CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21829

PHARMACOLOGY REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neuropharmacological Drug Products (HFD-120)
Center for Drug Evaluation and Research

Date: March 1, 2006

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 21-829 (rotigotine, Neupro™)

Rotigotine (Neupro™) is intended for the treatment of Parkinson's disease. The nonclinical data on rotigotine have been reviewed by Paul Roney, Ph.D. (Pharmacology/Toxicology Review, 3/1/06). Based on this review, Dr. Roney has concluded that the NDA is approvable, but has identified three deficiencies that need to be addressed prior to approval:

(1) inadequate assessment of the potential for rotigotine to induce preneoplastic/neoplastic changes at the application site.

(2) inadequate assessment of genotoxic potential (i.e., in vivo micronucleus assay).

(3) inadequate assessment of the reproductive toxicology of rotigotine.

Comments:

(1) I concur with Dr. Roney's recommendation regarding the need for additional assessment of the application site effects of rotigotine prior to approval. Both carcinogenicity studies of rotigotine were conducted using subcutaneous dosing; therefore, the potential for preneoplastic/neoplastic changes with dermal application (i.e., the clinical route) was assessed only in 6-month studies in minipig.

Two dermal studies were conducted in minipig, both using a 10 cm^2 transdermal patch containing 4.5 mg of rotigotine. In the first "6-month" study, 2 rotigotine (or placebo) patches were applied to the same site daily; the study was terminated early (Day 65) due to unacceptable local toxicity (i.e., drug-related "severe purulent dermatitis, slight to

moderate lympho-histocytic or mix-cell infiltration and severe hyperplasia with pronounced hyperkeratosis...", as described in the sponsor's summary of toxicology). In the repeat 6-month study, one rotigotine (or placebo) patch was applied daily, with the application site rotated so that the same site was used only once every 8 days. According to Dr. Roney, no dose-limiting effects (systemic or local) were observed. Since this study represents the only assessment of application site toxicity (i.e., preneoplastic/neoplastic changes), it is particularly important that a maximum tolerated (or maximum feasible) dose be tested. The 10 cm^2 patch (containing 4.5 mg of rotigotine) is the lowest dose clinical patch; therefore, there is no safety margin between the dose successfully tested for 6 months in minipig and the 30 cm^2 transdermal patch delivering the maximum recommended clinical dose of 6 mg/day.

The local effects reported in the 6-month study were reported as minimal to mild, and were similar in incidence and severity with rotigotine and placebo. Therefore, it is likely that higher doses could be tested, either by more frequent application to a particular site or by using a higher dose patch.

2. I concur with Dr. Roney's conclusion that the in vivo micronucleus assay in mouse conducted using i.v. dosing may not have optimally exposed bone marrow to drug, considering the differences in pharmacokinetics between i.v. bolus and s.c. dosing. (The subcutaneous route in animals was demonstrated to adequately mimic the pattern of drug exposure following transdermal application in humans.) Maximizing the dose (and, presumably, exposure to bone marrow) is of particular importance given the relative insensitivity of the in vivo assay. However, since rotigotine has been adequately assessed in two 2-year carcinogenicity studies, I would not recommend requiring the sponsor to repeat the in vivo micronucleus assay prior to approval. However, since (1) neither carcinogenicity study evaluated tumorigenic potential at the application site, (2) rotigotine was positive (for mutagenicity and clastogenicity, in the absence and presence of metabolic activation) in the in vitro mouse lymphoma tk assay, (3) there is not complete concordance between the results of the standard battery of genotoxicity studies and carcinogenicity studies, and (4) genotoxicity study results are described in label, it is not unreasonable to ask for a repeat in vivo micronucleus assay as a phase 4 commitment. (The sponsor should be asked to provide a time line for study conduct and submission of the final study report.) Alternatively, if the sponsor can provide data to justify the use of i.v. bolus administration, then a repeat study would not been needed.

3. I concur with Dr. Roney's conclusion that the potential for rotigotine to adversely affect reproduction has not been adequately assessed. Specifically, it would appear that there is reasonable concern that the fetal evaluations in embryofetal development studies in rat, mouse, and rabbit were inadequate, based on the paucity of findings (particularly, malformations) in drug-treated and concurrent control animals. (Historical control data were similarly bereft of findings.) If the sponsor cannot provide data to document the sensitivity of the method used to detect, in particular, a wide range of fetal malformations in these studies, then either the mouse or rat embryofetal development study would need to be repeated (only one rodent study is required). The rabbit study will need to be repeated even if the methodology is validated since sufficiently high doses were not

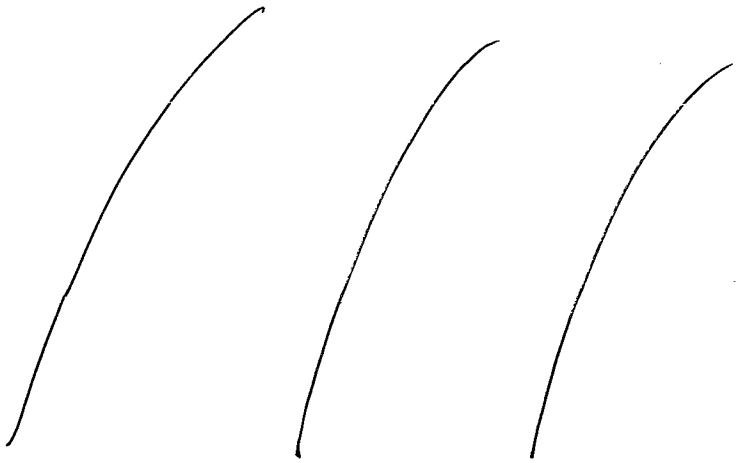
tested. Dr. Roney has recommended that this issue be addressed prior to approval; however, considering the indication (Parkinson's disease) and the age range of the intended patient population, I would recommend that the sponsor be asked to address this as a phase 4 commitment. The sponsor should propose a time line for study conduct and submission of final report(s).

There is one additional issue that was discovered late in the review cycle. According to the chemistry reviewer (David Claffey, Ph.D.), the formation of a known drug product degradant, also results in formation of The sponsor has proposed a limit of in the drug product; however, formation of the as not addressed by the sponsor. Stability data indicates the presence of and, therefore, the at in the drug product after 24 months.

Published literature indicates that may be potent mutagens

Therefore, simply lowering the limit in the drug product specification to below the qualification threshold (i.e., may not be adequate. If is genotoxic, then the limit would have to be set at a level that would result in a daily dose of ≤ /day. Therefore, the sponsor may either specify this lower limit or demonstrate that this degradant does not exhibit genotoxic potential, i.e., negative in the Ames assay and either an in vitro chromosomal aberration assay in mammalian cells or an in vitro mouse lymphoma tk assay (with colony sizing). If is negative in these in vitro assays, then a specification limit of would be acceptable. A limit > would require documentation that the degradant has been qualified in either animals (i.e., a 3-month toxicity study in one species) or humans. This issue would need to be addressed prior to approval.

