ADENOMA C CELL bilat	0	0	0	1	0	0.5000	0.6918	n/a	n/a
5	Back	35	FIBROMA	0	0	0	1	0	0.5000	0.6918	n/a	n/a
5	Back	64	OSTEOSARCOMA	0	0	0	0	1	0.2632	0.1198	0.5000	0.2525
5	Back	79	TRICHOFOLLICULOMA	0	1	0	0	0	1.0000	0.9098	1.0000	0.9088
51	Injection site (I)	56	MALIGFIBRO HISTRIOCYTOMA (MFH)	1	0	0	0	0	n/a	n/a	n/a	n/a

Organ No. Code	Organ Name	Tumor Code	Tumor Name	CTRL1	CTRL2	LOW	MED	HIGH	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic)	Pair-wise P-Value (Exact)	Pair-wise P-Value (Asymptotic)
7	Brain (cerebellum)	18	ASTROCYTOMA	0	0	1	0	2	0.1242	0.1396	0.2500	0.1193
8	Brain (cerebrum)	18	ASTROCYTOMA	0	0	1	0	1	0.3014	0.3121	0.5000	0.2525
8	Brain (cerebrum)	37	GRANULAR CELL TUMOR	1	0	0	0	0	n/a	n/a	n/a	n/a
8	Brain (cerebrum)	61	MIXED GLIOMA	1	1	0	0	0	1.0000	0.9150	1.0000	0.9088
8	Brain (cerebrum)	63	OLIGODENROGLIOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
9	Caecum	42	HAEMANGIOSARCOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
9	Caecum	45	HISTIOCYTIC SARCOMA	0	1	0	0	0	1.0000	0.9150	1.0000	0.9088

*Control Group 1 given for information only; trend test with control group 2 (vehicle), low, medium, and high dose groups; Pair-wise comparison between control group 2 and high dose.

Toxicokinetics: Weeks 26, 52 and 104. 2, 4, 8, 24, 31 and 48 hours post dose; also 6 hours post dose for prolactin serum levels.

SPM 962 [mg/kg b.w.]	C_{max} [ng/mL]					
	TW 26	TW 52	TW 104	TW 26	TW 52	TW 104
	males			females		
0.3	1.29	1.84	1.90	1.18	1.54	2.78
1.0	5.13	4.24	3.48	2.42	3.08	1.73
3.0	8.03	5.86	10.2	8.16	3.56	6.12

SPM 962 [mg/kg b.w.]	AUC_{0-t} [h * ng/mL]					
	TW 26	TW 52	TW 104	TW 26	TW 52	TW 104
	males			females		
0.3	32.9	31.9	42.5	24.6	34.9	28.8
1.0	90.8	94.3	103	64.1	72.6	63.8
3.0	261	206	328	197	139	203

TW = test week

Figure 82, from page 77 of Report —12485/99

Mean plasma concentration of prolactin [ng/ml] (6 h after application)				
SPM962 dose [mg/kg b.w.]	Sex	Week 26	Week 52	Week 104
NaCl	males	28.54	21.01	439.67
vehicle		30.07	39.75	49.87
0.3		3.72	14.47	11.27
1.0		1.26	6.63	69.62
3.0		0.85	0.95	8.04
NaCl	females	66.84	214.68	660.93
vehicle		231.0	314.6	611.40
0.3		95.13	129.74	71.45
1.0		74.63	24.73	172.90
3.0		6.62	23.24	12.92

Figure 83, from page 75 of Report — 12485/99

APPEARS THIS WAY
ON ORIGINAL

2.6.6.5.3 6-Month Study On Potential Preneoplastic Changes From SPM 962 Patch By Dermal Administration To Minipigs

Study no.: Report No. 16332/02
1633202-study-report.pdf
Conducting laboratory and location:

Date of study initiation:
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity:
Formulation/vehicle:

Animals: minipig / Göttingen minipigs

Methods

Doses: Pigs (6/sex) were administered two 10 cm^2 patches/day, one placebo (right side) and one containing 4.5 mg rotigotine (left side). Patches were kept in place for 24 hours. Seven application sites/side were used. The daily application site was rotated so that each site was used once per week. Application sites were shaved every week.

Study design: Pigs were treated for six months (184 days). At the end of this time, the pigs were sacrificed and subjected to a gross pathological evaluation. Histopathology was performed on 3 locations/application site (21 specimens/dose). Additional histopathology examinations were made of 3 locations on shaved skin exposed to curafix and 3 locations on shaved skin without exposure to curafix. The following organs were removed and fixed in 10% buffered formalin:

Lymphonodus cervicalis superficialis left and right (2)
Lymphonodus subiliacus left and right (2)
Eye left (1)
Epididymis right (1)
Testicle right (1)
Ovary right (1)
Pieces of the uterus

These tissues were not examined unless the sponsor requests the examination in the future.

Results:
Eschers were observed at both the placebo and rotigotine patch sites in both males and females. The incidence of eschers was similar in both placebo and rotigotine sites.

No clinical signs were noted in the pigs.

No effects on pig body weight or food consumption were observed.

Histopathological examinations did not reveal any drug-related dermal toxicity. The severity of the lesions was similar between rotigotine and placebo sites.

All skin localisations exposed to SPM 962 patch (—), the SPM 962 placebo patch, the untreated shaved skin exposed to Curafix® or the untreated shaved skin without exposure to Curafix® revealed similar histomorphological findings in form of minimal to mild superficial purulent dermatitis, epithelial hyperplasia, hyperkeratosis and lympho-histiocytic infiltration.

The skin tissue showed a normal histomorphological appearance in all minipigs. Minimal to mild hyperkeratosis was noted at all skin localisations. A minimal to mild focal hyperplasia of the epidermis, superficial purulent dermatitis and minimal to mild lympho-histiocytic infiltrations in the subepithelial layer (corium) were observed in some skin localisations. There was no difference in the type of changes observed between the animals treated with SPM 962 patch (—), SPM 962 placebo patch, untreated shaved skin exposed to Curafix® or the untreated skin localisations without exposure to Curafix®. However, the incidence of localisations with changes was highest for the SPM 962 patch (—) or the SPM 962 placebo patch treated application sites due to the repeated mechanical exposure to the patch. Correlating changes were noted for the untreated shaved skin exposed to Curafix® or the untreated skin.

Figure 84, from page 448 of — Report 16332/02

Number of skin localisations with pathological findings for all animals per sex

Diagnosis	SPM 962 patch —		SPM 962 placebo patch		Untreated skin with exposure to Curafix®		Untreated skin without exposure to Curafix®	
	male	female	male	female	male	female	male	female
Superficial purulent dermatitis	14	7	15	5	2	1	1	1
Epithelial hyperplasia	21	27	21	30	3	6	0	1
Hyperkeratosis	40	26	41	30	4	3	0	5
Lympho-histiocytic infiltr.	13	25	10	32	3	4	1	2

Figure 85, from page 449 of — Report 16332/02

2.6.6.6 Reproductive and developmental toxicology

2.6.6.6.1 Examination of the Influence of SPM 962 on the Fertility and Early Embryonic Development to Implantation of Sprague-Dawley Rats by Subcutaneous Administration to Animals of the F0-Generation, Separate Male and Female Study Segment I Study

Study no.: 12018/99
Location: -1201899-study-report.pdf
Conducting laboratory and location:

Date of study initiation: January 31, 2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 99040145 / 99040143

Methods

Doses:

Group	SPM 962	
	dose in mg/kg b.w./ day, s.c.	volume in ml/kg/ b.w./day (concentration used)
both male and female animals placebo-treated		
1	0 (control)	1.5 (SPM 962 placebo)
female animals treated, male animals untreated		
2	1.5	0.3 (0.5%)
3	5	0.5 (1%)
4	15	1.5 (1%)
male animals treated, female animals untreated		
5	1.5	0.3 (0.5%)
6	5	0.5 (1%)
7	15	1.5 (1%)

Figure 86, from page 22 of Report 12018/99

Species/strain: Rat, Sprague-Dawley / CD(BR)
Number/sex/group: 20/sex/dose
Route, formulation, volume, and infusion rate: subcutaneous injection into the back region; 3 sites were rotated (neck region, just behind shoulder region, and caudal part of the back region)
Satellite groups used for toxicokinetics: not done

Study design: males dosed starting 70 days before mating, females dosed 14 days prior to mating and until gestation day 6; treated rats mated with untreated rats

Parameters and endpoints evaluated: Fertility and early fetal development (up to GD 13)

Results

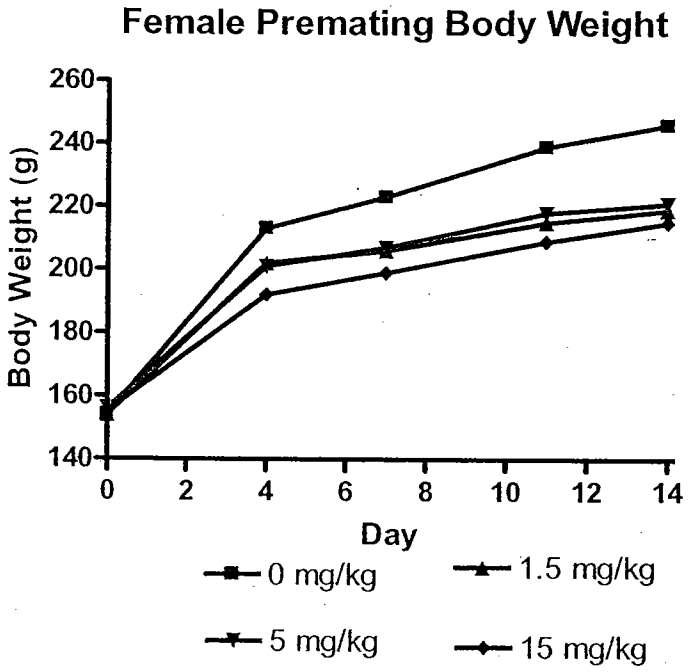
Mortality:
One 15 mg/kg male died

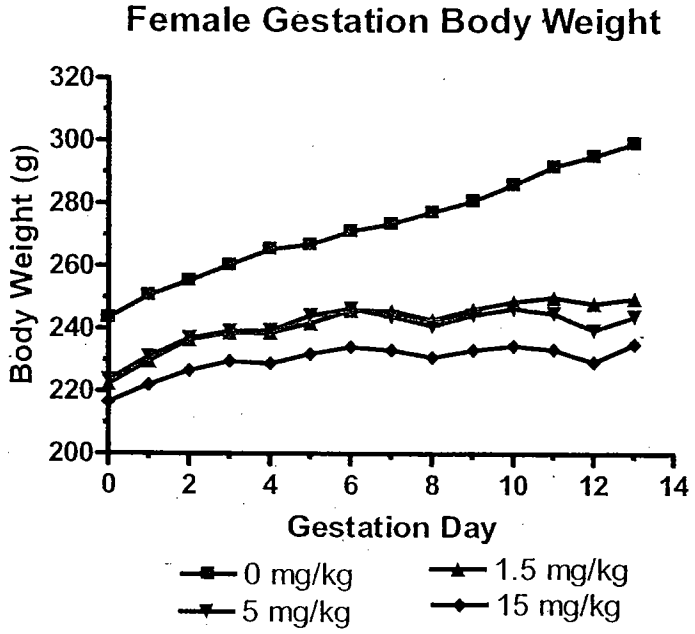
Clinical signs:
Restlessness was observed at 1.5 mg/kg and above (i.e., all doses) in both sexes.

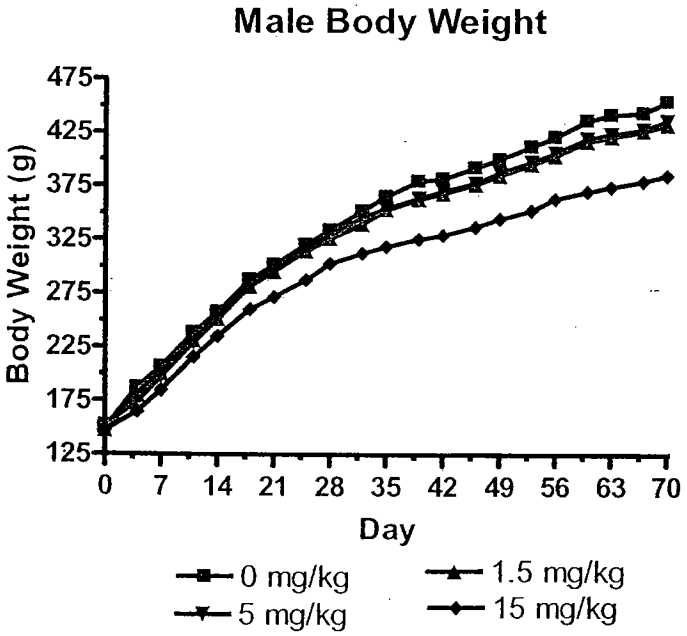
Body weight:

Mating Body Weights in g (% of control)

	0 mg/kg	1.5 mg/kg	5 mg/kg	15 mg/kg
Male	454 (100%)	432 (95%)	436 (96%)	385 (85%)
Female	246 (100%)	219 (89%)	221 (90%)	215 (87%)







Food consumption:
Increased relative food consumption was observed in females post-mating; the significance of this finding is uncertain.
No effects were observed on females or male rats during the pre-mating period.

Prolactin Levels: 5 females/dose

Mean (SEM) Prolactin Plasma Concentrations in ng/ml in Female Rats

Dose	Predose	Day 2	Day 13	GD 13
0 mg/kg	114 (39.8)	114 (77.9)	25 (8.8)	13 (4.9)
1.5 mg/kg	97 (26.8)	22 (13.4)	12 (3.3)	58 (19.5)
5 mg/kg	67 (51.3)	8 (0.6)	6 (0.1)	135 (62.7)
15 mg/kg	82 (39.1)	8 (0.6)	7 (0.2)	9 (1.4)*

*N=4

Toxicokinetics:

Mean (SEM) Rotigotine concentrations in ng/ml at 6 hours post dosing

Dose	Males		Females	
	Day 2	Day 70	Day 2	Day 12
1.5 mg/kg	3.12 (0.43)	4.99 (0.08)	4.34 (3.06)	4.72 (0.50)
5 mg/kg	9.74 (1.8)	10.92 (1.87)	10.38 (1.73)	13.98 (1.45)
15 mg/kg	40.88 (6.11)	53.02 (4.97)	26.42 (3.12)	37.76 (3.45)

Necropsy:
Injection site edema was observed in all females at 15 mg/kg. No effects noted at lower doses.

No substance related systemic findings were noted.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

None of the female treated rats were pregnant at necropsy. This is likely due to the pharmacological action of rotigotine (prolactin lowering).

No adverse effects were observed on male fertility. However, the percentage of motile sperm in the epididymal cauda was decreased at 15 mg/kg. No effects were observed on testes or epididymis weights.

Criteria	Group 4 Control	Group 5 1.5 mg/kg	Group 6 5 mg/kg	Group 7 15 mg/kg
male rats evaluated	n = 20	n = 20	n = 20	n = 19
percentage of motile spermatozoa in the epididymal cauda	$\bar{x}$: 90.2 SD: 7.8 t: -	$\bar{x}$: 85.2 SD: 18.7 t: ns	$\bar{x}$: 88.3 SD: 9.8 t: ns	$\bar{x}$: 80.0 SD: 12.1 t: 3.159
spermatids per g testicular tissue X 10^6	$\bar{x}$: 76.4 SD: 13.9 t: -	$\bar{x}$: 76.3 SD: 19.3 t: ns	$\bar{x}$: 84.2 SD: 5.8 t: ns	$\bar{x}$: 83.8 SD: 9.6 t: ns

____: ($p \leq 0.01$). Dunnett or Student

Figure 87, from page 85 of Report — -12018/99

Key study findings:

1. No effects were observed on male fertility, although sperm motility was decreased at 15 mg/kg. Fertility is an insensitive endpoint in rats so the potential for effects on male fertility is uncertain.
2. There was a trend to lower serum prolactin levels in rotigotine treated females rats. However there was a wide variation in prolactin concentrations which prevented the changes from being statistically significant.
3. None of the treated female rats were pregnant at termination. This is most likely a pharmacological effect of rotigotine since rotigotine is a dopamine agonist which would be expected to lower prolactin levels.