Recommended wording for the action letter



3 Page(s) Withheld

_____ Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

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

/s/

Lois Freed
3/1/2006 04:51:23 PM
PHARMACOLOGIST

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

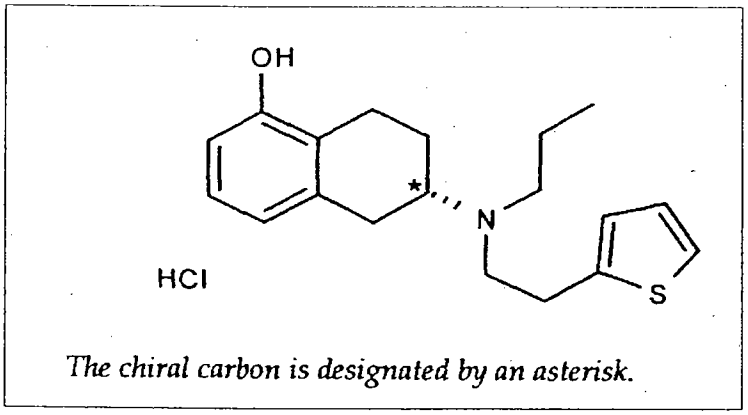
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-829
Review number: 1
Sequence number/date/type of submission: January 28, 2005
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Schwarz-Pharma

Reviewer name:	**Paul L. Roney, Ph.D., D.A.B.T.**
Division name:	**Division of Neurology Products**
HFD #:	**120**
Review completion date:	**February 28, 2006**

Drug:

Trade name: Neupro
Generic name: Rotigotine
Code name: **SPM-962, N-0923**
Chemical name: (-)-5,6,7,8-tetrahydro-6-{propyl-[2-(2-thienyl)ethyl]amino}-1-naphthalenol hydrochloride
CAS registry number: NA
Molecular formula/molecular weight: $C_{19}H_{25}NOS.HCl$ / 351.93
Structure:



The chiral carbon is designated by an asterisk.

Relevant INDs/NDAs: IND 47,852

Drug class: Dopamine D2 Agonist

Intended clinical population: Patients with early Parkinson's disease

Clinical formulation: Transdermal Patch containing 4.5, 9.0, 13.5 [redacted] 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.

Route of administration: Dermal Patch

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

APPEARS THIS WAY
ON ORIGINAL

TABLE OF CONTENTS

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary
See page 196.

2.6.2.2 Primary pharmacodynamics

2.6.2.2.1 Mechanism of action

2.6.2.2.1.1 [redacted] Methods Only Data Report on Compounds Sca-14 to Sca-17 for Schwarz Pharma Ag
Study Report 2292 [redacted] Receptor Screen
Phtox2292-study-report.pdf

Rotigotine was incubated with a variety of receptors in a screening assay. Rotigotine bound to the human Dopamine D3 and D_{2L} receptors with an IC_{50}'s of 2.61 and 59 nM, respectively. A list of significant binding results (ie IC_{50}<1000 nM) is presented below. It is interesting to note that rotigotine has a greater affinity for the adrenergic alpha-2 receptor (IC_{50} = 33 nM) than for the dopamine D_{2L} receptor.

List of significant in vitro binding results in ascending order by Ki

Receptor	Species	Ki	IC_{50} (nM)
Dopamine D_3	Human	0.885 nM	2.61
Dopamine D_{2L}	Human	0.02 uM	59
Adrenergic Alpha-2, Non-Selective	Rat	0.03 uM	33
Dopamine D_{2S}	Human	0.07 uM	195
Sigma, Non-Selective	Guinea Pig	0.13 uM	135
Dopamine Transporter	Human	0.291 uM	366
Dopamine D_1	Human	0.352 uM	900

	CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION -100 -50 0 50 100 %	IC_{50}	K_i	n_H
♦	203500	Adrenergic α_1, Non-Selective	34840	rat	2	10 µM	88	0.623 µM	0.165 µM	0.65
♦					2	1 µM	54			
					2	0.1 µM	27			
					2	10 nM	3			
♦	203900	Adrenergic α_2, Non-Selective	34844	rat	2	10 µM	107	0.033 µM	0.03 µM	0.835
♦					2	1 µM	97			
♦					2	0.1 µM	70			
					2	10 nM	28			
	204410	Adrenergic, Norepinephrine Transporter								
♦			35327	hum	2	10 µM	85			
♦			35524	hum	2	30 µM	94	1.24 µM	1.23 µM	0.785
♦					2	10 µM	85			
♦					2	3 µM	67			
					2	1 µM	41			
					2	0.3 µM	30			
					2	0.1 µM	10			
♦			35789	hum	2	10 µM	84	1.17 µM	1.16 µM	0.761
♦					2	3 µM	69			
					2	1 µM	43			
					2	0.3 µM	29			
					2	0.1 µM	13			
♦			36104	hum	2	10 µM	80	1.82 µM	1.8 µM	0.769
♦					2	3 µM	60			
					2	1 µM	33			
					2	0.3 µM	29			
					2	0.1 µM	4			
♦	219500	Dopamine D_1	34586	hum	2	10 µM	89			
♦			35303	hum	2	10 µM	87	1.22 µM	0.477 µM	0.884
♦					2	3 µM	72			
					2	1 µM	42			
					2	0.3 µM	21			
					2	0.1 µM	12			
					2	0.03 µM	9			
♦			35497	hum	2	30 µM	96	0.94 µM	0.368 µM	0.813

*Batch: Represents compounds tested concurrently in the same assay(s).

♦ Denotes item meeting criteria for significance

†Results with ≥ 50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

R=Additional Comments

gp=gp; hum=human

Figure 1, from page 79 of Report 2292 — Receptor Screen

CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC_{50}	K_i	n_H
						% -100 -50 0 50 100			
♦ 219500	Dopamine D_1	35497	hum	2	10 µM	81	0.94 µM	0.368 µM	0.813
♦				2	3 µM	75			
♦				2	1 µM	53			
				2	0.3 µM	26			
♦		35768	hum	2	10 µM	94	0.541 µM	0.212 µM	0.92
♦				2	3 µM	83			
♦				2	1 µM	66			
				2	0.3 µM	31			
				2	0.1 µM	23			
♦ 219600	Dopamine D_{2s}	35304	hum	2	1 µM	88	0.07 µM	0.023 µM	0.769
♦				2	0.3 µM	74			
♦				2	0.1 µM	57			
				2	0.03 µM	37			
				2	10 nM	17			
				2	3 nM	6			
♦		35498	hum	2	1 µM	92	0.063 µM	0.021 µM	0.848
♦				2	0.3 µM	83			
♦				2	0.1 µM	54			
				2	0.03 µM	36			
				2	10 nM	19			
♦		35769	hum	2	1 µM	92	0.042 µM	0.014 µM	0.779
♦				2	0.3 µM	84			
♦				2	0.1 µM	64			
				2	0.03 µM	43			
				2	10 nM	26			
♦ 219700	Dopamine D_{2s}	35305	hum	2	10 µM	97			
♦		35499	hum	2	10 µM	94	0.143 µM	0.051 µM	0.734
♦				2	3 µM	91			
♦				2	1 µM	82			
♦				2	0.3 µM	63			
				2	0.1 µM	42			
				2	0.03 µM	26			
♦		35770	hum	2	3 µM	91	0.184 µM	0.066 µM	0.831
♦				2	1 µM	82			
♦				2	0.3 µM	59			

*Batch: Represents compounds tested concurrently in the same assay(s).