2.6.6.6.2 Examination of the Influence of SPM 962 on the Fertility and Early Embryonic Development to Implantation of Mice by Subcutaneous Administration to the Female Animals of the F0 Generation - In Accordance with the ICH Guideline 4.1.1 - Segment I Study

Study no.: 17203/03
Location: 1720303-study-report.pdf
Conducting laboratory and location: [redacted]

Date of study initiation: November 17, 2003
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 20302045, 20302046, 2017057, 20107058

Methods

Doses: See table below

Group	SPM 962 dose or administration volume				Number and sex of mice (for animal nos. see table below)	
	treatment from 2 weeks until 4 days before mating		treatment from 3 days before mating until day 7 of gestation			
	[mg/kg b.w.]	[mL/kg b.w.] [concentration used]	[mg/kg b.w.]	[mL/kg b.w.] [concentration used]	Main study	Satellites
males untreated, females treated						
1	0 (control)	9 SPM 962 placebo	0 (control)	6.5 SPM 962 placebo	20 m 20 f	24 m 24 f
2	10	2 (0.5%)	6	6.5 (0.1%)	20 m 20 f	24 m 24 f
3	30	3 (1.0%)	6	6.5 (0.1%)	20 m 20 f	24 m 24 f
4	90	9 (1.0%)	6	6.5 (0.1%)	20 m 20 f	24 m 24 f

Group	Animal nos. and sex		
	Main study	Satellites	
		Kinetics	Prolactin
1	1 - 20 m 21 - 40 f	161 - 172 m 185 - 196 f	173 - 184 m 197 - 208 f
2	41 - 60 m 61 - 80 f	209 - 220 m 233 - 244 f	221 - 232 m 245 - 256 f
3	81 - 100 m 101 - 120 f	257 - 268 m 281 - 292 f	269 - 280 m 293 - 304 f
4	121 - 140 m 141 - 160 f	305 -316 m 329 - 340 f	317 - 328 m 341 - 352 f

m: male
f: female

Figure 88, from page 28 of Report 17203/03

Species/strain: Mouse, CD-1 / Icr: CD1

Number/sex/group: 20/sex/dose

Route, formulation, volume, and infusion rate: subcutaneous injection into three sites in the back region (neck region, just behind the shoulder region, caudal part of the back region).

Satellite groups used for toxicokinetics: 24/sex/group

Study design: males were untreated, females dosed 14 days prior to mating through gestation day 7; females were sacrificed on GD 14

Parameters and endpoints evaluated: Fertility and early fetal development (up to GD 14)

Results

Mortality:
No treatment related mortality was observed. One group 2 female died due to accident.

Clinical signs:

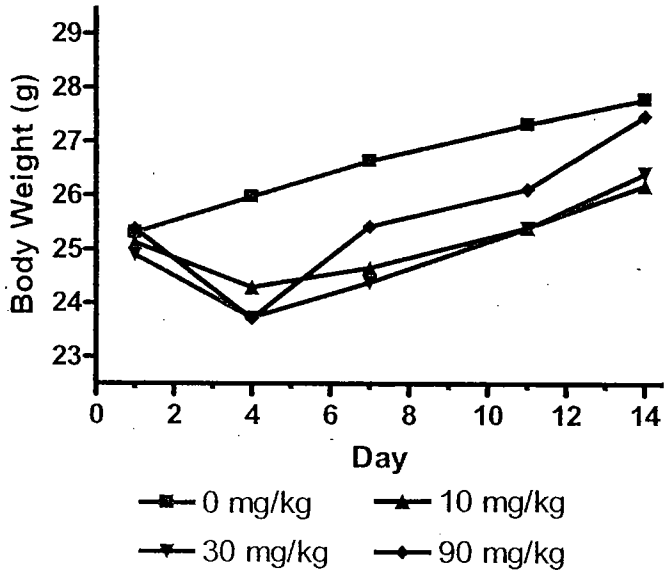
Group / Dose level	Symptoms / Criteria	No. of affected females during premating	during mating	during gestation
1 (Control)	Local intolerance reactions: wheal(s)	9 of 20	11 of 20	14 of 20
2 (10/6 mg/kg)	Local intolerance reactions: wheal(s)	0 of 20	0 of 20	0 of 20
3 (30/6 mg/kg)	Local intolerance reactions: wheal(s)	0 of 20	0 of 20	0 of 20
4 (90/6 mg/kg)	Local intolerance reactions: wheal(s)	6 of 20	10 of 20	13 of 20

Figure 89, from page 51 of Report — -17203/03

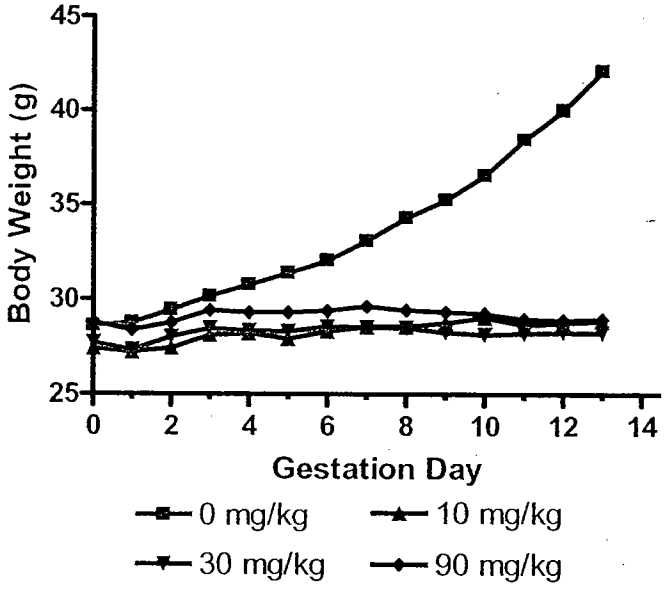
no significant clinical signs were observed.

Body weight:
Transient decrease in body weight followed by recovery; post mating body weight data are confounded by pregnancy status (among treated mice, only one low dose female was pregnant).

Premating Body Weights



Postmating Body Weights



Food consumption:
No significant effects

Prolactin Levels: Pooled samples (3 mice/pool, 4 pools/dose, 12 mice/dose) were used.

Prolactin Plasma Concentrations in ng/ml in Female Mice

Dose	Day 7		Gestation Day 7	
	Values	Mean (SEM)	Values	Mean (SEM)
0 mg/kg	/	4.67 (1.06)	/	7.92 (3.85)
10 mg/kg	/	3.79 (1.73)	/	2.83 (0.94)
30 mg/kg	/	1.49 (0.44)	/	1.84 (0.62)
90 mg/kg	/	4.61 (2.02)	/	5.98 (3.59)

Toxicokinetics: Blood was collected 8 hours post dosing. Pooled samples (3 mice/pool, 4 pools/dose, 12 mice/dose) were used.

Mean (SEM) Rotigotine concentrations in ng/ml at 6 hours post dosing

Dose	Day 7	Gestation Day 7
0 mg/kg	0.31 (0.05)	Not detected
10 mg/kg	21.4 (1.44)	12.2 (0.99)
30 mg/kg	61.8 (3.78)	9.10 (0.84)
90 mg/kg	120 (13.1)	15.8 (3.04)

Necropsy:
Injection site edema was observed in all females at 15 mg/kg. No effects noted at lower doses.

No substance related systemic findings were noted.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Rotigotine treated mice were not pregnant.

Key study findings:
Only one treated mouse was pregnant at necropsy. Sponsor attributed effect to inhibition of prolactin, although the data do not suggest that there was a consistent effect on prolactin levels. In addition, the sponsor did not provide literature references to support this conclusion.

2.6.6.2.3 Study of Embryo-Fetal Development In Rats With SPM 962 By Subcutaneous Administration – Segment II Study

Study No: and number: 12452/99
Site and testing facility: [redacted]

GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: 99080183 and 99080184/1

Methods

Doses: 0, 0.5, 1.5, and 5 mg/kg
Species/strain: Rat, Sprague-Dawley / [redacted] CD(BR)
Number/sex/group: 20 females/dose (actually, there were 25, 30, 30 and 35 females in the 0, 0.5, 1.5 and 5 mg/kg dose groups, respectively, but only 20 pregnant rats were included per dose)
Route, formulation, volume, and infusion rate: Subcutaneous injection into the back region; 3 sites were rotated (neck region, just behind shoulder region, and caudal part of the back region)
Satellite groups used for toxicokinetics: not done
Study design: Females administered rotigotine from Gestation Day 6 through 17
Parameters and endpoints evaluated: embryo-fetal development

Results

Mortality (dams):
None

Clinical signs (dams):
0.5 mg/kg and above- restlessness, increased locomotion
1.5 mg/kg- vaginal bleeding in 4/29 dams on GD 8, 9 or 10
5 mg/kg- vaginal bleeding in 21/28 dams on GD 8, 9, or 10

Body weight (dams):
Sponsor stated that there was decreased body weight gain at 1.5 and 5 mg/kg. This decrease is attributable to the high number of resorptions at these doses; sponsor did not analyze body weight data of pregnant animals separately; no difference in body weight of dams with viable offspring.

Food consumption (dams):
Decreased food consumption was observed at 1.5 mg/kg and above.

Toxicokinetics:
Not done

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Parameter		Group 1 Control	Group 2 0.5 mg/kg	Group 3 1.5 mg/kg	Group 4 5 mg/kg
Corpora lutea	total	306	326	467	409
	per dam	15.3	15.5	16.1	14.6
Implantation sites	total	297	311	**418	*382
	per dam	14.9	14.8	14.4	13.6
Resorptions	total	17	*31	**186	**382
	per dam	0.9	1.5	6.4	13.6
Early resorptions	total	15	24	*181	**382
	per dam	0.8	1.1	6.2	13.6
Late resorptions	total	2	7	*5	**0
	per dam	0.1	0.3	0.2	0.0
Live fetuses	total	280	*280	**232	**0
	per dam	14.0	14.0	14.5	0.0
Pre-implantation loss	mean %	2.9	4.1	9.6	6.1
Post-implantation loss	mean %	6.7	9.9	47.3	100.0

* Significantly different from the controls at $p \leq 0.05$
** Significantly different from the controls at $p \leq 0.01$

Figure 90, from page 24 of Report — 12452/99

Offspring (malformations, variations, etc.):
No drug-related external, visceral or skeletal abnormalities were observed.

Incidence of Fetal Visceral Variations

Type	0 mg/kg	0.5 mg/kg	1.5 mg/kg	5 mg/kg
Total Fetuses (Litters) Examined	140 (20)	140 (20)	116 (16)	0 (0)
4th Cerebral Ventricle Dilated	1 (1)	1 (1)	4 (3)	0 (0)
Cerebellum; Hemorrhage	0 (0)	1 (1)	0 (0)	0 (0)
Renal Pelvis Dilation	6 (5)	5 (5)	8 (7)	0 (0)
Kidney Hemorrhagic Focus	3 (3)	1 (1)	0 (0)	0 (0)
Liver Hemorrhagic Focus	6 (5)	7 (7)	5 (4)	0 (0)
Thoracic Cavity Hemorrhage	0 (0)	3 (2)	0 (0)	0 (0)
Total Variations	14 (11)	17 (11)	17 (11)	0 (0)

Incidence of Fetal Skeletal Variations (litters affected)

Type	0 mg/kg	0.5 mg/kg	1.5 mg/kg	5 mg/kg
Total Fetuses Examined	140 (20)	140 (20)	116 (16)	0 (0)
Skull, Interparietal not ossified	0 (0)	0 (0)	1 (1)	0 (0)
Wavy Ribs	4 (3)	1 (1)	1 (1)	0 (0)
Shortened Ribs	1 (1)	1 (1)	0 (0)	0 (0)
Bipartite Sternebra	3 (3)	4 (2)	0 (0)	0 (0)
Misaligned Sternebra (slight)	2 (2)	2 (1)	2 (2)	0 (0)
Total Variations	10 (9)	7 (4)	4 (4)	0 (0)

Incidence of Fetal Skeletal Retardations (litters affected)

Type	0 mg/kg	0.5 mg/kg	1.5 mg/kg	5 mg/kg
Total Fetuses Examined	140 (20)	140 (20)	116 (16)	0 (0)
5th Metacarpalia Not Ossified	5 (5)	2 (1)	2 (1)	0 (0)
Hyoid Not Ossified	21 (10)	26 (12)	14 (7)	0 (0)
Skull Incomplete Ossification	16 (9)	17 (9)	9 (5)	0 (0)
Sternebrae Incompletely Ossified or Reduced in Size	136 (20)	134 (20)	111 (16)	0 (0)
Thoracic Vertebral Bodies Incompletely Ossified or Misshapen	2 (2)	6 (5)	6 (4)	0 (0)
Total Retardations	136 (20)	134 (20)	111 (16)	0 (0)

Historical Control Data

1. General reproductive indices

Indices	M ± SD of the control groups (n = 30)	Range for control groups	M ± SD of groups not significantly influenced by any test compound (n = 52)	Range for the groups
No. of groups	30	-	52	-
No. of pregnant animals	704	16 - 24	1195	16 - 24
No. of living and dead fetuses	11135	-	18363	-
No. of living and dead fetuses per dam	15.4 ± 1.4	13.7 - 18.8	15.0 ± 1.9	12.6 - 16.4
No. of dead fetuses at laparotomy absolute per dam	6 0.01 ± 0.03	0 - 4 0 - 0.17	7 0.01 ± 0.03	0 - 3 0 - 0.18
No. of resorptions per dam	0.83 ± 0.15	0.6 - 1.1	0.76 ± 0.22	0.5 - 1.0
Rate of early resorptions in %	79.4 ± 12.1	56.3 - 87.6	89.1 ± 12.0	67.4 - 100
No. of implantations per dam	16.1 ± 1.7	14.1 - 17.9	16.4 ± 2.0	13.7 - 19.0
No. of corpora lutea per dam	16.6 ± 1.2	15.3 - 18.1	16.7 ± 1.2	14.7 - 19.5
No. of malformations total per dam in % mean %	113 15.60 ± 4.06 1.23 ± 0.44	0 - 6 0 - 30.0 -	171 14.19 ± 2.39 0.70 ± 0.37	0 - 5 0 - 25.0 -
No. of runts total per dam	30 0.04 ± 0.04	0 - 4 0 - 0.08	27 0.02 ± 0.03	0 - 4 0 - 0.21
Fetuses with skeletal retardations (mean %) variations (mean %)	88.2 ± 13.1 12.5 ± 6.6	63.0 - 97.1 0 - 23.9	90.7 ± 5.6 14.0 ± 7.4	62.7 - 98.6 0 - 26.5
Fetuses with visceral variations (mean %)	11.4 ± 6.4	5.9 - 21.8	11.6 ± 3.7	7.9 - 19.2
No. of twins, total	5	0 - 2	11	0 - 3
Sex ratio ♂:♀	1.07 ± 0.09	0.95 - 1.2	1.03 ± 0.11	0.89 - 1.14
Mean weight of fetuses in g	3.6 ± 0.3	3.4 - 4.0	3.8 ± 0.2	3.4 - 4.1
Mean weight of placentae in g	0.55 ± 0.06	0.47 - 0.60	0.53 ± 0.03	0.49 - 0.59

Figure 91, from page 355 of Report — 12452/99

4. Malformations

Indices	Data of control groups (n = 30)			Data of groups not significantly influenced by any test compound (n = 52)		
	no.	mean %	Range for control groups mean %	no.	mean %	Range for the groups mean %
No. of groups examined	30	-	-	52	-	-
No. of fetuses examined	11135	-	-	18383	-	-
No. of litters examined	704	-	-	1195	-	-
Acephalus, spina bifida, omphalocele, short extremities	1	0.01 ±0.10	0 - 0.4	0	-	-
Microcephaly	0	-	-	1	0.01 ±0.10	0 - 0.5
Meningoencephalocele or preliminary encephalocele	3	0.03 ±0.14	0 - 0.9	1	0.01 ±0.10	0 - 0.5
Meningoencephalocele, spina bifida, cleft palate	1	0.01 ±0.10	0 - 0.4	0	-	-
Open eyelid	0	-	-	2	0.02 ±0.10	0 - 1.1
Cleft palate	0	-	-	2	0.02 ±0.10	0 - 1.1
Agnathia	0	-	-	1	0.01 ±0.10	0 - 0.5
Brachygnathia	1	0.01 ±0.10	0 - 0.4	2	0.02 ±0.10	0 - 1.1
Brachygnathia, small oral opening, absent tongue, skeletal fusions	0	-	-	1	0.01 ±0.10	0 - 0.5
Thoracic situs inversus	1	0.01 ±0.10	0 - 0.9	1	0.01 ±0.10	0 - 0.8
Thoracic and abdominal situs inversus	0	-	-	1	0.01 ±0.10	0 - 0.5
Omphalocele	1	0.01 ±0.10	0 - 0.4	2	0.02 ±0.10	0 - 1.0
Omphalocele, short tail	0	-	-	1	0.01 ±0.10	0 - 0.5
Scoliosis	0	-	-	1	0.01 ±0.10	0 - 0.5
Short abdomen, short tail	22	0.20 ±0.44	0 - 1.90	31	0.17 ±0.36	0 - 2.0
Short abdomen, short tail, uni- or bilateral crossed leg position	27	0.24 ±0.60	0 - 2.42	33	0.18 ±0.35	0 - 2.0
Short abdomen, short tail, uni- or bilateral crossed leg position, anal atresia	0	-	-	1	0.01 ±0.10	0 - 0.5

Figure 92, from page 360 of Report — 12452/99

4. Malformations (cont.)

Indices	Data of control groups (n = 30)			Data of groups not significantly influenced by any test compound (n = 52)		
	no.	mean %	Range for control groups mean %	no.	mean %	Range for the groups mean %
Short abdomen, short tail, thoracic situs inversus	1	0.01 ±0.10	0 - 0.9	0	-	-
Short abdomen, short tail, cleft palate	0	-	-	1	0.01 ±0.10	0 - 0.5
Short tail	4	0.03 ±0.14	0 - 0.9	3	0.03 ±0.15	0 - 0.8
Crossed leg position	0	-	-	1	0.01 ±0.10	0 - 0.8
Hindlimbs shortened, unilateral club-foot	1	0.01 ±0.10	0 - 0.9	0	-	-
Generalized oedema	1	0.01 ±0.10	0 - 0.9	0	-	-
Haematoma* location in:						
- cerebral ventricle	1	0.01 ±0.10	0 - 0.9	2	0.02 ±0.13	0 - 1.1
- pericardium	4	0.05 ±0.12	0 - 1.1	9	0.05 ±0.11	0 - 0.8
- thoracic cavity	49	0.44 ±0.31	0 - 1.2	88	0.48 ±0.27	0 - 1.2
- abdominal cavity	3	0.03 ±0.14	0 - 0.9	3	0.03 ±0.15	0 - 0.9
Intestine shortened*	0	-	-	2	0.02 ±0.10	0 - 1.1

Figure 93, from page 361 of report — 12452/99

Key Study Findings

1. The doses used in this study were limited by the pharmacological action of rotigotine. Rats require prolactin for early embryonic/fetal development. Since rotigotine suppresses prolactin secretion, rotigotine adversely affected embryo-fetal development resulting in increased resorptions at 1.5 and 5 mg/kg/day.
2. No other effects were observed on embryo-fetal development.
3. This reviewer is concerned about the sensitivity of this study to detect an effect if one was present. No malformations were detected at any dose level. The historical control data suggest that about 1.23% of the 396 fetuses would be expected to have a malformation (expected number =5). In addition, the historical control data did not include any visceral or skeletal malformations. This suggests that the laboratory could not detect these malformations if they were present.

2.6.6.2.4 Study of Embryo-Fetal Development in CD-1 Mice With SPM 962 By Subcutaneous Administration

Study No: and number: 14011/01
Site and testing facility: [redacted]

GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: 20106915 and 20107016

Methods

Doses: 0, 10, 30 and 90 mg/kg
Species/strain: Mouse, CD-1. CD-1(ICR)BR
Number/sex/group: 20 females/dose (actually, there were 25/group, but only 20 pregnant mice were included per dose)
Route, formulation, volume, and infusion rate: Subcutaneous injection into the back region; 3 sites were rotated (neck region, just behind shoulder region, and caudal part of the back region)
Satellite groups used for toxicokinetics: not done
Study design: Females administered rotigotine from Gestation Day 6 through 15
Parameters and endpoints evaluated: embryo-fetal development

Results

Mortality (dams):
Three mice died at 90 mg/kg; no consistent necropsy findings were observed.

Dam No.	Day of Death (Gestation day)	Macroscopic findings
91	15	Stomach: multiple haemorrhagic foci (diameter approx. 0.5 - 1 mm) Intestines: inflated, empty
97	8	Thoracic cavity: filled with dark-red soft mass Small intestine: inflated
100	12	Vagina: haemorrhagic

Figure 94, from page 36 of Report 14011/01

Clinical signs (dams):

TABLE 2 SUMMARY OF MATERNAL CLINICAL SIGNS (systemic)

		TEST GROUP 1 Control	TEST GROUP 2 10 mg/kg	TEST GROUP 3 30 mg/kg	TEST GROUP 4 90 mg/kg
NO REMARKABLE OBSERVATIONS	N	19	20	17	0
	%	95.0	100.0	85.0	0.0
DAM DIED PREMATURELY	N	0	0	0	3
	%	0.0	0.0	0.0	13.0
HUNCHED POSTURE	N	0	0	0	23
	%	0.0	0.0	0.0	100.0
PILO-ERECTION	N	0	0	3	10
	%	0.0	0.0	15.0	43.5
PRELETHAL SYMPTOMS: ATAXIA, REDUCED MOTILITY AND/OR PILO-ERECTION	N	0	0	0	3
	%	0.0	0.0	0.0	13.0
RESTLESSNESS	N	0	0	0	23
	%	0.0	0.0	0.0	100.0

Figure 95, from page 48 of Report 14011/01

Wheals were observed in one 30 mg/kg dam and 16 90 mg/kg dams.

Body weight (dams):
Slightly decreased body weights at 30 and 90 mg/kg

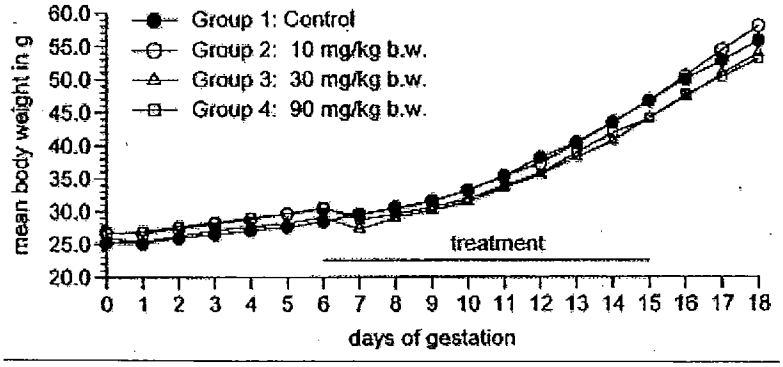


Figure 96, from page 34 of Report [redacted] -14011/01

Mean Body Weight in Grams (% of Control) in Treated Dams

Gestation Day	0 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
0	25.2 (100%)	26.8 (106%)	25.9 (103%)	26.6 (106%)
6	28.4 (100%)	30.3 (107%)	29.1 (102%)	30.6 (108%)
9	31.6 (100%)	31.5 (100%)	30.1 (95%)	30.6 (97%)
12	38.2 (100%)	37.3 (98%)	35.5 (93%)	35.8 (94%)
15	46.7 (100%)	46.8 (100%)	44.1 (94%)	44.1 (94%)
18	55.8 (100%)	58.0 (104%)	53.9 (97%)	53.1 (95%)

Food consumption (dams):
A transient decrease was observed in all drug treated groups on gestation day 7 (i.e, after the first dose of drug), then returned to control levels.

Toxicokinetics: Not done

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

TABLE 7 SUMMARY OF REPRODUCTION DATA

		TEST GROUP 1 Control	TEST GROUP 2 10 mg/kg	TEST GROUP 3. 30 mg/kg	TEST GROUP 4 90 mg/kg
Females Pregnant	N	20	20	20	23
Aborted	N	0	0	0	0
Premature Birth	N	0	0	0	0
Dams with Viable Fetuses	N	20	20	20	19
Dams with all Resorptions	N	0	0	0	1
Female Mortality	N	0	0	0	3
	%	0	0	0	12
Pregnant at C-section	N	20	20	20	20
	%	100	100	100	100
Corpora Lutea	MEAN	13.1	14.1	12.7	12.9
	S.D.	2.1	1.5	2.4	2.5
	TOTAL	261	281	253	258
Implantation Sites	MEAN	12.9	14.1	12.5	12.7
	S.D.	2.1	1.5	2.3	2.6
	TOTAL	258	281	250	254
Pre-implantation Loss	MEAN%	1.1	0.0	1.0	1.6
	S.D.	2.8	0.0	2.5	4.5
Post-implantation Loss	MEAN%	2.6	2.0	2.4	10.4
	S.D.	3.6	3.2	3.8	22.8

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05 ** = P<0.01 (Fisher or Chi-square test)

Figure 97, from page 54 of Report — 14011/01

Total Number Post Implantation Loss (Resorptions) of implants

Type	0 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
Early	2 (0.8%)	4 (1.3%)	2 (0.9%)	19 (7.8%)
Late	5 (1.8%)	2 (0.7%)	4 (1.4%)	4 (2.3%)
Total	7 (2.6%)	6 (2.0%)	6 (2.4%)	23 (10.1%)

12 of the 90 mg/kg early resorptions were observed in one dam (#79) with complete resorptions of all implants. Discounting that dam, there would have been 7 early resorptions (2.9%) at 90 mg/kg and 11 total resorptions (5.4%).

Offspring (malformations, variations, etc.):
No significant effects on fetal weight were observed.
The only external malformation was an encephalocele observed at 90 mg/kg; no external variations were observed.

No skeletal or visceral malformations were observed.

Incidence of Fetal Visceral Variations

Type	0 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
Total Fetuses (Litters) Examined	125 (20)	137 (20)	122 (20)	116 (19)
4th Cerebral Ventricle Dilated	0	1	1	1
Renal Pelvis Dilation	0	0	1	1
Liver Hemorrhagic Focus	0	3	2	1
Misplaced Kidney	1	0	0	1
Thoracic Cavity Hemorrhage	2	0	0	1
Total Variations	3	4	4	5

All variations occurred in separate litters.

Incidence of Fetal Skeletal Variations (litters affected)

Type	0 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
Total Fetuses (Litters) Examined	126 (20)	138 (20)	122 (20)	116 (19)
Accessory 14th Ribs	9 (7)	12 (7)	12 (8)	10 (6)
Fused Ribs (slight)	0 (0)	1 (1)	0 (0)	0 (0)
Shortened Ribs	1 (1)	0 (0)	0 (0)	0 (0)
Bipartite Sternebra	41 (13)	35 (14)	37 (14)	43 (15)
Misaligned Sternebra (slight)	0 (0)	0 (0)	0 (0)	1 (1)
Total Variations	44 (15)	44 (16)	44 (16)	51 (16)

Incidence of Fetal Skeletal Retardations (litters affected)

Type	0 mg/kg	10 mg/kg	30 mg/kg	90 mg/kg
Total Fetuses (Litters) Examined	126 (20)	138 (20)	122 (20)	116 (19)
Cervical Vertrebeal Bodies Not Ossified	2 (1)	0 (0)	1 (1)	0 (0)
Cervical Vertrebral Bodies Reduced in Size	4 (2)	5 (2)	**11 (5)**	**23 (6)**
Skull Incomplete Ossification	2 (2)	4 (2)	2 (2)	3 (3)
Sternebrae Incompletely Ossified	23 (12)	27 (17)	26 (13)	**37 (15)**
Sternebrae Reduced in Size	1 (1)	2 (1)	0 (0)	1 (1)
Tails Not Ossified	50 (13)	60 (14)	55 (13)	**66 (15)**
Total Retardations	59 (16)	75 (19)	68 (16)	**80 (18)**

Values in **Bold** significantly different from controls ($p<0.05$)

Historical Control Data

4. Malformations

Indices	Data of control groups n = 4			Data of groups not significantly influenced by any test compound n = 12		
	no.	mean%	range for control groups mean %	no.	mean %	range for the groups mean %
No. of groups examined	4	-	-	12	-	-
No. of fetuses examined	1050	-	-	3158	-	-
No. of litters examined	90	-	-	268	-	-
A. External findings						
Head						
Encephalocele	2	0.3 ± 0.5	0.0 - 1.0	2	0.3 ± 0.5	0.0 - 1.0
Mouth and jaw						
Brachygnathia	0	0.0 ± 0.0	0.0 - 0.0	0	0.0 ± 0.0	0.0 - 0.0
Cleft palate	2	0.3 ± 0.5	0.0 - 1.0	5	0.4 ± 0.9	0.0 - 1.4
Extremities						
Malrotated limbs	0	0.0 ± 0.0	0.0 - 0.0	0	0.0 ± 0.0	0.0 - 0.0
Micromelia	0	0.0 ± 0.0	0.0 - 0.0	4	0.3 ± 0.6	0.0 - 1.9
Tail						
Rudimentary	0	0.0 ± 0.0	0.0 - 0.0	0	0.0 ± 0.0	0.0 - 0.0
Brachycaudia	0	0.0 ± 0.0	0.0 - 0.0	0	0.0 ± 0.0	0.0 - 0.0
B. Skeletal findings#						
Skull						
Frontal, Parietal, Interparietal, Supraoccipital bones:						
Incomplete ossification	0	0.0 ± 0.0	0.0 - 0.0	0	0.0 ± 0.0	0.0 - 0.0
Extremities						
Individual bones:						
Incomplete ossification	0	0.0 ± 0.0	0.0 - 0.0	0	0.0 ± 0.0	0.0 - 0.0
C. Visceral findings#						
Liver						
Displaced	0	0.0 ± 0.0	0.0 - 0.0	0	0.0 ± 0.0	0.0 - 0.0
Intestines						
Displaced	0	0.0 ± 0.0	0.0 - 0.0	0	0.0 ± 0.0	0.0 - 0.0

Figure 98, from page 294 of Report — 14011/01

Key Study Findings

1. Mortality was observed at 90 mg/kg.
2. Slightly reduced body weight (about 6%) observed at 30 and 90 mg/kg.
3. Increased early resorptions were observed at 90 mg/kg, primarily due to one dam with complete resorption of all implants.
4. No effects on fetal viability or fetal weight were observed.
5. No significant increase in malformations or variations was observed.
6. A increase in the incidence of skeletal retardation was observed at 90 mg/kg.
7. This reviewer is concerned about the sensitivity of this study to detect an effect if one was present. Only one malformation was observed in this study. In addition, the historical control data did not include any visceral or skeletal malformations. This suggests that the laboratory could not detect these malformations if they were present.