♦ Denotes item meeting criteria for significance

†Results with ≥ 50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

R=Additional Comments

gp=gp; hum=human

Figure 2, from page 80 of Report 2292 — Receptor Screen

	CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC50	Ki	nH
							-100 -50 0 50 100 % ↓ ↓ ↓ ↓ ↓			
	219700	Dopamine D_{2S}	35770	hum	2	0.1 µM	37	0.184 µM	0.066 µM	0.831
					2	0.03 µM	20			
♦			36055	hum	2	3 µM	87	0.256 µM	0.092 µM	0.87
♦					2	1 µM	79			
♦					2	0.3 µM	56			
					2	0.1 µM	21			
					2	0.03 µM	22			
♦	219800	Dopamine D_3	35306	hum	2	10 µM	100			
♦			35771	hum	2	0.03 µM	89	2.68 nM	0.909 nM	0.884
♦					2	10 nM	77			
♦					2	3 nM	54			
					2	1 nM	26			
					2	0.3 nM	14			
					2	0.1 nM	9			
♦			36056	hum	2	0.03 µM	91	2.4 nM	0.815 nM	0.804
♦					2	10 nM	74			
♦					2	3 nM	54			
					2	1 nM	35			
					2	0.3 nM	15			
♦			36277	hum	2	0.03 µM	92	2.74 nM	0.93 nM	0.982
♦					2	10 nM	75			
♦					2	3 nM	53			
					2	1 nM	32			
					2	0.3 nM	3			
	219900	Dopamine $D_{4.2}$	35307	hum	2	10 µM	38			
	220000	Dopamine $D_{4.4}$	35308	hum	2	10 µM	39			
	220100	Dopamine $D_{4.7}$	35503	hum	2	10 µM	37			
♦	220200	Dopamine D_5	35310	hum	2	10 µM	75			
♦			35504	hum	2	30 µM	90	1.46 µM	0.39 µM	0.623
♦					2	10 µM	82			
♦					2	3 µM	56			
					2	1 µM	39			
					2	0.3 µM	29			
					2	0.1 µM	22			
♦			35775	hum	2	30 µM	92	1.81 µM	0.485 µM	0.732

*Batch: Represents compounds tested concurrently in the same assay(s).

♦ Denotes item meeting criteria for significance

†Results with ≥ 50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

R=Additional Comments

gp=gp; hum=human

Figure 3, from page 81 of Report 2292 — Receptor Screen

	CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION -100 -50 0 50 100 %	IC_{50}	K_i	n_H
♦	220200	Dopamine D_5	35775	hum	2	10 µM	78	1.81 µM	0.485 µM	0.732
♦					2	3 µM	55			
					2	1 µM	42			
					2	0.3 µM	21			
♦			36060	hum	2	30 µM	87	3.15 µM	0.841 µM	0.817
♦					2	10 µM	73			
					2	3 µM	49			
					2	1 µM	23			
					2	0.3 µM	19			
♦	220320	Dopamine Transporter	35328	hum	2	10 µM	97			
♦			35523	hum	2	3 µM	84	0.349 µM	0.277 µM	0.833
♦					2	1 µM	70			
					2	0.3 µM	46			
					2	0.1 µM	31			
					2	0.03 µM	8			
					2	10 nM	1			
♦			35790	hum	2	10 µM	94	0.423 µM	0.336 µM	0.935
♦					2	3 µM	88			
♦					2	1 µM	66			
					2	0.3 µM	46			
					2	0.1 µM	18			
♦			36105	hum	2	10 µM	98	0.328 µM	0.26 µM	1.06
♦					2	3 µM	93			
♦					2	1 µM	75			
					2	0.3 µM	47			
					2	0.1 µM	23			
♦	239500	Histamine H_1, Central	34852	gp	2	10 µM	58	7.17 µM	2.39 µM	1.01
					2	1 µM	12			
					2	0.1 µM	2			
					2	10 nM	-6			
♦	252010	Monoamine Transporter	35312	rabbit	2	10 µM	51			
♦			35506	rabbit	2	100 µM	99	9.26 µM	7.69 µM	1.25
♦					2	30 µM	84			
					2	10 µM	48			
					2	3 µM	20			

*Batch: Represents compounds tested concurrently in the same assay(s).

♦ Denotes item meeting criteria for significance

†Results with ≥ 50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

R=Additional Comments

gp=gp; hum=human

Figure 4, from page 82 of Report 2292 — Receptor Screen

	CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION -100 -50 0 50 100 %	IC_{50}	K_i	n_H
	252010	Monoamine Transporter	35506	rabbit	2	1 µM	13	9.26 µM	7.69 µM	1.25
					2	0.3 µM	1			
♦			35783	rabbit	2	100 µM	100	7.42 µM	6.16 µM	1.08
♦					2	30 µM	80			
♦					2	10 µM	56			
					2	3 µM	30			
					2	1 µM	10			
♦			36061	rabbit	2	100 µM	96	10.4 µM	8.64 µM	0.994
♦					2	30 µM	77			
					2	10 µM	40			
					2	3 µM	26			
					2	1 µM	15			
♦	252700	Muscarinic M_2	34862	hum	2	10 µM	63	4.97 µM	1.77 µM	0.729
					2	1 µM	23			
					2	0.1 µM	8			
					2	10 nM	-3			
♦	260210	Opiate κ	34864	hum	2	10 µM	78	2.51 µM	1 µM	0.855
					2	1 µM	28			
					2	0.1 µM	10			
					2	10 nM	8			
♦	260410	Opiate µ	34865	hum	2	10 µM	79	1.99 µM	0.808 µM	0.665
					2	1 µM	32			
					2	0.1 µM	20			
					2	10 nM	3			
	271000	Serotonin 5-HT_1, Non-Selective								
♦			34866	rat	2	10 µM	76	1.07 µM	0.456 µM	0.47
					2	1 µM	49			
					2	0.1 µM	20			
					2	10 nM	16			
♦	274020	Serotonin Transporter	34621	hum	2	10 µM	73			
♦			35329	hum	2	100 µM	91	4.75 µM	2.53 µM	1.03
♦					2	30 µM	86			
♦					2	10 µM	72			
					2	3 µM	37			
					2	1 µM	12			

*Batch: Represents compounds tested concurrently in the same assay(s).

♦ Denotes item meeting criteria for significance

†Results with ≥ 50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

R=Additional Comments

gp=gp; hum=human

Figure 5, from page 83 of Report 2292 — Receptor Screen

CAT. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC50	Ki	nH
						% -100 -50 0 50 100			
274020	Serotonin Transporter	35329	hum	2	0.3 µM	13	4.75 µM	2.53 µM	1.03
♦		35522	hum	2	100 µM	93	4.74 µM	2.52 µM	0.813
♦				2	30 µM	82			
♦				2	10 µM	68			
				2	3 µM	34			
				2	1 µM	28			
♦		36106	hum	2	100 µM	95	5.72 µM	3.04 µM	0.843
♦				2	30 µM	81			
♦				2	10 µM	57			
				2	3 µM	41			
				2	1 µM	17			
♦	278300 Sigma, Non-Selective	34871	gp	2	10 µM	104	0.135 µM	0.13 µM	1.12
♦				2	1 µM	91			
				2	0.1 µM	41			
				2	10 nM	7			
♦	279500 Sodium Channel, Site 2	34851	rat	2	10 µM	81	2.16 µM	1.94 µM	0.886
				2	1 µM	32			
				2	0.1 µM	9			
				2	10 nM	0			

Figure 6, from page 84 of Report 2292 — Receptor Screen

APPEARS THIS WAY
ON ORIGINAL

2.6.2.2.1.2 In Vitro Pharmacology Study of SPM962 HCl

Study Report 5405

— 5405-study-report.pdf

Rotigotine (1 uM) was incubated with a variety of receptors in a screening assay.

Effects of SPM 962 HCl on the specific radioligand binding to the receptors studied and IC_{50} values for the reference compounds

Receptors	SPM 962 HCl	Reference compounds		
	1 µM		IC_{50} (nM)	*(nH)*
A_1 *(h)*	-	DPCPX	5.7	*(0.8)*
A_{2A} *(h)*	-	NECA	27	*(0.7)*
A_{2B} *(h)*	-	NECA	505	*(0.7)*
A_3 *(h)*	-	IB-MECA	2.2	*(1.0)*
ADO transporter	-	NBTI	0.31	*(1.0)*
α_{1A}	86	WB 4101	0.70	*(1.3)*
α_{1B}	52	spiperone	2.1	*(1.3)*
α_{2A} *(h)*	55	yohimbine	7.5	*(1.1)*
α_{2B}	90	yohimbine	10	*(1.3)*
α_{2C} *(h)*	78	yohimbine	5.8	*(1.0)*
β_1 *(h)*	-	atenolol	2,350	*(0.9)*
β_2 *(h)*	-	ICI 118551	2.8	*(1.3)*
β_3 *(h)*	-	cyanopindolol	18	*(0.5)*
NE transporter *(h)*	12	protriptyline	18	*(1.0)*
BZD (central)	-	diazepam	19	*(1.4)*
BZD (peripheral)	-	PK 11195	36	*(2.4)*
B_1	-	desArg^9[Leu^8]-BK	33	*(0.7)*
B_2 *(h)*	-	NPC 567	6.8	*(0.8)*
CB_1 *(h)*	-	WIN 55212-2	23	*(1.3)*
CB_2 *(h)*	-	WIN 55212-2	23	*(0.5)*
D1 *(h)*	73	SCH 23390	0.76	*(1.2)*
D2 *(h)*	83	(+)butaclamol	4.2	*(0.9)*
D3 *(h)*	103	(+)butaclamol	18	*(1.4)*
D4.4 *(h)*	96	clozapine	86	*(1.1)*
D4.2 *(h)*	95	clozapine	155	*(1.2)*
D4.7 *(h)*	96	clozapine	135	*(1.0)*
D5 *(h)*	83	SCH 23390	1.4	*(1.0)*
DA transporter *(h)*	36	GBR12909	9.1	*(2.8)*
$GABA_A$	-	muscimol	29	*(1.1)*
$GABA_B$	13	baclofen	215	*(0.4)*
GABA transporter	-	nipecotic acid	5,060	*(0.6)*
AMPA	-	L-glutamate	915	*(0.7)*
Kainate	-	kainic acid	14	*(0.7)*
NMDA	-	CGS 19755	335	*(1.0)*

Figure 7, from page 16 of Report — 5405

Receptors	SPM 962 HCl	Reference compounds		
	1 µM		IC_{50} (nM)	*(nH)*
Glycine (strychnine-sensitive)	-	strychnine	10	*(1.0)*
Glycine (strychnine-insensitive)	-	glycine	311	*(0.6)*
TNF-α *(h)*	-	TNF-α	0.15	*(0.9)*
H_1 (central)	56	pyrilamine	1.2	*(0.9)*
H_1 (peripheral)	11	pyrilamine	5.7	*(0.8)*
H_2	19	cimetidine	1,220	*(0.6)*
H_3	-	(R)α-Me-histamine	1.5	*(1.0)*
I_2 (central)	-	idazoxan	7.6	*(0.6)*
I_2 (peripheral)	-	idazoxan	12	*(0.8)*
M_1 *(h)*	11	pirenzepine	15	*(1.0)*
M_2 *(h)*	44	methoctramine	32	*(1.0)*
M_3 *(h)*	-	4-DAMP	2.6	*(1.6)*
M_4 *(h)*	39	4-DAMP	1.5	*(1.2)*
M_5 *(h)*	-	4-DAMP	1.9	*(1.3)*
Choline transporter	-	hemicholinium-3	7.3	*(0.8)*
NK_1 *(h)*	18	[Sar^9,$Met(O_2)^{11}$]-SP	2.8	*(1.0)*
NK_2 *(h)*	-	[Nle^{10}]-NKA(4-10)	10	*(0.8)*
NK_3 *(h)*	-	[$MePhe^7$]-NKB	6.1	*(1.5)*
Y_1 *(h)*	-	NPY	0.32	*(1.1)*
Y_2 *(h)*	-	NPY	0.74	*(0.8)*
N (neuronal) (α-BGTX-insensitive)	-	nicotine	8.0	*(0.8)*
δ *(h)*	-	DPDPE	4.6	*(1.3)*
κ	45	U 50488	0.57	*(0.7)*
µ *(h)*	46	DAMGO	1.4	*(1.0)*
PCP	25	MK 801	4.1	*(1.1)*
$5\text{-}HT_{1A}$ *(h)*	97	8-OH-DPAT	0.47	*(0.8)*
$5\text{-}HT_{1B}$	-	serotonin	33	*(0.9)*
$5\text{-}HT_{1D}$	40	serotonin	3.9	*(1.2)*
$5\text{-}HT_{2A}$ *(h)*	-	ketanserin	2.2	*(1.1)*
$5\text{-}HT_{2B}$ *(h)*	21	serotonin	118	*(0.9)*
$5\text{-}HT_{2C}$ *(h)*	-	mesulergine	1.6	*(1.2)*
$5\text{-}HT_3$ *(h)*	-	MDL 72222	7.1	*(0.9)*
$5\text{-}HT_4$	-	serotonin	192	*(0.9)*

Figure 8, from page 17 of Report — 5405

Receptors	SPM 962 HCl	Reference compounds		
	1 µM		IC_{50} (nM)	*(nH)*
5-HT_{4E} *(h)*	-	serotonin	418	*(0.8)*
5-HT_{5A} *(h)* (5-ht_{5A})	29	serotonin	465	*(0.7)*
5-HT_6 *(h)*	-	serotonin	443	*(1.0)*
5-HT_7 *(h)*	76	serotonin	0.93	*(1.0)*
5-HT transporter *(h)*	-	imipramine	8.3	*(1.1)*
σ (non-selective)	58	haloperidol	37	*(0.8)*
σ_1	58	haloperidol	6.6	*(1.1)*
σ_2	53	haloperidol	100	*(0.8)*
Ca^{2+} channel (L; DHP site)	-	nitrendipine	0.94	*(1.3)*
Ca^{2+} channel (L, diltiazem site)	-	diltiazem	36	*(0.9)*
Ca^{2+} channel (L, verapamil site)	-	D 600	26	*(0.7)*
Ca^{2+} channel (N)	-	ω-conotoxin GVIA	0.0065	*(1.6)*
K^+_{ATP} channel	-	glibenclamide	2.3	*(1.4)*
K^+_V channel	-	α–dendrotoxin	0.93	*(1.2)*
SK^+_{Ca} channel	-	apamin	0.011	*(1.0)*
Na^+ channel (site 1)	-	tetrodotoxin	11	*(0.9)*
Na^+ channel (site 2)	23	veratridine	3,930	*(0.7)*
Cl^- channel	-	picrotoxinin	295	*(1.0)*

For SPM 962 HCl, the results are expressed as a percent inhibition of control specific binding (mean values ; n = 2).
The symbol - indicates an inhibition of less than 10%.