2.6.6.2.5 Study Of Embryo-Fetal Development In Rabbits With SPM 962 By Subcutaneous Administration

Study No: and number: 12453/99
Site and testing facility: [redacted]
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: 99080184/2 and 99080185/1 + 2

Methods

Doses: 0, 1, 5, and 15 mg/kg

Species/strain: Rabbit, Himalayan

Number/sex/group: 20 females/dose

Route, formulation, volume, and infusion rate: Subcutaneous injection into the back region; 3 sites were rotated (neck region, just behind shoulder region, and caudal part of the back region)

Satellite groups used for toxicokinetics: not done

Study design: Females administered rotigotine from Gestation Day 6 through 20; rabbits were sacrificed on day 29

Parameters and endpoints evaluated: embryo-fetal development

Results

Mortality (dams):
None

Clinical signs (dams):
Increased restlessness and locomotion at 5 and 15 mg/kg; No clinical signs at 1 mg/kg

TABLE 1 [redacted] SUMMARY OF MATERNAL CLINICAL SIGNS (local)

		TEST GROUP 1 Control	TEST GROUP 2 1 mg/kg	TEST GROUP 3 5 mg/kg	TEST GROUP 4 15 mg/kg
NO REMARKABLE OBSERVATIONS	N	18	13	14	15
	%	90.0	65.0	70.0	75.0
INJECTION SITE: ESCHAR FORMATION	N	1	4	5	1
	%	5.0	20.0	25.0	5.0
INJECTION SITE: REDDENED	N	0	0	0	1
	%	0.0	0.0	0.0	5.0
INJECTION SITE: HAEMORRHAGIC	N	1	3	0	0
	%	5.0	15.0	0.0	0.0
INJECTION SITE: HAEMORRHAGIC/ESCHAR FORMATION	N	0	0	1	1
	%	0.0	0.0	5.0	5.0
INJECTION SITE: THICKENED	N	0	0	0	2
	%	0.0	0.0	0.0	10.0

Figure 99, from page 37 of Report — 12453/99

Body weight (dams):
No significant effects

Figure 1 Body weight of female animals daily mean values per group

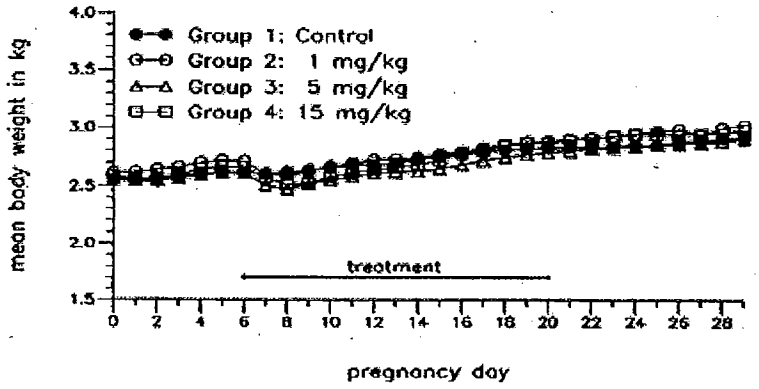


Figure 100, from page 29 of Report — 12453/99

Food consumption (dams):
A transient decrease was observed in all drug treated groups on gestation day 7 (i.e., after the first dose of drug), which then returned to control levels.

Toxicokinetics: Not done

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

APPEARS THIS WAY
ON ORIGINAL

Parameter		Group 1 Control	Group 2 1 mg/kg	Group 3 5 mg/kg	Group 4 15 mg/kg
Corpora lutea	total	157	165	145	162
	per dam	7.9	8.3	7.3	8.5
Implantation sites	total	107	125	108	113
	per dam	5.4	6.3	5.4	5.9
Resorptions	total	4	4	4	5
	per dam	0.2	0.2	0.2	0.3
Early resorptions	total	3	2	4	4
	per dam	0.2	0.1	0.2	0.2
Late resorptions	total	1	2	0	1
	per dam	0.1	0.1	0.0	0.1
Live fetuses	total	103	121	104	108
	per dam	5.2	6.1	5.2	5.7
Dead fetuses	total	0	0	0	0
Pre-implantation loss	mean %	28.6	22.9	26.1	29.0
Post-implantation loss	mean %	3.5	2.6	4.5	4.3

Figure 101, from page 31 of Report — -12453/99

Offspring (malformations, variations, etc.):
No significant effects on fetal weight were observed.
No skeletal malformations were observed.

Incidence of External Malformations/Variations

Type	0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg
Total Fetuses (Litters) Examined	103 (20)	121 (20)	104 (20)	108 (19)
Hyperflexion of the Paw	**0 (0)**	**0 (0)**	**2 (1)**	**2 (2)**
Omphalocele	**1 (1)**	**0 (0)**	**0 (0)**	**1 (1)**
Misplaced Kidney	3 (1)	0 (0)	0 (0)	0 (0)
Total Malformations	1 (1)	0 (0)	2 (1)	3 (3)
Total Variations	3 (1)	0 (0)	0 (0)	0 (0)

Malformations are in **Bold**

Incidence of Fetal Skeletal Variations (litters affected)

Type	0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg
Total Fetuses (Litters) Examined	103 (20)	121 (20)	104 (20)	108 (19)
Caudal Vertebral Body Fused	0 (0)	1 (1)	0 (0)	1 (1)
Skull, Parietal Area Not Ossified	0 (0)	1 (1)	1 (1)	1 (1)
Sternebrae Fused	8 (7)	6 (4)	4 (4)	14 (8)
Misaligned Sternebra (slight)	0 (0)	0 (0)	1 (1)	1 (1)
Total Variations	8 (7)	8 (6)	5 (5)	16 (10)

Incidence of Fetal Skeletal Retardations (litters affected)

Type	0 mg/kg	1 mg/kg	5 mg/kg	15 mg/kg
Total Fetuses (Litters) Examined	103 (20)	121 (20)	104 (20)	108 (19)
Hyoid Not Ossified	0 (0)	3 (1)	1 (1)	1 (1)
Sternebrae Incompletely Ossified	62 (19)	60 (17)	64 (17)	59 (17)
Total Retardations	62 (19)	60 (17)	64 (17)	60 (17)

Historical Control Data

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4. Malformations

Indices	Data of control groups (n = 33)			Data of groups not significantly influenced by any test compound (n = 58)		
	no.	mean %	Range for control groups mean %	no.	mean %	Range for the groups mean %
No. of groups examined	33	-	-	58	-	-
No. of fetuses examined	2810	-	-	4978	-	-
No. of litters examined	455	-	-	820	-	-
Acephalia, omphalocele, obstetrician's hand, crossed legs	-	-	-	-	-	-
Acephalia, obstetrician's hand	-	-	-	-	-	-
Encephalocele, obstetrician's hand	-	-	-	-	-	-
Encephalocele, brachygnathia	-	-	-	-	-	-
Preliminary encephalocele, cleft lips, open eylelid, aplasia of distal abdomen, abdominal wall and hindlimbs, rudimentary forelimbs	-	-	-	-	-	-
Preliminary encephalocele, obstetrician's hand, crossed legs	-	-	-	1	0.03 ± 0.21	0-1.3
Encephalocele, short tail	-	-	-	-	-	-
Encephalocele, omphalocele	-	-	-	2	0.05 ± 0.30	0-2.6
Encephalocele or preliminary encephalocele	-	-	-	4	0.10 ± 0.32	0-1.6
Opaque eye lenses	-	-	-	-	-	-
Brachygnathia	1	0.04 ± 0.21	0-1.4	-	-	-
Cleft palate	1	0.03 ± 0.21	0-1.3	-	-	-
Cleft palate, cleft lip (paramedial)	-	-	-	-	-	-
Spina bifida	1	0.03 ± 0.28	0-1.4	-	-	-
Spina bifida, scoliosis, omphalocele, aplasia of forelimbs	-	-	-	1	0.03 ± 0.21	0-1.3
Spina bifida, kyphosis, aplasia of forelimbs and sternum, aplasia of abdominal wall	-	-	-	-	-	-

Figure 102, from page 258 of Report 12453/99

Indices	Data of control groups (n = 33)			Data of groups not significantly influenced by any test compound (n = 58)		
	no.	mean %	Range for control groups mean %	no.	mean %	Range for the groups mean %
Spina bifida, aplasia of sternum, forelimbs and abdominal wall	-	-	-	-	-	-
Aplasia of hindlimbs and distal abdomen and abdominal wall, obstetrician's hand, shortened and fused phalanges	1	0.03 ± 0.21	0-1.3	-	-	-
Omphalocele	9	0.34 ± 0.50	0-3.0	13	0.31 ± 0.48	0-2.1
Omphalocele, obstetrician's hand	-	-	-	2	0.05 ± 0.30	0-2.6
Obstetrician's hand	-	-	-	1	0.03 ± 0.21	0-1.3
Crossed legs	-	-	-	1	0.03 ± 0.21	0-1.3
Generalized oedema, obstetrician's hand, crossed legs	-	-	-	1	0.03 ± 0.21	0-1.3
Chicken breast, asymmetric/bifurcate ribs and vertebrae	1	0.03 ± 0.21	0-1.3	-	-	-
Several ribs fused	-	-	-	2	0.04 ± 0.18	0-1.0
Subcutaneous haematoma	1	0.03 ± 0.21	0-1.3	-	-	-
Haematoma inside pericardium	1	0.05 ± 0.31	0-1.6	-	-	-

Figure 103, from page 259 of Report — 2453/99

Key Study Findings

1. No significant toxicity was observed in the dams. Higher doses could have been used.
2. No significant effects were observed in the fetuses.
3. There was an unusually low incidence of observations in this study and in the historical control groups.

2.6.6.2.6 Examination of SPM-962 for Effects on the Pre- and Postnatal Development (Including Maternal Function) Following subcutaneous Administration to the Dams of Rats of the F_0-Generation

Study No: and number: 12787/99
Site and testing facility:

GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers:

Methods

Doses: 0, 0.1, 0.3, 1.0 mg/kg/day
Species/strain: Rat, Sprague-Dawley, CD BR
Number/sex/group: 24 dams/dose, only the first 20 dams at each dose were used for evaluation of post natal development. 1 pup/sex/litter were used for F1 fertility
Route, formulation, volume, and infusion rate: subcutaneous injection
Satellite groups used for toxicokinetics: not done
Study design: Dams dosed Gestation Day 6 through Post Natal Day 21
Parameters and endpoints evaluated: dam pregnancy, pup survival and fertility

Results

F_0 in-life:
No mortality or clinical signs were observed in the dams.
Decreased body weight was observed at 1 mg/kg during lactation, which appeared to be due to decreased weight gain during lactation; body weights at the end of lactation were comparable, but this appeared to be due to low dose dams losing weight.

Figure 1 Body weight of female animals daily mean values per group F_0—generation

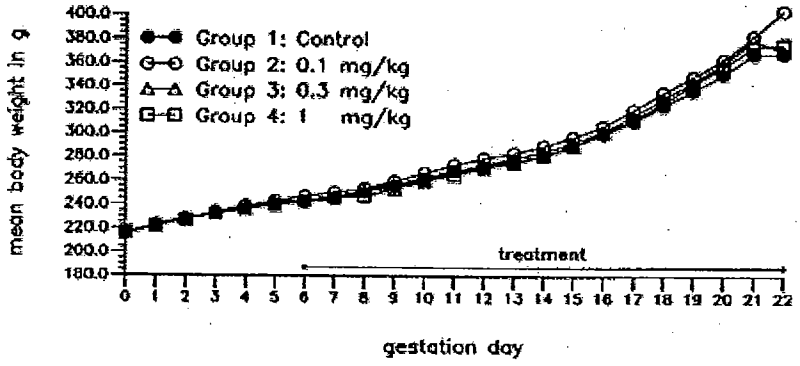

Figure 104, from page 34 of Report -12787/99

Figure 2 Body weight of female animals daily mean values per group F_0–generation

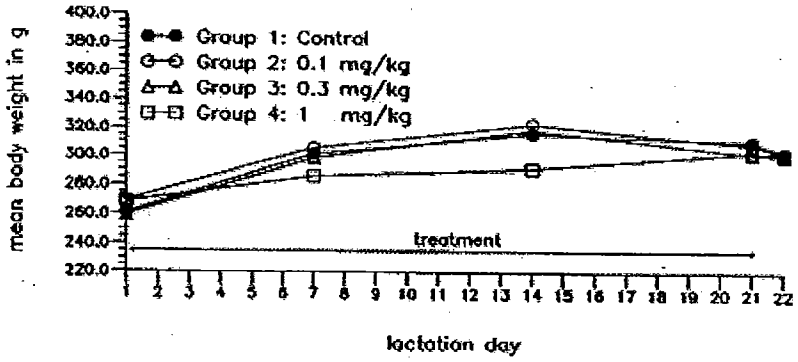

Figure 105, from page 35 of Report — 12787/99

4 dams at 1 mg/kg had complete litter loss.

Pup viability parameters

	0 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Gestation Length (days)	21.3	21.5	21.4	**21.7**
Birth Index (%pups born/implant scars)	94.9	93.0	95.1	92.0
Live Birth Index (%live pups born/total pups born)	98.6	99.6	99.7	98.6
Viability Index (%pups alive on PND4/pups born alive)	98.3	98.9	98.6	**82.4**
Lactation Index (%pups alive on PND21/pups alive PND4)	99.7	99.4	99.7	**69.8**
Overall Survival (%pups alive on PND21/pups born)	96.8	97.8	98.0	**64.5**

Values in **Bold** are significantly different from controls ($p<0.05$)

The presence of milk in the pups' stomachs was examined on the first four post natal days. Decreased milk production was observed at 1 mg/kg.

Incidence of Inadequate Lactation

Post Natal Day	0 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Day 1	0/20	0/20	1/20	1/24
Day 2	1/20	0/20	1/20	5/24
Day 3	1/20	0/20	0/20	6/24
Day 4	0/20	0/20	0/20	5/24

The number of dams allowing single pups to be left cold was also evaluated during the first four postnatal days. No effects on maternal behavior were observed.

Incidence of Dams allowing single pups to be cold

Post Natal Day	0 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Day 1	1/20	0/20	0/20	2/24
Day 2	0/20	0/20	1/20	1/24
Day 3	0/20	0/20	0/20	1/24
Day 4	1/20	0/20	0/20	0/24

F_0 necropsy:
No effects

F_1 physical development:
Decreased litter weight (probably related to inadequate milk production) was observed at 1 mg/kg from Day 4 through weaning. Body weight decrements at 1 mg/kg were observed throughout the study in the F1 generation.

TABLE 9 F_1-Generation. Group Mean Pup Weight (g) ± Standard Deviation - Summary

Day of Age	Group/Dose Level of the dams of the F_0-generation (mg SPH 962/kg b.w.)			
	(Control)	(0.1 mg/kg)	(0.3 mg/kg)	(1 mg/kg)
<u>males</u>				
1	6.52 ± 0.62	6.73 ± 0.63	6.62 ± 0.59	6.70 ± 0.68
4	8.76 ± 1.05	9.49 ± 0.98	9.12 ± 1.03	<u>7.30 ± 1.63</u>
7	13.12 ± 1.60	13.69 ± 1.50	13.22 ± 1.41	<u>8.96 ± 2.10</u>
14	26.63 ± 2.98	27.51 ± 3.10	26.17 ± 3.13	<u>11.69 ± 3.22</u>
21	44.01 ± 5.41	45.61 ± 5.74	43.00 ± 6.42	<u>15.70 ± 5.64</u>
22 - 27 *	51.80 ± 5.73	54.66 ± 6.52	50.92 ± 7.05	<u>24.06 ± 4.89</u>
<u>females</u>				
1	6.18 ± 0.62	6.41 ± 0.57	6.26 ± 0.52	6.31 ± 0.60
4	8.39 ± 1.00	9.12 ± 0.98	8.74 ± 1.01	<u>7.09 ± 1.21</u>
7	12.68 ± 1.60	13.29 ± 1.40	12.69 ± 1.46	<u>8.59 ± 1.82</u>
14	26.11 ± 2.77	26.60 ± 3.03	25.44 ± 3.26	<u>11.45 ± 2.91</u>
21	42.65 ± 4.97	43.71 ± 5.64	41.68 ± 6.64	<u>16.87 ± 4.63</u>
30 - 35 **	103.78 ± 9.92	107.89 ± 8.23	109.85 ± 9.36	<u>84.41 ± 7.82</u>

<u>____</u>: $p \leq 0.01$ (Student, Dunnett)