Figure 9, from page 18 of Report — 5405

APPEARS THIS WAY
ON ORIGINAL

2.6.2.2.1.3 In Vitro Pharmacology of SPM962: Determination of the Interaction of the Compound with Various Receptors

Study Report 5651

— 5651-study-report.pdf

Rotigotine was incubated with a variety of receptors in a screening assay.

IC_{50} and K_i values determined for SPM 962 HCl and the reference compounds at the receptors studied

Receptors		SPM 962 HCl			Reference compounds			
		IC_{50} (nM)	K_i (nM)	*(nH)*		IC_{50} (nM)	K_i (nM)	*(nH)*
α_{1A}		336	176	*(1.5)*	WB 4101	0.24	0.12	*(1.0)*
α_{1B}		664	273	*(1.1)*	spiperone	1.8	0.75	*(1.2)*
α_{2A} *(h)*		760	338	*(1.1)*	yohimbine	10	4.3	*(1.2)*
α_{2B}		68	27	*(0.6)*	yohimbine	7.0	2.8	*(1.2)*
α_{2C} *(h)*		329	135	*(0.8)*	yohimbine	5.0	2.1	*(0.9)*
D1 *(h)*		260	83	*(0.9)*	SCH 23390	0.78	0.25	*(1.1)*
D2 *(h)*	1st test	46	17	*(0.8)*	(+)butaclamol	6.7	2.4	*(1.4)*
	2nd test	29	10	*(0.9)*	(+)butaclamol	22	7.9	*(1.2)*
D3 *(h)*		3.2	0.71	*(1.0)*	(+)butaclamol	15	3.4	*(1.4)*
D4.4 *(h)*		35	15	*(0.9)*	clozapine	87	37	*(0.9)*
D4.2 *(h)*		20	3.9	*(0.7)*	clozapine	223	43	*(0.9)*
D4.7 *(h)*		23	5.9	*(0.7)*	clozapine	158	40	*(0.8)*
D5 *(h)*	1st test	14	6.3	*(0.5)*	SCH 23390	0.47	0.21	*(0.9)*
	2nd test	10	4.5	*(0.5)*	SCH 23390	0.58	0.26	*(0.7)*
H_1 (central)		775	330	*(0.9)*	pyrilamine	1.8	0.76	*(1.1)*
H_1 (peripheral)		3,440	549	*(0.9)*	pyrilamine	2.7	0.44	*(1.0)*
M_2 *(h)*		843	576	*(1.0)*	methoctramine	30	21	*(1.0)*
M_4 *(h)*		1,730	769	*(1.0)*	4-DAMP	1.8	0.80	*(1.3)*

Figure 10, from page 11 of Report — 5651

Receptors	SPM 962 HCl			Reference compounds			
	IC_{50} (nM)	K_i (nM)	*(nH)*		IC_{50} (nM)	K_i (nM)	*(nH)*
5-HT_{1A} *(h)*	64	30	*(1.0)*	8-OH-DPAT	0.84	0.39	*(1.3)*
5-HT_{1D}	1,390	853	*(1.2)*	serotonin	2.5	1.5	*(1.1)*
5-HT_{2B} *(h)*	4,290	1,950	*(1.2)*	serotonin	160	73	*(1.0)*
5-HT_{5A} *(h)* (5-ht_{5A})	1,520	797	*(1.0)*	serotonin	152	80	*(0.8)*
5-HT_7 *(h)*	243	86	*(1.0)*	serotonin	0.73	0.26	*(0.9)*
σ_1	237	149	*(1.2)*	haloperidol	4.2	2.6	*(1.2)*
σ_2	1,170	1,010	*(1.3)*	haloperidol	50	43	*(0.9)*
Na^+ channel (site 1)	N.C.	-	-	tetrodotoxin	13	4.0	*(0.9)*
Na^+ channel (site 2)	5,830	5,250	*(1.2)*	veratridine	3,840	3,460	*(1.1)*
NE transporter *(h)*	2,390	2,220	*(1.0)*	protriptyline	10	10	*(1.1)*
DA transporter *(h)*	1,810	826	*(0.9)*	GBR12909	7.8	3.6	*(3.1)*
5-HT transporter *(h)*	11,700	4,810	*(1.3)*	imipramine	5.5	2.3	*(1.0)*

N.C. : value not calculable because of no inhibition at the highest test concentration.

Figure 11, from page 12 of Report — 5651

2.6.2.2.1.4 In Vitro Pharmacology Study of SPM962 HCl

Study Report 5652

— /652-study-report.pdf

Rotigotine (1 uM) was investigated for its effects on a variety of in vitro cell biology systems in a screening assay.

At 1 uM, rotigotine substantially inhibited (>70%) the uptake of dopamine, norepinephrine and serotonin. Rotigotine did not inhibit the release of dopamine. Rotigotine had no effect on several 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.

Effects of SPM 962 HCl in the studied cell biology assays and IC_{50} values for the reference compounds

Assays	SPM 962 HCl	Reference compounds		
	1 µM		IC_{50}	*(nH)*
NOS constitutive *(h)* (endothelial)	-	L-NMMA	0.31 µM	*(0.8)*
NOS constitutive (cerebellar)	-	L-NMMA	0.69 µM	*(1.3)*
Caspase-3 *(h)*	-	Ac-DEVD-CHO	0.0012 µM	*(0.8)*
Catechol-O-methyltransferase	-	Ro 41-0960	0.12 µM	*(0.8)*
MAO-A *(h)*	-	clorgyline	0.037 µM	*(1.8)*
MAO-B *(h)*	-	deprenyl	0.028 µM	*(1.8)*
Phenylethanolamine N-methyl transferase	-	LY 78335	26 µM	*(0.9)*
Tyrosine hydroxylase	-	3-iodo L-tyrosine	1.0 µM	*(0.7)*
NE uptake	96	protriptyline	0.0017 µM	
DA uptake	80	GBR 12909	0.0024 µM	
5-HT uptake	72	imipramine	0.023 µM	
O_2^- secretion *(h)*	-	diphenyleneiodonium	0.79 µM	
Superoxide O_2^- scavenging/ xanthine oxidase	-	allopurinol	2.3 µM	
H_2O_2 secretion *(h)*	12	catalase	305 U/ml	
H_2O_2 scavenging	16	catalase	23 U/ml	
Lipid peroxidation	11	N-propyl gallate	2.5 µM	
ATPase (H^+/K^+)	-	omeprazole	4.3 µM	*(1.2)*
ATPase (Na^+/K^+)	-	ouabain	0.52 µM	*(0.5)*
Acetylcholinesterase *(h)*	13	neostigmine	0.030 µM	*(1.1)*
Carbonic anhydrase	-	acetazolamide	0.0031 µM	*(1.1)*

For SPM 962 HCl, the results are expressed as a percent inhibition of control activity (mean values ; n = 2).
The symbol - indicates an inhibition of less than 10%.

Figure 12, from page 12 of Report — 5652

Effects of SPM 962 HCl on the monoamine releases and EC_{50} values for the reference compounds

Assays	SPM 962 HCl	Reference compounds	
	1 µM		EC_{50} (µM)
DA release	-	amphetamine	1.6
NE release	-	amitriptyline	48
5-HT release	-	fenfluramine	0.44

For SPM 962 HCl, the results are expressed as a percent stimulation of basal activity (mean values ; n = 2).
The symbol - indicates a stimulation of less than 10%.

Figure 13, from page 13 of Report — 5652

2.6.2.2.1.5 *In Vitro* Pharmacology: Monoamine Uptake and Release Assays - Study of SPM 962
Study Report 6128a
— 6128a-study-report.pdf

The in vitro effects of rotigotine on neurotransmitter uptake and release were studied in rat synaptosomes derived from the hypothalamus (norepinephrine), corpora striatum (dopamine) and whole brain (serotonin).

Rotigotine inhibited the uptake of norepinephrine, dopamine and serotonin. It had no significant effects on neurotransmitter release.

	IC_{50} (nM) on Neurotransmitter Uptake	IC_{50} (nM) on Neurotransmitter Release
Dopamine	160	6,000
Norepinephrine	48	>30,000
Serotonin	710	15,000

2.6.2.2.1.6 Interaction of SPM 962 (Rotigotine) with Cloned Human Dopamine Receptors: Determination Of Agonistic/Antagonistic Activity (EC50)
Study Report 02/SP/01
02SP01-study-report.pdf

The in vitro effects of rotigotine on cloned human dopamine receptors (D1, D2L, D3, D4.4 and D5) was examined in CHO cells.

Rotigotine had dopamine agonist properties at all tested dopamine receptors. The rank order of potency (lowest EC50 to highest) was D3>D1>D5>D2L>D4.4.

classification	receptor	Dopamine EC50 (nM)	pEC50	Rotigotine EC50 (nM)	pEC50	EC50 Dopamine/ Rotigotine	Figure
D1-like	D1	4.18	8.38	0.952	9.02	4.4	Fig. 1A
	D5	2.00	8.70	1.39	8.86	1.4	Fig. 5A
D2-like	D2L	19.10	7.72	2.35	8.6	8.1	Fig. 2A
	D3	535.8	6.27	0.206	9.7	2600	Fig. 3A
	D4.4	12.89	7.89	4.10	8.4	3.1	Fig. 4A

Tab. 3: Summary of EC50 and pEC50 values of dopamine in comparison to rotigotine on human dopamine receptor D1, D2L, D3, D4.4 and D5.

Figure 14, from page 12 of Report 02/SP/01

2.6.2.2.2 Drug activity related to proposed indication

2.6.2.2.2.1 Final Report on the Actions of Rotigotine on Locomotor Activity and Disability Scores in MPTP Treated Common Marmosets Following Subcutaneous Administration

Study F-9462
F-9461--study-report.pdf

Four female common marmoset monkeys were administered 2 mg/kg MPTP subcutaneously for five days to induce a Parkinson's-like deficit in motor activity. After stabilization of motor activity (several weeks post MPTP exposure), monkeys were adminsistered 0.01875-0.3 mg/kg rotigoine by subcutaneous injection in an sterile water. The monkeys were monitored for motor activity and disability (Parkinson's-like symptoms) for 300 minutes following administration.

Rotigotine increased motor activity and decreased disability indicating that it has activity in this models of the motor deficits of Parkinson's disease.

Total locomotor counts in 300 minutes following subcutaneous administration of rotigotine in MPTP treated common marmosets. Doses are in mg/kg.

Animals	Vehicle	0.01875	0.0375	0.075	0.15	0.3
F23	436	5,469	7,936	5,414	7,620	12,087
3117	1,481	1,616	2,905	4,205	5,590	6,446
3132	476	3,835	6,029	6,242	11,012	11,006
3100	1,544	2,049	4,757	6,043	5,474	5,109

Figure 15, from page 22 of Report F-9462

Total disability scores in 300 minutes following administration of rotigotine in MPTP treated common marmosets. Doses are in mg/kg.

Animals	Vehicle	0.01875	0.0375	0.075	0.15	0.3
F23	128	88	84	70	53	25
3117	87	71	77	65	47	36
3132	131	97	88	63	48	41
3100	77	86	85	48	44	50

Figure 16, from page 23 of report F-9462

2.6.2.3 Secondary pharmacodynamics

2.6.2.3.1 Interaction of SPM 962 (Rotigotine) with cloned human non-dopamine receptors: Determination of agonistic/antagonistic activity

Study Report 02/SP/02
02sp02-study-report.pdf

The potential agonist and antagonist effects of rotigotine on the human adrenergic, muscarinic and serotonergic receptors were examined in transfected CHO-DUKX cell lines.

Rotigotine had antagonistic activities at the adrenergic alpha 2b and 2c receptors as well as the muscarinic-2 receptor. Rotigotine also had agonistic properties at the serotonin 1A receptor.

Table: agonist effects

Receptor	EC_{50} (nM)	% activation at 10 µM	pEC_{50}	Natural ligand	EC_{50} (nM)	pEC_{50}	Fig.
alpha 1A	26 ± 4	45 ± 2	7.6 ± 0.1	adrenaline	17 ± 1.4	7.8 ± 0.05	1 A/B
alpha 1B	no activity			adrenaline	37 ± 1.4	7.5 ± 0.05	2 A/B
alpha 2A	> 1000	56 ± 2	> 6.0	adrenaline	3 ± 0.5	8.5 ± 0.1	3 A/B
alpha 2B	> 1000	23 ± 5	> 6.0	adrenaline	54 ± 4.3	7.3 ± 0.05	4 A/B
alpha 2C	no activity			adrenaline	60 ± 4.6	7.3 ± 0.05	5 A/B
M1	no activity			acetylcholine	1107 ± 483	6.0 ± 0.2	6 A/B
M2	no activity			acetylcholine	62 ± 13	7.2 ± 0.1	7 A/B
5-HT1A	1040 ± 220	71 ± 7	6.0 ± 0.1	5-HT	15 ± 1.4	7.9 ± 0.3	8 A/B
5-HT1B	> 1000	28 ± 6	> 6.0	5-HT	14 ± 1.9	7.9 ± 0.05	9 A/B
5-HT1D	31*	36 ± 10	7.5*	5-HT	17 ± 10.4	7.9 ± 0.3	10 A/B
5-HT7	no activity			5-HT	25 ± 1.7	7.6 ± 0.0	11 A/B
H1	no activity			histamine	3730 ± 270	5.5 ± 0.05	12 A/B

Tab. 3: Summary of EC_{50}, pEC_{50} values and % activation at 10 µM concentrations of rotigotine in comparison to the natural ligand for the respective receptor. Values are expressed as ± SD (n=2). Percent activation has been calculated using the maximum activation of the natural ligand as 100 %. The most significant activities of rotigotine are marked yellow.