* = cleavage of the balanopreputial gland
** = vaginal opening

Figure 106, from page 64 of Report 12787/99

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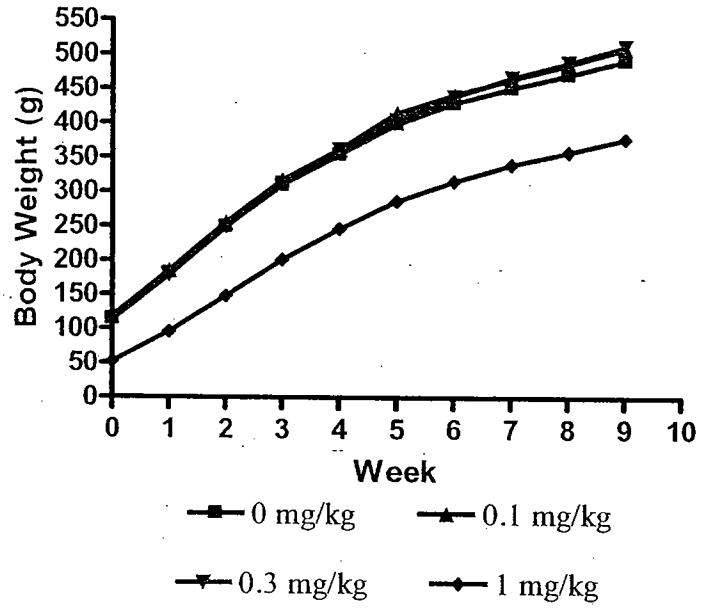
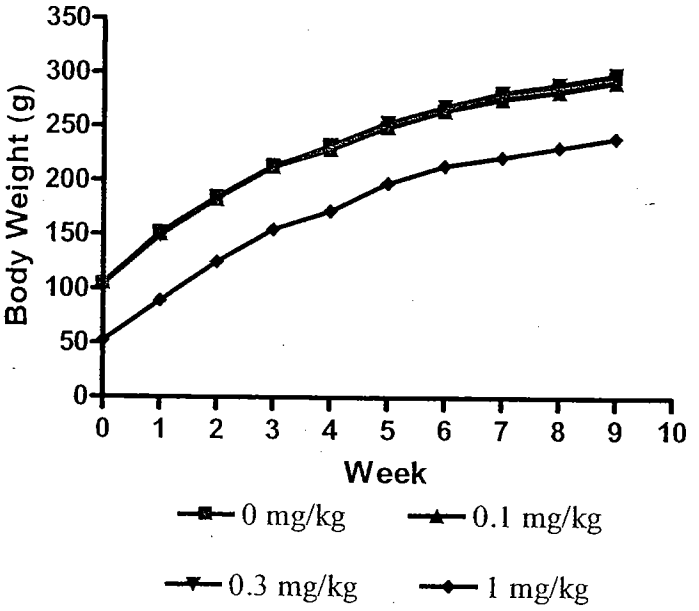
Male Post Weaning Body Weight



Female Post Weaning Body Weight



Mean (SD) Post Natal Day of Morphological Landmarks

Criteria	0 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Pinna detachment	3.6 (0.6)	3.1 (0.5)	3.2 (0.6)	3.5 (0.7)
Upper incisor eruption	12.3 (0.7)	11.8 ((0.9)	12.1 (0.8)	11.9 (0.8)
Ear opening	13.4 (0.6)	13.0 (0.6)	13.2 (0.6)	**14.9 (1.1)**
Eye opening	15.0 (0.6)	14.6 (0.5)	14.5 (0.5)	**16.2 (0.9)**
Balano-preputial gland cleavage	22.8 (0.4)	22.9 (0.6)	22.7 (0.6)	**24.8 (1.7)**
Vaginal opening	32.5 (0.8)	32.5 (0.8)	33.1 (0.9)	**39.0 (4.6)**

Values in **Bold** significantly different from control ($p<0.05$).

F_1 behavioral evaluation:

A large percentage of 1 mg/kg pups (70.4 vs 5.4% in control) did not have an auditory startle reflex on day 14. This may be related to the delayed ear opening (average ear opening on day 14.9) observed at this dose level.

Pups were tested for passive avoidance learning on PND 27 and memory on PND 34. Slight decreases in passive avoidance learning and memory were observed at 1 mg/kg, but there was wide variation in the ability of pups to learn so that it is difficult to assess the significance of this finding.

Percent failing to meet the success criteria (SD)

	0 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Passive Avoidance Learning	23.6 (22.1)	10.5 (14.8)	23.5 (24.3)	37.7 (26.5)
Passive Avoidance Memory	15.0 (19.2)	10.8 (10.1)	12.4 (13.8)	21.6 (20.6)

Pups were tested in the Open Field on post natal day 27 (only time point examined). Pups were placed in the circular open field (size not specified, but divided into 36 sectors) and observed for 3 minutes. Decreased activity was observed in 1 mg/kg pups.

Open Field Behavior Mean (SD)

Criteria	0 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Pups Tested	131	142	145	85
Latency to leave central area (sec)	3.4 (8.0)	3.0 (3.8)	3.9 (15.3)	3.6 (4.3)
Grooming incidents/pup	0.8 (1.0)	0.8 (1.1)	0.9 (1.1)	**1.4 (1.0)**
Rearing incidents/pup	8.6 (3.8)	8.6 (4.0)	9.7 (5.2)	**5.6 (3.2)**
Number of Sectors entered/pup	65.2 (28.8)	71.9 (28.1)	65.4 (31.9)	**38.7 (24.3)**
Defecation/pup	0.3 (1.0)	0.5 (1.1)	0.5 (1.1)	**1.0 (1.7)**

Values in bold are statistically significant ($p<0.05$)

F_1 reproduction: The female and male pups closest to the median in each litter were selected for assessing reproduction capabilities. The rats were mated with a pup from another litter at the same dose level (brother-sister pairings were not allowed).

Figure 7 Body weight of female animals daily mean values per group F_1-generation

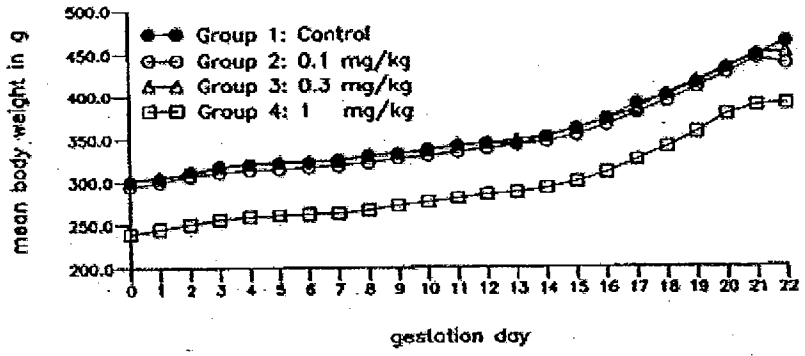


Figure 107, from page 43 of Report — 12787/99

TABLE A4 F_2-Generation, Survival Indices

Criteria		Group/Dose level of the dams of the F_2 generation (mg SPM 9677/kg b.w.)			
		1 (Control)	2 (0.1 mg/kg)	3 (0.3 mg/kg)	4 (1 mg/kg)
Birth index	Mean litter index (%)	92.4	98.3	99.1	101.4
	Number losing >2 implants	2	1	0	0
	Number of litters	19	20	20	19
Live birth index	Mean litter index (%)	99.4	97.8	99.4	97.9
	Number losing >1 pup	0	1	0	0
	Number of litters	19	20	20	19
Viability index (day 0-4)	Mean litter index (%)	98.1	97.0	95.8	92.3
	Number losing >3 pups	0	1	1	2
	Number of litters	19	20	20	19
Lactation index (days 4-21)	Mean litter index (%)	95.1	93.6	95.5	88.2
	Number losing >1 pup	3	3	2	4
	Number of litters	19	20	20	19
Overall survival index (birth-21)	Mean litter index (%)	93.1	89.5	91.0	82.7
	Number losing >4 pups	1	2	2	3
	Number of litters	19	20	20	19

____: $p \leq 0.01$ (Student, Dunnett)

Figure 108, from page 313 of Report — 12787/99

F_2 findings:

TABLE A7 F_2-Generation, Group Mean Pup Weight (g) ± Standard Deviation - Summary

Day of Age	Group/Dose level of the dams of the F_0-generation (mg SPH 962/kg b.w.)			
	1 (Control)	2 (0.1 mg/kg)	3 (0.3 mg/kg)	4 (1 mg/kg)
males				
1	6.91 ± 0.86	6.71 ± 0.65	6.47 ± 0.57	6.60 ± 0.68
4	9.26 ± 2.03	8.83 ± 1.91	8.85 ± 1.04	8.45 ± 1.82
7	13.50 ± 3.05	12.89 ± 2.64	13.11 ± 1.68	12.31 ± 2.29
14	24.98 ± 6.30	23.77 ± 4.71	24.12 ± 4.22	22.72 ± 4.06
21	41.46 ± 10.99	40.47 ± 9.07	40.38 ± 7.12	37.67 ± 7.50
females				
1	6.54 ± 0.73	6.31 ± 0.61	6.22 ± 0.59	6.15 ± 0.61
4	8.85 ± 2.05	8.41 ± 1.71	8.61 ± 1.17	8.01 ± 1.76
7	12.98 ± 3.23	12.29 ± 2.69	12.74 ± 1.89	11.84 ± 2.33
14	24.86 ± 6.26	22.68 ± 4.90	23.21 ± 4.43	21.16 ± 4.19
21	41.84 ± 10.65	39.05 ± 8.34	39.40 ± 7.24	35.86 ± 6.71

____: $p \leq 0.01$ (Student, Dunnett)

Figure 109, from page 317 of Report — -12787/99

No external abnormalities were observed in F2 offspring.

Key Study Findings:

1. Normal maternal behavior was observed in rats at all doses.
2. Decreased milk production was observed at 1 mg/kg (high dose).
3. Increased mortality with lower body weight was observed in the offspring of the 1 mg/kg rats. In addition, there were delays in physical landmarks at 1 mg/kg.
4. The effects on physical landmarks in the 1 mg/kg pups may be due to inadequate nutrition during lactation. A cross fostering study would allow one to determine whether the effects were due to effects of the drug on the pup or dam.
5. Decrements in learning and memory were observed in 1 mg/kg pups. Decreased activity in the open field test was also observed in 1 mg/kg pups.
6. Decreased survival was noted in F2 offspring of the 1 mg/kg F1 offspring.

2.6.6.7 Local tolerance

2.6.6.7.1 Local Tolerance Study of SPM 962 Patch By Epicutaneous Administration For 4 Weeks To Rabbits With Intact And Scarified Skin

Study No: 12155/99

Conducting laboratory and location: [redacted]

Date of study initiation: May 20, 1999

GLP compliance: Yes

QA- Reports Yes (X) No ( ):

Methods: Himalayan Rabbit

Dosing: two 10 sq cm patch/rabbit (one on intact and one on abraded skin)

Drug, lot#, radiolabel (if applicable), and % purity:

Formulation/vehicle: dermal patch applied to intact and abraded skin, 22 hours/day for four weeks

Observations and times: clinical signs, mortality, body weight, local tolerance, skin histopathology

Results:

Slight increase in local irritation was associated with SPM 962 patch

Erythema Scores After Removal of the Patch

Intact Skin				Scarified Skin			
Male		Female		Male		Female	
Control	Active	Control	Active	Control	Active	Control	Active
0.68	1.11	0.63	1.14	0.55	1.11	0.64	1.09

Scale: 0=no erythema; 1=slight erythema; 2=well-defined erythema

No effect on body weight.

2/4 male rabbits had pituitary weights 8-50 times control values

No effect on skin histopathology.

2.6.6.8 Special toxicology studies

2.6.6.8.1 Phototoxicity Study of SPM 962 Patch After Repeated Epicutaneous Application To Guinea-Pigs

Study No: 12206/99
Conducting laboratory and location:

Date of study initiation: August 9, 1999
GLP compliance: Yes
QA- Reports Yes (X) No ( ):

Methods: male Dunkin-Hartley guinea pigs
Dosing: Dermal patch was applied for 24 hours; 8-methoxypsoralen was administered 30 minutes prior to UV irradiation; Dosing was repeated twice at weekly intervals (except group 4) for a total of three applications.

Group	Substance	Number of animals (animal nos.)	UV irradiation
1	untreated control	5 (1 - 5)	yes
2	placebo control (SPM 962 placebo patch)	5 (6 - 10)	yes
3	SPM 962 patch	5 (11 - 15)	yes
4	positive control 40 mg 8-methoxypsoralen[6]/kg b.w. p.o.	5 (16 - 20)	yes
5	irritation control (SPM 962 patch)	5 (21 - 25)	no

Drug, lot#, radiolabel (if applicable), and % purity: WE 10887 (corresponding to LTS batch no. 829021)
Formulation/vehicle: 10 sq cm Dermal patch containing 4.5 mg SPM 962
Observations and times: guinea pigs were observed 4, 24, and 48 hours post irradiation
Results:
SPM 962 and the negative controls did not induce erythema or edema following repeated application; the positive control 8 methoxypsoralen induced erythema and edema.
Summary:
Key finding(s):
SPM 962 was not phototoxic under these test conditions.

2.6.6.8.2 Photosensitisation Test of SPM 962 Patch After Repeated Epicutaneous Application to Guinea Pigs

Study No: 12207/99
Conducting laboratory and location: [redacted]

Date of study initiation: August 30, 1999
GLP compliance: Yes
QA- Reports Yes (X) No ( ):

Methods: Female Dunkin-Hartley Guinea pigs were used
Dosing:

Group	Substance	UV-irradiation during stages 1 to 3
1	untreated control	yes
2	placebo control (SPM 962 placebo patch)	yes
3	SPM 962 patch	yes
4	positive control {1 ml 5% 2,2'-thio-bis(4,6-dichlorophenol)}[5]	yes
5	irritation control (SPM 962 patch)	no

Guinea pigs were exposed to patch for 23 hours followed by 1 hour exposure to UV-A and UV-B radiation for five consecutive days. In addition, Freund's Complete Adjuvant was administered on three times at 48 hour intervals. After a 10 day induction period, this procedure was repeated twice (two exposures); after an additional 13 day period, guinea pigs were challenged with test compound and placebo for 3 applications.
Drug, lot#, radiolabel (if applicable), and % purity: WE 10887 (corresponding to LTS batch no. 829021)
Formulation/vehicle: dermal patch

Results:
SPM 962 and negative controls did not induce photosensitization in this assay. The positive control was positive.

2.6.6.8.3 Examination of SPM 962 Free Base in the Skin Sensitisation Test in Guinea-Pigs According to Magnusson and Kligman (Maximisation Test)

Study No: and number: # 11623/98
Site and testing facility:

GLP compliance: Yes
QA- Report Yes (X) No ( ):
Lot and batch numbers: WE 6765

Methods:

- Species/strain: Guinea Pig, Dunkin-Hartley
- Doses employed: 0, 0.1 ml of 0.1% SPM 962 solution (0.3 mg/kg) (1st induction); 0, 1% in vasaline (2nd induction)
- Route of Administration: intracutaneously, topical
- Rationale: standard protocol/ also route of exposure
- Number of animals/sex/dosing group 3 males/dose
- Endpoints: sensitization upon topical challenge
- Observations: erythema and edema formation
- Timing: challenge 5 days post induction

Overall Summary:

SPM 962 did not induce sensitization in the Guinea pig maximization test.

2.7 OVERALL CONCLUSIONS AND RECOMMENDATIONS

2.7.1 Introduction

Parkinson's disease is a neurodegenerative disease characterized by bradykinesia, muscular rigidity, resting tremors, and postural instability. If left untreated, it will progress to a rigid akinetic state in which the patient is incapable of taking care of him/herself. Pathologically, Parkinson's disease is characterized by a progressive loss of neurons marked by the presence of Lewy bodies. It has been proposed that the disease progresses from the dorsal motor nucleus of the vagas nerve upward through the lower brainstem and basal forebrain until it reaches the cerebral cortex. The characteristic clinical features of Parkinson's disease result from the progressive loss of dopaminergic neurons in the substantia nigra resulting in decreased dopaminergic tone.[1] Since the cause of this loss is unknown at present, current therapy for Parkinson's disease utilizes substances that increase dopaminergic tone (e.g., ropinirole, pramipexole) or increase the amount of dopamine available at the receptor site (e.g. levodopa, COMT inhibitors, MAO-B inhibitors). Other potential therapies which aim to modulate dopaminergic tone include adrenergic alpha-2 antagonists and adenosine A2 antagonists. Despite the use of these drugs, patients may still experience "off" episodes in which their muscle movement is slow or frozen. These off episodes are thought to be the result of deficient dopaminergic tone. Rotigotine is a non-ergoline dopamine agonist similar to ropinirole and pramipexole, proposed for the treatment of Parkinson's disease.

2.7.2 Pharmacology of Rotigotine

The affinity of rotigotine for neuroreceptors has been examined in a series of in vitro assays (see pages 8, 15, 18). Rotigotine has a high affinity for Dopamine D2, D3 D4 and D5 receptors. It also has significant binding at the adrenergic alpha-2 receptor (IC50=33nM). Rotigotine did not have significant in vitro binding to other receptor types (IC50 > 100 time the affinity for dopamine D2 receptor), including the adenosine, beta-adrenergic, histamine, monoamine transporter, muscarinic, serotonin (except the 5HT1A and 5HT7 receptors). In addition, rotigotine did not inhibit a series of enzymes (see page 19). In particular, rotigotine did not inhibit enzymes involved in the metabolism of dopamine including Catechol-O-methyltransferase (converts dopamine to 3-methoxytyramine), MAO-B (converts dopamine to DOPAC) and Tyrosine hydroxylase (converts tyrosine to DOPA, the dopamine precursor). L-aromatic amino acid decarboxylase (responsible for the conversion of DOPA to dopamine) was not examined in this study. The effects of rotigotine on neurotransmitter release and uptake was examined (see page 21). Rotigotone weakly inhibited the reuptake of dopamine, norepinephrine or serotonin (IC50's = 160, 48 and 710 nM, respectively). Rotigotine had no significant effect on the release of these neurotransmitters. Since rotigotine interacts with the dopamine receptors at about 1 nM, the effects on neurotransmitter uptake are not expected to contribute to the clinical efficacy of the drug.

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[1] Braak, H, K Del Tredici, U Rub, RAI de Vos, ENH Jansen Steur and E Braak (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:297-211.

The significance of the in vitro binding was examined in cloned human receptors expressed in cell lines. Rotigotine was an agonist at human dopamine receptors with EC50's between 0.2 and 4.1 nM (see page 21). The significance of the binding to non-dopamine receptors was further examined in in vitro test systems (see page 24). Rotigotine was an antagonist at the adrenergic alpha-2B and -2C receptors (IC50 = 394 and 588 nM, respectively). Rotigotine was an agonist at the serotonin 1A receptor, although the EC50 was relatively high (EC50 = 1,040 nM). Although adrenergic alpha-2 receptor antagonists have been proposed as a potential therapies for Parkinson's disease, the significance of these findings for rotigotine are uncertain. It had relatively weak activity in an in vitro model. It inhibited the adrenergic alpha-2A, alpha-2B and alpha-2C receptors by 0, 63 and 64%, respectively, at 10 uM (see page 24).

Rotigotine had improved motor function and reduced disability in a monkey model of Parkinson's disease at doses as low as 0.01875 mg/kg subcutaneously in water (see page 22), suggesting that it could provide symptomatic relief of the dopamine deficits associated with Parkinson's disease. On the other hand, rotigotine did not prevent dopaminergic cell death at doses up to 3 mg/kg in MPTP-treated mice, suggesting that it does not have neuroprotective properties (see page 26).

Rotigotine has been examined in a series of safety pharmacology studies examining its effects on the neurological, cardiovascular, respiratory, renal and gastrointestinal systems.

Rotigotine was pro-convulsant in a series of safety pharmacology studies. It increased the number of convulsions induced by sub-seizurogenic electroshock treatment in mice at subcutaneous doses as low as 0.1 mg/kg; it increase the mortality at 0.5 mg/kg (see ← 12022/99, see page 31). Similarly, it increased the number of convulsions and deaths in mice administered seizurogenic doses of pentylenetetrazol at subcutaneous dose of 0.1 mg/kg rotigotine (— study 12083/99, see page 32). These studies suggest that rotigotine has the potential to lower seizure threshold. The sponsor states that the dose dependent effects on convulsive activity are consistent with stimulation of central dopamine receptors (page 15 of Pharmacology Nonclinical Summary). The Sponsor did not provide any references to support this statement. This reviewer identified two studies that suggested that dopamine agonists (pergolide, apomorphine, quinpirole, SKF38393) either had no effect or an anticonvulsant effect.[2,3] In addition, pramipexole at doses up to 3 mg/kg did not lower the threshold for pentylenetetrazol-induced seizures in mice (page 13 of the pharmacology/toxicology review by Dr Steele (NDA 20-667)). This reviewer could not identify any published studies on the effects of ropinirole on seizure threshold.

The effects of rotigotine (0, 0.1, 05. and 1 mg/kg subcutaneously) on mouse behavior was examined in two studies — Studies 12019/99 and 12020/99, see page 28). No effects were observed at 0.1 mg/kg. At 0.5 and 1 mg/kg, increased activity (restlessness) was observed in both studies. This is an expected pharmacological action of a dopamine agonist such as rotigotine.

Rotigotine (up to 10 mg/kg subcutaneously) had no consistent effect in the rotorod test (Study D00.272/2, see page 30). Rotigotine did not affect reserpine induced

[2] Ogren, SO and B Pakh (1993) Effects of Dopamine D1 and D2 Receptor Agonists and Antagonists on Seizures Induced by Chemoconvulsants in Mice. Pharmacol. Toxicol. 72(4-5):213-20.
[3] Helton, DR, DL Modlin and PD Williams (1992) Behavioral Characterization of the New Potent Nonselective Dopamine Agonist Pergolide. Arzneimittelforschung 42(7):885-90.

hypothermia in mice (Study — 12025/99, see page 33) nor hexobarbital sleeping time in mice (Study — 12021/99, see page 34).

Rotigotine had no effect on cardiovascular parameters (ECG, heart rate and blood pressure) or respiratory parameters (respiration rate, minute volume, tidal volume) at subcutaneous doses up to 4 mg/kg in anesthetized monkeys — Study 12026/99, see page 35). Rotigotine decreased the heart rate and blood pressure of anesthetized rats by about 10% starting at 0.05 mg/kg intravenously (Study —-88001/A, see page 36). Rotigotine inhibited the hERG channel in two separate studies (Study 020317 — and E-01-001-013, see page 36) with IC50's of 0.5 and 0.15 uM. Rotigotine also prolonged the action potential duration in isolated canine Purkinje fibers at 100 nM and above (study 20020509PECM, see page 37) and the guinea pig papillary muscle at 100 nM and above (— Study 12027/99, see page 39). Rotigotine had no significant effects on the L-type calcium inward current in guinea pig myocytes (Study 16116/02, see page 40) or on the SCN5A sodium channel (study E-01-014-004, see page 41). The positive effects in the hERG channel and the prolongation of the action potential suggest that rotigoitne may have effects on the QT-interval, although the lack of effects in the in vivo monkey cardiovascular study suggests a lower potential for effects.

The effect of rotigotine (0, 0.1, 0.5 and 1 mg/kg subcutaneously) on kidney function was examined in saline loaded rats (study — 12029/99, see page 41). Rotigotine inhibited the excretion of urine at 0.1 mg/kg and above during the first hour following administration. A no-effect dose was not observed in this study. This study suggests that rotigotine could have an anti-diuretic effect.

Rotigotine had no effect on intestinal motility at doses up to 1 mg/kg subcutaneously in mice (study — 12028/99, see page 44). In contrast, rotigotine inhibited the contractile properties of acetylcholine and histamine on the guinea pig ileum with EC50's of 350 and 140 ng/ml, respectively (study — 12030/99, see page 44).

In summary, rotigotine exhibits the pharmacological properties expected of a dopamine agonist. The primary issues of concern in the safety pharmacology studies are its potential for cardiovascular effects via inhibition of the hERG channel, for lowering seizure thresholds and its anti-diuretic effects on the kidney.

2.7.3 Pharmacokinetics of Rotigotine

The pharmacokinetics of rotigotine in humans has been reviewed by Dr Ronald Kavanaugh. In humans, rotigotine is administered via a dermal patch due to low oral bioavailability. The transdermal patch contains 4.5, 9.0 or 13.5 mg and providing nominal delivery to the skin of 2, 4 or 6 mg of rotigotine per day, respectively. The quantitative composition per cm^2 is identical for all strengths. The different strengths correspond to patch sizes of 10, 20 or 30 cm^2 respectively. At the maximum proposed dose, the steady state plasma levels are approximately 0.75 ng/ml (AUC(0-24) = 18 ng-hr/ml). The major metabolites observed in humans were the sulfate conjugate (approximately 2.9-times the parent compound exposure), the despropyl sulfate conjugate (approximately 1.2-times the parent compound exposure). The desthienyl sulfate conjugate and glucuronide conjugate were also observed, but at lower concentrations than the parent compound (0.67- and 0.56-times the parent compound exposure, respectively).

The pharmacokinetics of rotigotine were examined following oral, subcutaneous and intravenous administration in male Sprague-Dawley rats (Study — 11640/98, see

page 45). In this study, rotigotine had poor oral bioavailability (less than 1%). This poor bioavailability was attributed to rapid metabolism. In order to obtain sustained pharmacological actions, it was necessary to develop alternative routes of administration. Dermal patches were not considered suitable for the nonclinical studies. Subcutaneous injections were considered the most appropriate route of exposure for rotigotine. The Sponsor investigated a series of formulations. An oily crystal suspension (0.5-2% rotigotine, [redacted] was determined to be the most appropriate vehicle. Higher bioavailability was obtained with another vehicle (clear solution), but rotigotine was removed rapidly from the plasma even though higher plasma levels were obtained. In contrast, relatively constant plasma levels were obtained using the oily vehicle. The Sponsor examined several concentrations of rotigotine in the oily vehicle (Study [redacted] 1865/98, see page 46). It was determined that slightly higher bioavailability was observed using a higher concentration of rotigotine in the vehicle. At 6 mg/kg, the AUC(0-24)'s were higher than the AUC(0-24) in humans at the maximum recommended dose (401-616 ng-hr/ml in rats versus 18 ng-hr/ml in humans). It was concluded that subcutaneous injections using the oily vehicle is an appropriate method in rats.

The suitability of the oily formulation was also examined in monkeys ([redacted] study 11932/99, see page 47). In this study, rotigotine was administered in an aqueous formulation by intravenous infusion or subcutaneous injection at 1 mg/kg or in the oily vehicle by subcutaneous injection at 4 mg/kg. There was a rapid elimination of rotigotine from the plasma when it was administered in the aqueous formulation by both routes of administration. In contrast, there were sustained rotigotine levels following subcutaneous injection in the oily vehicle. The AUC(0-24) was 128 ng-hr/ml was higher than the AUC(0-24) in humans at the maximum recommended dose ([redacted] hr/ml). It was concluded that subcutaneous injections using the oily vehicle is an appropriate method in monkeys.

The distribution of radiolabeled rotigotine has been studies in albino Sprague-Dawley and pigmented Lister hooded rats following intravenous infusion (Study [redacted] 1017, see page 53). Highest concentrations of radiolabel were observed in the intestines, liver, urine, kidney and adrenals, which reflects the rapid metabolism and excretion of rotigotine. High concentrations were also observed in the eye of the pigmented rats. Radiolabel was also observed in the brain at about twice the concentration of whole blood (plasma was not examined).

The distribution of radiolabeled rotigotine (1 mg/kg IV infusion) has been studies in cynomolgus monkeys (Study [redacted] 1014-A1, see page 48). Highest concentrations of radiolabel were observed in the intestines, liver, kidney and adrenals, which reflects the rapid metabolism and excretion of rotigotine. High concentrations were also observed in the eye and prostate. Radiolabel concentrations in the brain were about half the concentration in the plasma.

The metabolism of rotigotine has been examined in Sprague-Dawley rats (Study [redacted] 1028, see page 59). In rats, rotigotine was rapidly and extensively metabolized following intravenous five minute infusion of 1.5 mg/kg radiolabeled rotigotine. At 15 minutes post infusion, less than 4% of circulating radiolabel was parent rotigotine in males and females. Rotigotine was not detected in the plasma of females at 1 or 4 hours. The primary metabolite identified was desthienyldespropyl rotigotine sulfate. Despropyl

rotigotine sulfate and rotigotine sulfate were also observed in the first 15 minutes following injection. Multiple minor metabolites (less than 5% of circulating radiolabel) were also identified. Eight and six metabolites were present at least 5% of total circulating radiolabel at 5 minutes in males and females, respectively. In addition, 30% of circulating radiolabel in males was minor metabolites (defined as peaks that accounted for less than 5% of total radioactivity). The metabolism data following subcutaneous injection could not be interpreted due to the low levels of circulating radiolabel and the numerous metabolites made it difficult to determine individual metabolite peaks (page 60 of report). Rotigotine was detected only at 2 hours in male rats; it was not detected at the other time points. In summary, rotigotine is rapidly metabolized in rats through sulfation and through removal of the propyl and thienyl moieties.

The metabolism of rotigotine has been examined in cynomolgus monkeys (Study 1028, see page 59). In monkeys, rotigotine was rapidly and extensively metabolized following intravenous 60 minute infusion of 1 mg/kg radiolabeled rotigotine. At 15 minutes post infusion, less than 10% of circulating radiolabel was parent rotigotine. The primary metabolites were desthienyldespropyl rotigotine sulfate and desthienyl rotigtoine sulfate. Despropyl rotigotine sulfate and rotigotine sulfate were s also observed in the first 15 minutes following injection. A similar pattern was observed following subcutaneous injection. Rotigotine was present at less than 10% of circulating radiolabel. The primary metabolite was desthienyl rotigotine sulfate, although desthienyldespropyl rotigotine sulfate was present at about the same concentration as parent rotigotine. In summary, rotigotine is rapidly metabolized in monkeys through sulfation and through removal of the propyl and thienyl moieties.

The proposed metabolic scheme in rats and monkeys is presented below. The profiles are essentially identical in both species. The glucuronide conjugate of rotigotine was not detected in monkey plasma, but it was observed in monkey urine. It is reasonable to assume that the glucuronide conjugate was formed in vivo, but the levels were below the levels of quantification in this study. The metabolism of rotigotine is similar in humans, rats and monkeys. The in vivo metabolism of rotigotine has not been determined in mice or rabbits.

Metabolic map in rat plasma. Structures in brackets not observed. Study No. — 1028.

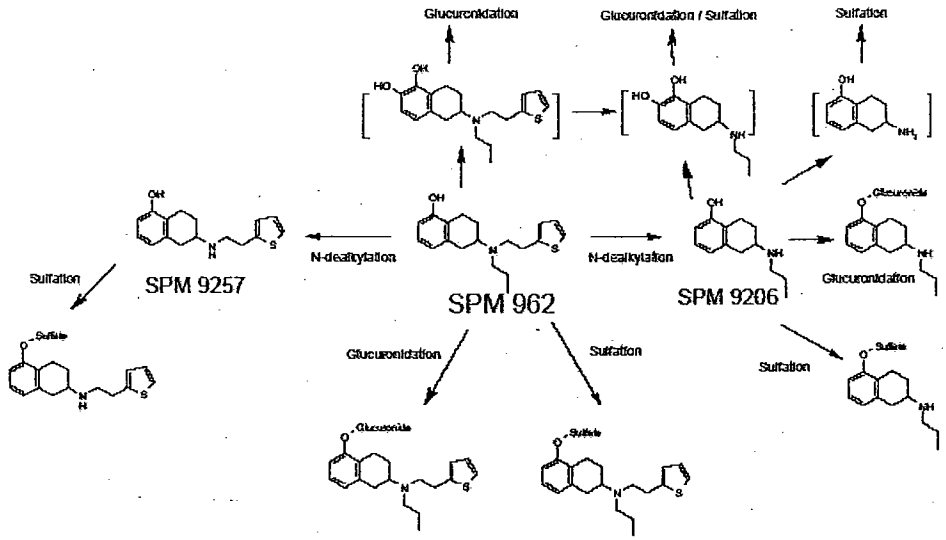

Figure 110, from page 75 of Sponsor's Tabulated Summary of Pharmacokinetics

In rats, rotigotine was primarily excreted via the feces (74%) (Study — 1028, see page 59). In monkeys, rotigotine was excreted about equally in the urine and feces (Studies — 1014 and — 1028, see pages 48 and 59, respectively).

In summary, rotigotine has poor oral bioavailability in the rat and monkey. When administered parenterally, it is rapidly metabolized via sulfation and dealkylation. It is excreted via the feces and urine. An oily vehicle was developed that permitted the study of relatively constant plasma levels of rotigotine as would be expected from dermal administration in the clinic. These results of the pharmacokinetic studies are broadly consistent with the pharmacokinetics in humans suggesting that the rat and monkey are appropriate species for examining the potential toxicity of rotigotine.