* saturation could not be reproduced in a second experiment.

Figure 17, from page 14 of Report 02/SP/02

Table: antagonist effects

Receptor	IC_{50} (nM)	% inhibition at 10 µM	pKi	Reference antagonist EC_{80} [concentration] of agonist	IC_{50} (nM)	pKi	Fig.
alpha 1A	740 ± 252	28 ± 3	7.4 ± 0.2	Prazosin [300 nM] adrenaline	5.8 ± 2.1	9.5 ± 0.2	1 C/D
alpha 1B	2700 ± 690	77 ± 3	6.5 ± 0.1	Prazosin [300 nM] adrenaline	0.6 ± 0.58	10.7 ± 0.8	2 C/D
alpha 2A	no inhibition			Yohimbine [10 nM] [30 nM] adrenaline	2.9 14.7	9.0 ± 0.2	3 C/D
alpha 2B	394 ± 168	64 ± 7	7.3 ± 0.2	Yohimbine [300 nM] adrenaline	38.3 ± 6	8.3 ± 0.05	4 C/D
alpha 2C	588 ± 116	63 ± 13	7.2 ± 0.1	Yohimbine [500 nM] adrenaline	17.5 ± 3.7	8.8 ± 0.2	5 C/D
M1	3100 ± 190	49 ± 1	6.4 ± 0.1	Scopolamine [5 µM] [10 µM] acetylcholine	1.7 3.0	9.6 ± 0.2	6 C/D
M2	776 ± 207	82 ± 3	6.9 ± 0.2	Scopolamine [300 nM] acetylcholine	5.3 ± 1.2	9.1 ± 0.2	7 C/D

Figure 18, from page 15 of Report 02/SP/02

5-HT1A	no inhibition			WAY100635 [100 nM] [500 nM] 5-HT	3.2 7.6	9.7 ± 0.2 8 C/D
5-HT1B	no inhibition			Methiothepin [100 nM] [300 nM] 5-HT	70.4 108.0	8.2 ± 0.2 9 C/D
5-HT1D	no inhibition			Methiothepin [30 nM] [300 nM] 5-HT	42.0 257.6	7.9 ± 0.2 10 C/D
5-HT7	no inhibition			Clozapine [100 nM] [300 nM] 5-HT	44.8 40.9	8.3 ± 0.2 11 C/D
H1	2440 ± 1050	55 ± 2	6.6 ± 0.2	Pyrilamine [30 µM] histamine	9.0 ± 3.3	9.1± 0.2 12 C/D

Tab. 4: Summary of IC_{50} values and % inhibition at 10 µM concentrations of rotigotine in comparison to a reference antagonist for the respective receptor. EC_{50} values were calculated from the corresponding dose-response experiment. pKi values were calculated using the equation of Cheng and Prusoff {pKi = –log IC_{50}/[1+ used concentration of agonist/EC_{50}]}. Values are expressed as ± SD (n=2). The most significant activities of rotigotine are marked yellow.

Figure 19, from page 16 of Report 02/SP/02

2.6.2.3.2 Efficacy of rotigotine in an acute MPTP mouse model - Reduction of cell death and increase of tyrosine hydroxylase staining in western analysis
Study Report 03-SP-04
03sp04-study-43po45.pdf

C57BL/6 mice (12 males/group) were injected subcutaneously with rotigotine or vehicle. After 18 hours they were injected with MPTP (ip, 4 x 20 mg/kg at 2 hour intervals). Treatment groups are detailed below. Mice were sacrificed 24-27 hours after the first MPTP treatment. Brains were removed and analyzed for cell death (fluoro-jade staining), dopaminergic cell number (tyrosine hydroxylase positive neurons) and western blot analysis.

#group	treatment		
S01-1	vehicle	+	saline
S01-2	vehicle	+	MPTP
S01-3	0.3mg/kg rotigotine	+	MPTP
S01-4	1 mg/kg rotigotine	+	MPTP
S01-5	3 mg/kg rotigotine	+	MPTP
S01-6	3 mg/kg rotigotine	+	saline

Figure 20, from report 03-SP-04

This is a difficult study to interpret. Even though quantitative measurements were made (number of TH-positive cells), the means were not generally presented. Based on the available data, MPTP did not reduce the number of TH-positive neurons (less than 10% change) in the substantia nigra. Based on the investigator's evaluation of the data in the striatum, rotigotine did not protect dopaminergic cells from the adverse effects of MPTP. Rotigotine had no discernible effect on MPTP impairment of motor activity. Rotigotine had no clear effect on tyrosine hydroxylase content in the striatum or substantia nigra. One of the two examiners reported decreased fluoro jade staining in the mesencaphalon at 3 mg/kg rotigotine suggesting that there was decreased cell death after MPTP at the high rotigotine dose.
Two group 6 mice died after saline treatment.

2.6.2.4 Safety pharmacology

2.6.2.4.1 Neurological effects

2.6.2.4.1.1 Neuropharmacological Screening of Mice According to Irwin Following Subcutaneous Administration of SPM 962

Study — Report No. 12019/99 (GLP)

— 1201999-study-report.pdf

Rotigotine was administered to CD-1 mice (8 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously. Mice were evaluated at 15, 30, 60 and 120 minutes post injection in the Irwin method.

No changes in behavior were observed at 0 and 0.1 mg/kg.

Incidence of Behavioral Changes Following Dosing

	0.5 mg/kg				1 mg/kg			
Time (min)	15	30	60	120	15	30	60	120
Restlessness	8/8	8/8	0/8		8/8	8/8	7/8	0/8
Diminished alertness	8/8	8/8	0/8	0/8	8/8	8/8	7/8	0/8
Bradypnea	8/8	0/8	0/8	0/8	8/8	8/8	0/8	0/8
Hypergesia	0/8	0/8	0/8	0/8	1/8	0/8	0/8	0/8
Tremors	0/8	0/8	0/8	0/8	1/8	0/8	0/8	0/8

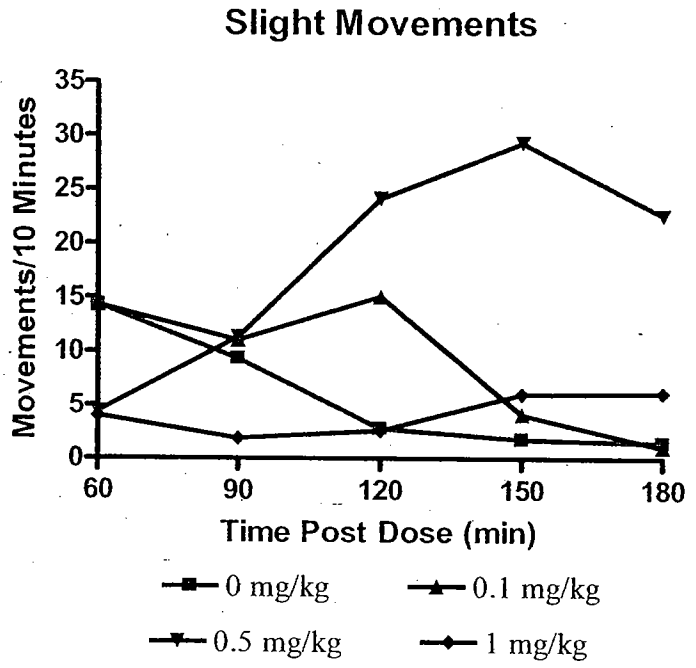
2.6.2.4.1.2 Examination of the Influence of SPM 962 on the Spontaneous Motility of Mice Following Subcutaneous Administration