2.7.4 Toxicology of Rotigotine

The toxicity of rotigotine has been examined in a three month dose range finding study in CD-1 mice (— 12015/99, see page 68). In this study, mice were administered 0, 3, 10, 30 or 60 (later raised to 90) mg/kg/48 hours by subcutaneous injection in the oily vehicle. No treatment related mortality was observed in this study. Restlessness was observed at 30 mg/kg and above. Decreased body weight was observed at all doses, but all doses appeared to be equally affected. Decreased hematocrit and hemoglobin were observed at 30 and 60/90 mg/kg, but the effects at 30 mg/kg were greater than the effects at 60/90 mg/kg. Increased ALT, AST and BUN were observed at 3 mg/kg and above in both males and females, but no changes in liver histopathology were observed. Decreased albumin (10 mg/kg and above), cholesterol (3 mg/kg and above) and triglyceride levels (3 mg/kg and above) were also observed. Local gross pathology changes included injection site edema at the high dose. In addition, hemorrhagic fluid was observed in the peritoneal cavity of 9/20 high dose mice, although the source of the fluid could not be determined. Decreased prolactin levels were observed in males on

Days 3 and 90 (all doses), but decreased prolactin levels were only observed in high dose females on Day 3. No changes in prolactin levels were observed in females on Day 90. The toxicokinetic monitoring was inadequate for determining the AUC(0-48) (only three time points were examined), but the 48 hour rotigotine concentration (36 ng/ml) at the high dose was approximately 48 times the anticipated human plasma steady state concentration (0.75 ng/ml). A NOEL was not observed in this study, however the toxicities observed (restlessness, body weight decrease, hematology and clinical chemistry changes) are relatively minor and easy to monitor in the clinic.

The toxicity of rotigotine via dermal exposure was examined in Sprague Dawley rats (study 0436RD15.001, see page 75). In this study, a 5 cm^2 patch containing 0 or 8.4 mg rotigotine was applied to the shaved skin of the rat for 22 hours/day for 28 days. The estimated delivered dose was 1.7 mg/day or 8.5 and 10 mg/kg for males and females, respectively. Moderate irritation was observed at the application site of both the control and rotigotine patches. A transient decrease was observed in male body weights, but they had returned to control levels by the end of the study. No other adverse effects were observed in this study, although only limited histopathological examinations (exposed skin and underlying structure) were performed. Rotigotine was rapidly eliminated from the plasma following removal of the patch (6- to 12-fold reductions in plasma concentrations were observed in the two hours following removal of the patch). The minimum plasma level observed in this study (5 ng/ml) was about 6.7-fold higher than the human steady state concentration (0.75 ng/ml). The utility of this study is limited by its short duration (27 days) and small number of rats/group (5/sex/group) and the limited number of doses used.

The toxicity of rotigotine via subcutaneous exposure was examined in a 6 month rat study — 12482/99, see page 79). In this study, Sprague-Dawley rats (20/sex/dose) were administered 0, 0.5, 2.5 or 12.5 mg/kg/48 hours via subcutaneous injection. Two rats died at the high dose. Restlessness and increased water intake was observed at 2.5 mg/kg and above. Decreased body weight was observed at 2.5 mg/kg and above. No effects were observed on hematology examinations. Increased BUN, ALP and AST were observed at 2.5 and 12.5 mg/kg. There were also single rats that had increases in ALT levels at 0.5 and 12.5 mg/kg. No effects on liver histopathology were observed. Decreased cholesterol and triglyceride levels were observed at 2.5 mg/kg and 12.5 mg/kg. Increased ovary weight with enlarged corpora lutea were observed at 2.5 mg/kg and above. This change was attributed to the prolactin lowering effects observed at these doses. Retinal degeneration was observed at 12.5 mg/kg (the eyes of the lower dose groups were not examined). Retinal degeneration has been observed in long term carcinogenicity studies with other dopamine agonists, although it is unusual to detect it in a six month study. The NOEL in this study was 0.5 mg/kg. The steady state plasma rotigotine concentration at 0.5 mg/kg (0.86 ng/ml) is approximately equal to the human steady state concentration (0.75 ng/ml) at the maximum recommended dose.

Since rotigotine has the potential to be administered with Sinemet (levodopa/carbidopa), the potential interaction between rotigotine and Sinemet was examined in a three month interaction study in Sprague-Dawley rats — -16083/02, see page 88). In this study, rats were administered 0, 1, 3 or 10 mg/kg rotigotine subcutaneously in combination with 150 mg/kg Sinemet (120 mg/kg levodopa/30 mg/kg carbidopa). Additional groups were administered 10 mg/kg rotigotine alone or vehicles

alone. Treatment with Sinemet was begun three weeks prior to treatment with rotigotine. Six deaths (1 male and 5 females) were observed in the 30 rats treated with the combination of 10 mg/kg rotigotine and Sinemet. No deaths were observed at the other doses. Restlessness was observed in all rats treated with the combination of rotigotine and Sinemet. Decreased body weight (>10%) was observed in rats administered 10 mg/kg rotigotine in the presence or absence of Sinemet. Increases in AST were observed in rats administered 10 mg/kg rotigotine in the presence or absence of Sinemet. Dose dependent decreases in triglyceride levels were observed in rotigotine treated rats. No significant histopathology signs were observed. In conclusion, rotigotine and Sinemet interacted to cause deaths in rats; death was not observed with either drug alone. This effect was most likely due to an additive effect on dopamine receptors. No unexpected toxicological findings were observed. No significant effects were noted on the toxicokinetics of rotigotine or Sinemet.

The chronic toxicity of rotigotine has been examined in a 12 month study in cynomolgus monkeys (study — 12483/99, see page 96). In this study, the monkeys were administered 0, 0.25, 1 or 4 mg/kg by subcutaneous injection in an oily vehicle. The high dose was increased in increments to 16 mg/kg, but the monkeys did not tolerate 16 mg/kg (one monkey died). Two monkeys died when the administered dose was 13 mg/kg. No deaths were observed at 10 mg/kg. Restlessness was observed at 4 mg/kg and above; hyperactivity was observed when the high dose was 13 and 16 mg/kg. No significant effects were observed on body weight. Increased reticulocytes were observed in high dose females, but not in males. Individual monkeys in the mid and high dose groups had elevated AST and ALT levels, but no effects on liver histology were observed. Histopathological changes were confined to granulation tissue at the injection site of control and rotigotine treated animals. The NOEL in this study was 1 mg/kg. The AUC(0-24) at this dose was approximately 34 ng-hr/ml, which is twice the AUC(0-24) observed at the maximum recommended human dose.

Since rotigotine has the potential to be administered with Sinemet (levodopa/carbidopa), the potential interaction between rotigotine and Sinemet was examined in a three month interaction study in cynomolgus monkeys (— 16084/02, see page 101). In this study, monkeys were administered 0, 0.25, 1 or 4 mg/kg rotigotine subcutaneously in combination with 100 mg/kg Sinemet (80 mg/kg levodopa/20 mg/kg carbidopa). Additional groups were administered 4 mg/kg rotigotine alone or vehicles alone. Treatment with Sinemet was begun three weeks prior to treatment with rotigotine. Restlessness was observed in monkeys administered Sinemet alone. The incidence of restlessness was increased with increasing rotigotine dose in Sinemet treated monkeys. Restlessness was not observed in monkeys administered rotigotine alone. No effects on body weight, ECG parameters or hematology parameters were observed. Sporadic increases in AST and creatine phosphokinase levels were observed at various time points in monkeys administered 4 mg/kg rotigotine with Sinemet. Histopathological changes were confined to granulation tissue at the injection site. No systemic changes in histopathology were observed. No unexpected toxicological findings were observed. No significant effects were noted on the toxicokinetics of rotigotine or Sinemet. In summary, rotigotine increased the Sinemet induced restlessness in monkeys, most likely through an additive effect on dopaminergic receptors. No additional toxicological or toxicokinetic interactions were observed.

Other potential toxic endpoints have also been examined in a series of special studies. Rotigotine was not phototoxic when administered to the skin of guinea pigs via patch (—12206/99, see page 193). Rotigotine was not a photosensitizer when administered to the skin of guinea pigs via a dermal patch (— 12207/99, see page 194). Rotigotine did not induce a hypersensitivity reaction when tested in the guinea pig maximization test (— -11623/98, see page 195). Rotigotine was mildly irritating to the skin of rabbits when administered as a patch for 22 hours/day for four weeks, but had no effect on skin histopathology (— 12155/99, see page 192).

In summary, the toxicity of rotigotine has been examined in mice, rats and monkeys. Plasma levels in these species exceeded those anticipated at the maximum recommended human dose suggesting that adequate exposure had been achieved. The primary toxicity observed in these studies could be attributed to excessive dopamine stimulation. The primary toxicologic finding was increased restlessness and hyperactivity at high doses of rotigotine. Serum prolactin levels were decreased which resulted in reproductive organ changes in rats. Sporadic increases were observed in plasma AST and ALT levels, although no liver toxicity was observed. The significance of this finding is uncertain. Decreases in serum cholesterol, albumin and triglycerides were also frequently observed in these studies. No additional toxicity studies are recommended at this time.

Retinal degeneration was observed in the six month rat study at 12.5 mg/kg. Other dopamine agonists (e.g., ropinirole and pramipexole) are known to cause retinal degeneration after prolonged exposure (i.e., two years). Rotigotine did not cause retinal degeneration in the rat carcinogenicity study at doses up to 3 mg/kg. This is considered a dopamine class effect and should be mentioned in the label.

2.7.5 Genotoxicity of Rotigotine

The potential for rotigotine to induce mutations in bacteria was examined in *Salmonella typhimurium* (strains TA98, TA100, TA102, TA1535 and TA1537) as well as *Escherichia coli* WP2 uvrA (Study —301-DIS-002-95, see page 107). These strains detect a variety of mutation types including frameshift mutations (TA98, TA1537), GC base pair substation (TA100, TA1535) and AT base pair substitutions (TA102 and E coli). Both the plate incorporation assay and the preincubation assay were used in this study. The high doses in these studies were limited by the toxicity of rotigotine; severe toxicity was observed at doses as low as 100 ug/plate in the absence of S9 and 167 ug/plate in the presence of S9. The sponsor evaluated at least four doses in each strain. No significant increase in mutant frequency was observed. This study was not conducted in accordance with GLP standards. The sponsor did not determine the stability, homogeneity or accuracy of the dose preparations. Since a sufficient concentration was administered to induce toxicity in this test system, this reviewer considers this to be an adequate assay.

The potential for rotigotine to induce genetic damage in mammalian in vitro was examined in L5178Y mouse lymphoma cells (Study —-R 990310, see page 112). The cells were incubated with rotigotine for 3 and 24 hours in the absence of metabolic activation and 3 hours in the presence of metabolic activation. In the presence of metabolic activation, rotigotine increased the mutation frequency by approximately 3.9-fold (525 versus 135 mutations/million cells in control) and 3.3-fold (526 versus 158

mutations/million cells in control). These results were obtained at doses which cause minimal cytotoxicity (plating efficiencies were 87% and 85%, respectively). The effect was dose dependent. Colony sizing suggested that there was an increase in the number of small colonies suggesting a clastogenic effect. An increased incidence of mutations was also observed in the absence of metabolic activation in cultures exposed for 24 hours. A two-fold increase in mutation frequency was observed at the second highest dose evaluated, while a 1.6 –fold increase was observed at the highest dose. The four highest doses had mean mutation frequencies that were above the historical control range. Plating efficiencies were 79% at all dose levels, suggesting that the results were not due to excessive cytotoxicity. This reviewer considers this to be a positive result. Finally, there were no consistent increases in cells exposed for three hours in the absence of metabolic activation, although there were frequent increases above the historical control range. Relatively low cytotoxicity was observed in this assay (plating efficiency at the highest evaluated dose (25 ug/ml) was above 88%). This reviewer considers this portion of the assay to be equivocal. In summary, rotigotine was positive in this assay system in the presence and absence of metabolic activation. Colony sizing suggest that the effect was due to a clastogenic effect.

The potential for rotigotine to induce genetic damage in bone marrow cells in vivo was examined in the mouse micronucleus assay (Study 0309FD15.001, see page 119). In this study, male and female mice were administered 0, 1, 5 or 15 mg/kg rotigotine intravenously. The high dose was selected based on solubility considerations. No changes in micronuclei or P/E ratio in the bone marrow were observed. This reviewer does not regard this as an adequate study. Rotigotine is administered as a dermal patch which would result in sustained plasma levels. The intravenous doses used in the present study would result in a rapid clearance of drug due to the known short half life of rotigotine. The sponsor needs to repeat this study using subcutaneous injections at higher doses.

The potential for rotigotine to induce genetic damage in the liver in vivo was examined in the unscheduled DNA synthesis assay in rats (Study [redacted] 990704, see page 123). In this study, male and female rats were administered 0, 6.25 or 12.5 mg/kg rotigotine intravenously. The high dose was selected based on lethality at the 20 mg/kg intravenous dose. No changes in DNA synthesis in the liver were observed. This reviewer does not regard this as an adequate study. Rotigotine is administered as a dermal patch which would result in sustained plasma levels. The intravenous doses used in the present study would result in a rapid clearance of drug due to the known short half life of rotigotine. Since this assay is regarded as a secondary in vivo test, it is not necessary for this study to be repeated.

2.7.6 Carcinogenicity of Rotigotine

CD-1 mice were administered rotigotine subcutaneously at doses of 0 (saline), 0 (oily vehicle), 3, 10 or 30 mg/kg/48 hours for two years (Study 12484/99, see page 125). There were no drug-related effects on survival rate (36, 30, 36, 38, 34% in males; 24, 42, 46, 28, 32% in females). Body weight tended to be lower in the 10- and 30-mg/kg groups (compared to vehicle controls), but was not significantly affected in either males (8-9% at the HD) or females (4-6% at the HD). Injection site findings included fluid-filled blisters,

edema, and fibrosis, and were likely attributable to vehicle. No significant increase in tumor incidence was observed in either male or female mice.

Sprague-Dawley rats were administered rotigotine subcutaneously at doses of 0 (saline), 0 (oily vehicle), 0.3, 1 or 3 mg/kg/48 hours for two years (Study —12484/99, see page 139). There were no drug-related effects on survival rate (70, 78, 74, 72, and 80% in males; 46, 50, 56, 64, and 56% in females). Dose-related decreases in body weight (compared to vehicle controls) were observed in both males (8-17% at the HD) and females (7-9% at the HD). Injection site findings included fluid-filled blisters, edema, and fibrosis, and were likely attributable to vehicle. Systemic non-neoplastic effects included uterine hyperplasia in the mid and high dose groups and hemometra and pyometra at the high dose. An increased incidence of vaginal squamous epithelium with keratin was observed in all rotigotine treated rats. In males, a significant increase was observed in the incidence of testicular Leydig cell adenomas at all dose levels. In females, a biologically significant increase in uterine tumors (adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma) was observed at all dose levels. No significant increase in any other tumors was observed in either male or female rats.

The Leydig cell adenomas observed in the rat carcinogenicity study are not believed to be clinically relevant. It is known that compounds that disrupt the hypothalamic-pituitary-testis axis can induce Leydig cell adenomas in rats. Rotigotine, like other dopamine agonists (ropinirole, pramipexole), disrupts this axis by lowering prolactin levels. Since prolactin is required for maintenance of testicular luteinizing hormone receptors, prolonged dopamine stimulation results in a decrease in the concentration of luteinizing hormone receptors. This causes a decrease in testosterone production leading to increased luteinizing hormone production. The increased luteinizing hormone is thought to result in Leydig cell hyperplasia and adenoma formation. Numerous compounds (e.g., androgen receptor antagonists, 5alpha-reductase inhibitors, testosterone biosynthesis inhibitors, aromatase inhibitors, gonadotropin releasing hormone inhibitors) have been identified which disrupt the hypothalamic-pituitary-testes axis leading to Leydig cell hyperplasia and adenoma formation in rats (for a comprehensive review see Cook et al., 1999[4]). This mechanism is unique to rats and not considered relevant for humans[5] Similar conclusions were reached in the previous NDA reviews of dopamine agonists (see the pharmacology/toxicology reviews of pramipexole by Dr Steele (NDA 20-667) and ropinirole by Dr Ault (NDA 20-658)).

Similarly, the uterine tumors observed in the rat carcinogenicity study are not believed to be clinically relevant. The uterine tumors are considered to be secondary to the prolactin lowering effects of rotigotine. The decreased prolactin levels in aged rats create an imbalance in the progesterone/estrogen levels resulting in endometrial stimulation. This mechanism is unique to rats and not considered relevant for humans[6]. Similar conclusions were reached in the previous NDA reviews of dopamine agonists

[4] Cook, JC, GR Klinefelter, JF Hardisty, R. Sharpe and PMD Foster (1999) Rodent Leydig Cell Tumorigenesis: A Review of the Physiology, Pathology, Mechanism and Relevance to Humans. Crit Rev Toxicol 29(2):169-261.

[5] Clegg, ED, JC Cook, RE Chapin, PMD Foster and GP Daston (1997) Leydig Cell Hyperplasia and Adenoma Formation: Mechanisms and Relevance to Humans. Reprod. Toxicol. 11(1):107-121.

[6] Slison, RH, CC Capen and DE Prentice (1994) Neoplastic Lesions of Questionable Significance to Humans. Toxicol. Pathol. 22(2)179-186.

(see the pharmacology/toxicology reviews of pramipexole by Dr Steele (NDA 20-667) and ropinirole by Dr Ault (NDA 20-658)).

Unlike other recently approved dopamine agonists (pramipexole, ropinirole), rotigotine did not cause retinal atrophy in the rat carcinogenicity study. This is a surprising finding since it has been proposed that dopamine agonists have been proposed to cause retinal atrophy by an uncertain mechanism in rats. On the other hand, retinal degeneration was observed in the chronic rat study which used higher doses of rotigotine (12.5 mg/kg in the chronic study versus 3 mg/kg in the carcinogenicity study).

Rotigotine is administered in a patch directly to the skin. To examine the potential for a direct tumorigenic effect on the skin, the sponsor conducted a six month dermal toxicity study in minipigs (study — 16332/02, see page 155). In this study, minipigs (six/sex) were administered 4.5 mg rotigotine in a 10 cm^2 patch rotated around seven sites/week for six months. No increase in preneoplastic foci was observed at the patch application sites. This reviewer is concerned that the sponsor did not use the maximum tolerated dose in this study. It would have been desirable if the sponsor had used a patch with a higher concentration of rotigotine than would be used in the clinic. It is recommended that this study be repeated with higher doses of rotigotine.

2.7.7 Reproductive Toxicity of Rotigotine

The effects of rotigotine on fertility and early embryonic development were examined in rats (Studies — 12018/99 and — 17203/03, see pages 157, 162, respectively). In the first study (— 12018/99), rotigotine was administered to male and female Sprague-Dawley rats at 0, 1.5, 5, or 15 mg/kg/day by subcutaneous injection starting 70 and 14 days prior to mating, respectively. Treated rats were mated with untreated rats. The female rats were sacrificed on gestation day 13. Restlessness was observed at 1.5 mg/kg and above in both sexes. Significantly decreased body weight was observed in high dose males (-15% compared to controls) and at all doses in females (-10-13%). Rotigotine did not affect male fertility, although sperm motility? was reduced in high dose males. In contrast, none of the females were pregnant at gestation day 14. This is probably due to the decreased prolactin levels associated with rotigotine administration in this study. Elevated prolactin is required for implantation in rats.

A second study (— 17203/03) was conducted using CD-1 mice. In this study, female mice (20/group) were administered 0, 10, 30 or 90 mg/kg/day from premating day 14 through premating day 4. From premating day 3 through gestation day 7, mice were administered 0 (control) or 6 mg/kg/day (all rotigotine treated mice). Mice were sacrificed on gestation day 14. Only one low dose female was pregnant; all mid and high dose females were not pregnant. The sponsor attributed pregnancy losses to decreased prolactin level. However, there was no consistent decrease in plasma prolactin levels in the rotigotine treated mice on day 7 of gestation.

The effect of rotigotine on embryo-fetal development was examined in rats (Study — 12452/99, see page 166). In this study, female rats were administered 0, 0.5, 1.5 and 5 mg/kg/day subcutaneously in oily vehicle from gestation day 6 through 17. Lower doses were used due to the known prolactin lowering effects of dopamine agonists which interferes with implantation in rats. Nevertheless, there was complete resorption of embryos in the dams administered 5 mg/kg/day. There was a significant increase in early

resorptions in dams administered 1.5 mg/kg with 14/30 rats having complete resorptions of all embryos. No effects on fetal body weight were observed. No external, visceral or skeletal malformations were observed. It is surprising that no malformations were observed any of the 396 fetuses examined in this study. No visceral or skeletal malformations were reported in the laboratories historical control data of 11,135 control fetuses and 18,363 fetuses from groups not significantly influenced by any test compound (pages 360 and 361 of the original report). For instance, historical control data for this strain of rat suggests that approximately 0.5% of fetuses would be expected to have hypoplastic renal pelvis[7], but no instances of this observation were in the historical control data base (expected number = 56 and 92 in control and unaffected treated groups, respectively. This suggests that this laboratory is unable to detect these visceral and skeletal malformations.

The same laboratory examined the effect of rotigotine on embryo-fetal development in the CD-1 mouse (Study — 14011/01, see page 172). In this study, mice were administered 0, 10, 30 or 90 mg/kg/day subcutaneously from gestation day 6 through 15. Three deaths occurred at 90 mg/kg. Slightly decreased body weight was observed on gestation day15 at 30 and 90 mg/kg (-6% compared to controls in both groups). No significant effects on fetal weight were observed. One 90 mg/kg dam had complete resorption of all embryos. One 90 mg/kg fetus had exencephaly. No visceral or skeletal malformations were observed. The laboratory's historical control database did not have any visceral or skeletal malformations among the 1050 control fetuses and 3,156 fetuses from groups not significantly influenced by any test compound (page 294 of the original report). This reviewer was unable to identify an appropriate historical control database for this strain of mouse. This suggests that this laboratory is unable to detect these visceral and skeletal malformations.

The same laboratory examined the effect of rotigotine on embryo-fetal development in the Himalayan rabbit (Study — 12453/99, see page 178). In this study, rabbits were administered 0, 1, 5 or 15 mg/kg/day subcutaneously from gestation day 6 through 20. Increased restlessness was observed at 5 and 15 mg/kg. No effects on embryo-fetal survival or body weight were observed. No significant increase in malformations was observed. This reviewer is concerned about this laboratory's ability to detect adverse effects if one is present. Unfortunately, this reviewer was unable to identify an historical control database for this strain of rabbit.

The effect of rotigotine on pre and postnatal development was examined in Sprague-Dawley rats (Study — 12787/99, see page 184). In this study, rats were administered 0.1, 0.3, and 1 mg/kg/day from gestation day 6 through post natal day 21. These doses are considered adequate since 5 mg/kg resulted in complete resorption of embryos when administered starting on gestation day 6. Increased mortality and decreased body weight were observed in pups from 1 mg/kg treated dams, which were attributed to decreased milk production by the dams. This is an expected pharmacological action of rotigotine based on its prolactin lowering effects. The decreased body weights persisted throughout the study in 1 mg/kg pups (out to post natal

[7] MARTA/MTA (1996) Historical Control Data (1992-1994) for Developmental and Reproductive Toxicity Studies Using the Crl:CD (SD)BR Rat. Available at http://www.criver.com

week 10). Developmental delays (ear and eye opening, balano-preputial gland cleavage, vaginal opening) were observed in the 1 mg/kg pups, which may be secondary to the slower growth rate of the pups. This was observed in ropinirole treated pups, although the degree of the delays was much smaller (0.6 day delay in vaginal opening in ropinirole treated pups versus 6.5 days in rotigotine treated pups). The 1 mg/kg pups had decreased performance on learning and memory tasks. The effect was not observed in ropinirole treated offspring. Decreased F2 pup survival was observed in the offspring of the 1 mg/kg F1 pups. In summary, perinatal rotigotine exposure had persistent effects on the offspring. Persistent decreases in body weight can not be entirely ascribed to inadequate lactation. Decreased pup weight during lactation was observed following perinatal exposure to ropinirole, but the pups recovered body weight during the post weaning period.

APPEARS THIS WAY
ON ORIGINAL

2.7.8 Recommendations

The sponsor has submitted studies that address most of the nonclinical needs for drug approval. There are three deficiencies in the data that the sponsor needs to address prior to approval.

1. The sponsor has not adequately examined the potential for rotigotine to induce preneoplastic lesions in the pig. The study needs to be repeated using patches containing higher concentrations of rotigotine.
2. The sponsor has not adequately evaluated the in vivo genotoxicity of rotigotine. The sponsor has submitted two in genotoxicity studies which used intravenous administration of rotigotine. Since rotigotine is administered as a dermal patch, it is desirable that the in vivo genotoxicity assay use a similar route of exposure. In this case, the Sponsor should repeat the in vivo micronucleus assay using subcutaneous injections as was used in the repeat dose toxicity studies.
3. The Sponsor has not adequately examined potential reproductive toxicity of rotigotine. The available data suggest that the laboratory which conducted the reproductive toxicity studies could not adequately evaluate the reproductive toxicity.

Based on these considerations, this reviewer considers this application to be **Approvable**.

1 Page(s) Withheld

_____ Trade Secret / Confidential

1 Draft Labeling

_____ Deliberative Process

APPENDIX/ATTACHMENTS

Executive CAC Meeting Minutes November 16, 1999- Dose Selection for Rat and Mouse Carcinogenicity Studies

Executive CAC
Date of Meeting **November 16, 1999**

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
Al DeFelice, Ph.D., HFD-110, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Team Leader
Paul Roney, Ph.D., HFD-120, Presenting Reviewer

Author of Minutes: Paul Roney, 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 # **IND 47,852**
Drug Name: **SPM 962 (N-0923)**
Sponsor **Schwarz Pharma**

Mouse Dose Selection

Sponsor's proposed subcutaneous doses are 1, 3, and 10 mg/kg every other day. The high dose was chosen based rough fur and restlessness observed at 30 mg/kg in the 90 day dose range finding study. The Division noted that the rough fur and restlessness observed at 30 mg/kg was a transient observation that was not observed after Day 33 of the study. The Division recommended that the high dose be raised to 60 mg/kg, based on edema and local effects observed at 90 mg/kg in the dose range finding study. The Committee expressed concern that the 60 mg/kg dose would be too high based on the local effects observed at 60/90 mg/kg. The Committee therefore recommends doses of 3, 10 and 30 mg/kg every other day.

The committee also noted that this compound would be a good candidate for a Tg.AC study instead of a two year study, should the sponsor decide to use this option.

The protocol calls for histopathology to only be conducted on the high dose and control groups.

Rat Dose Selection

The sponsor proposed subcutaneous doses of 0.3, 1, and 3 mg/kg every other day. The high dose was selected based on clinical signs and body weight decreases observed at 10 mg/kg in the range-finding study. The Division expressed concern about low multiple of the rat plasma levels versus the proposed human therapeutic plasma level. The Division proposed a high dose of 7.5 mg/kg every other day to permit a greater coverage of the proposed human therapeutic levels. The Committee concurred with the sponsor's selected high dose, due to a clear effect on reduction of body weight gain at (17% decrease in body weight at 10 mg/kg vs 7% decrease in body weight at 3 mg/kg) and potential liver toxicity (increased liver enzymes in females at 30 mg/kg). However, the committee could not be confident of the other doses, since the clinical chemistry assessments yielded results that were somewhat erratic, and the sponsor had not done histopathology at the lower dose levels. For example, there could be an effect such as the liver toxicity at the other levels as suggested by the clinical chemistry observations.

The Committee further noted changes in cholesterol, especially in females, and in clotting times. These effects are suggestive of possible effects on the liver.

The protocol calls for histopathology to only be conducted on the high dose and control groups.

Figure 111, Page 1 of November 16, 1999 Exec CAC Meeting Minutes

Executive CAC Recommendations and Conclusions:

1. The Committee did not concur with the doses proposed by the sponsor for the mouse sudy, but could concur with subcutaneous doses of 3, 10, and 30 mg/kg every other day for the mouse study
2. The Committee also noted that the mouse study would be a good candidate for a Tg.AC study instead, if the sponsor wishes to consider that instead of a 2-year study.
3. The Committee offered conditional concurrence with doses of 0.3, 1, and 3 mg/kg every other day for the rat study. Absent the histopathology, the committee could not be confident that the doses proposed would not result in toxicity that could compromise the study and its interpretation. The concurrence is conditional upon adequate completion of the study, or pending additional information supporting the utility of these doses (i.e. histopathology of the middle and low doses that could explain the clinical chemistry signs suggestive of effects on the liver).
4. For both studies, if the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:
   (a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups
   (b) for an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group
   (c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,
   (d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

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Figure 112, Page 2 of November 16, 1999 Exec CAC Meeting Minutes

Executive CAC Meeting Minutes January 31, 2005- Evaluation of Results for Rat and Mouse Carcinogenicity Studies

Executive CAC
Date of Meeting: January 31, 2006

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Joseph Contrera, Ph.D., OPS, Member
Abby Jacobs, Ph.D., OND IO, Member
Barry Rosloff, Ph.D., DPP, Alternate Member
Lois Freed, Ph.D., DNP, Supervisor
Paul Roney, Ph.D., DNP, Presenting Reviewer

Author of Draft: Paul Roney, Ph.D.

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

NDA # 21-829
Drug Name: Rotigotine
Sponsor Schwarz Pharma

Background Information

Mouse Carcinogenicity Study

CD-1 mice were administered rotigotine subcutaneously at doses of 0 (saline), 0 (oily vehicle), 3, 10 or 30 mg/kg/48 hours for two years. There were no drug-related effects on survival rate (36, 30, 36, 38, 34% in males; 24, 42, 46, 28, 32% in females). Body weight tended to be lower in the 10- and 30-mg/kg groups (compared to vehicle controls), but was not significantly affected in either males (8-9% at the HD) or females (4-6% at the HD). Injection site findings included fluid-filled blisters, edema, and fibrosis, and were likely attributable to vehicle. No significant increase in tumor incidence was observed in either male or female mice.

Rat Carcinogenicity Study

Sprague-Dawley rats were administered rotigotine subcutaneously at doses of 0 (saline), 0 (oily vehicle), 0.3, 1 or 3 mg/kg/48 hours for two years. There were no drug-related effects on survival rate (70, 78, 74, 72, and 80% in males; 46, 50, 56, 64, and 56% in females). Dose-related decreases in body weight (compared to vehicle controls) were observed in both males (8-17% at the HD) and females (7-9% at the HD). Injection site findings included fluid-filled blisters, edema, and fibrosis, and were likely attributable to vehicle. In males, a statistically and biologically significant increase was observed in the incidence of testicular Leydig cell adenomas at all dose levels. In females, a statistically and biologically significant increase in uterine tumors (adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma) was observed in the mid and high dose

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groups, and the increased incidence in the low dose group was also considered biologically significant because of its rarity in historical controls. No significant increase in any other tumors was observed in either male or female rats.

Executive CAC Recommendations and Conclusions:

Mouse:

The Committee concluded that the mouse study was an adequate evaluation of the carcinogenic potential of rotigotine and that it was negative for drug-related neoplasms.

Rat:

The Committee concluded that the rat study was an adequate evaluation of the carcinogenic potential of rotigotine and that it was positive for drug-related neoplasms:

(a) testicular Leydig cell adenomas were increased in male rats at all doses. The Committee considered this finding to be of questionable clinical significance because the endocrine mechanisms believed to be involved in the production of Leydig cell hyperplasia and adenomas in rats are not relevant to humans.

(b) uterine tumors (adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma) were increased in female rats at all doses.

The Committee noted that plasma exposures (AUC) at the LD and MD were lower or similar to that expected in humans at proposed therapeutic doses.

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

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/PRoney, DNP
/TWheelous, DNP
/ASeifried, OND IO

Figure 114, Page 2 of January 31, 2005 Exec CAC Meeting Minutes

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/s/

Paul Roney
3/1/2006 04:13:55 PM
PHARMACOLOGIST

Lois Freed
3/1/2006 04:41:21 PM
PHARMACOLOGIST
Please see separate memo for comments.

MEMORANDUM
Feb. 27, 2006

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-829

I have reviewed the nonclinical information on Neupro® (Rotigotine) provided by Dr. Paul Rooney and my conclusion is that the marketing application is approvable. If the sponsor provides sufficient data to support an approval action, the carcinogenicity and reproductive toxicology sections of the proposed label

Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluations II & III

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/s/

Kenneth Hastings
2/27/2006 03:22:53 PM
PHARMACOLOGIST