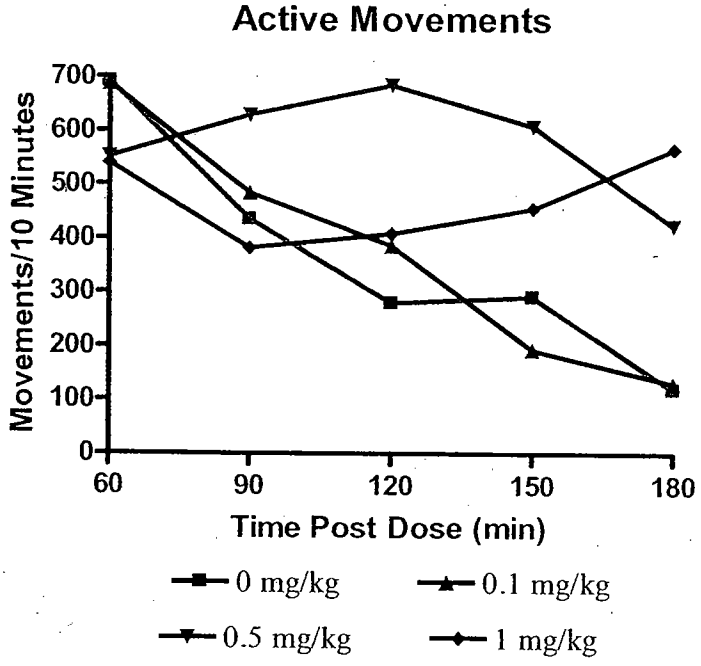
Study — Report No. 12020/99 (GLP)

— 202099-study-report.pdf

Rotigotine was administered to CD-1 mice (10 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously. Mice were evaluated at 15, 30, 60 and 120 minutes post injection in an open field. Movements were recorded for 10 minute intervals for 60 minutes followed by 10 minute intervals at 90. 120, 150 and 180 minutes post dose. Slight and active movements were recorded electronically. Slight movements were defined as the mouse moving without changing its position. Slight movements included grooming and stereotypic behavior. Active movement involved the animal changing its position.

Increased movements were observed at 0.5 and 1 mg/kg.


Slight Movements
Movements/10 Minutes
35
30
25
20
15
10
5
0
60
90
120
150
180
Time Post Dose (min)
0 mg/kg
0.1 mg/kg
0.5 mg/kg
1 mg/kg


Active Movements
Movements/10 Minutes
700
600
500
400
300
200
100
0
60
90
120
150
180
Time Post Dose (min)
0 mg/kg
0.1 mg/kg
0.5 mg/kg
1 mg/kg

2.6.2.4.1.3 SPM 962, SPM 9141, SPM 9206 and SPM 9257 Evaluation in the Rotarod Test in the Rat (S.C. Administration)

Study D00.272/2 (GLP)

D002722-study-report.pdf

Rotigotine and three metabolites (SPM 9141, SPM 9257 and SPM 9206) were evaluated in the rotarod test in male Wistar Han rats (10/dose). Diazepam (4 mg/kg) was the positive control. Rats were dosed subcutaneously (0, 0.5, 1, 5 and 10 mg/kg) 30 minutes prior to being placed on a rod rotating at 12 turns/minute. The time the rats were able to remain on the rod was recorded (maximum time was 3 minutes). In a separate study, rats were treated with 0.5 or 5 mg/kg sc 15, 30, 60 and 120 minutes prior to testing. It did not appear that the rats were trained on the rotarod prior to testing. Study personnel were blinded as to the treatment groups they were evaluating.

Effect of Rotigotine and Metabolites on Drop-off Time (seconds ± SEM (% change from control) in the Rat Rotarod Test 30 Minutes after Subcutaneous Injection

Dose (mg/kg)	Rotigotine	SPM 9141	SPM 9206	SPM 9257
Vehicle	141.0 ± 13.0	141.0 ± 13.0	141.0 ± 13.0	141.0 ± 13.0
0.5	107.2 ± 23.7 (-24%)	97.9 ± 19.0 (-31%)	132.5 ± 20.0 (-6%)	108.1 ± 17.8 (-23%)
1	140.5 ± 14.3 (0%)	**93.0 ± 14.9 (-34%)**	147.0 ± 17.5 (+4%)	110.6 ± 23.5 (-22%)
5	**81.6 ± 18.6 (-42%)**	**67.8 ± 17.0 (-52%)**	147.2 ± 18.0 (+4%)	**62.3 ± 10.9 (-56%)**
10	97.3 ± 18.7 (-31%)	99.2 ± 16.5 (-30%)	150.7 ± 15.6 (+7%)	**69.4 ± 18.7 (-51%)**
Diazepam	**49.2 ± 18.0 (-65%)**			

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

Effect of 5 mg/kg SC Rotigotine and Metabolites on Drop-off Time (seconds ± SEM (% change from control) in the Rat Rotarod Test at Various Times after Injection

Time (min)	Vehicle	Rotigotine	SPM 9141	SPM 9206	SPM 9257
15	130.7 ± 17.5	99.2 ± 20.9 (-24%)	**69.6 ± 19.9 (-47%)**	161.6 ± 10.4 (+24%)	88.4 ± 20.9 (-32%)
30	145.4 ± 16.6	135.1 ± 18.9 (-7%)	**68.5 ± 20.8 (-53%)**	107.8 ± 24.9 (-26%)	**78.9 ± 15.8 (-46%)**
60	133.7 ± 17.3	126.1 ± 19.9 (-6%)	**75.7 ± 16.3 (-43%)**	92.7 ± 24.1 (-31%)	**56.2 ± 15.9 (-58%)**
120	146.6 ± 19.1	163.7 ± 16.3 (+12%)	137.8 ± 18.3 (-6%)	127.4 ± 17.6 (-13%)	91.4 ± 25.1 (-38%)

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

Effect of 0.5 mg/kg SC Rotigotine and Metabolites on Drop-off Time (seconds ± SEM (% change from control) in the Rat Rotarod Test at Various Times after Injection

Time (min)	Vehicle	Rotigotine	SPM 9141	SPM 9206	SPM 9257
15	130.7 ± 17.5	133.1 ± 13.8 (+17%)	80.7 ± 18.2 (-38%)	117.8 ± 17.7 (-10%)	126.8 ± 19.7 (-3%)
30	145.4 ± 16.6	152.1 ± 14.5 (+5%)	**85.7 ± 20.1 (-41%)**	122.9 ± 21.8 (-15%)	**52.5 ± 16.4 (-64%)**
60	133.7 ± 17.3	106.0 ± 19.3 (-21%)	107.2 ± 22.6 (-20%)	111.8 ± 24.9 (-16%)	101.6 ± 22.4 (-24%)
120	146.6 ± 19.1	112.8 ± 20.2 (-23%)	131.4 ± 23.4 (-10%)	125.7 ± 19.7 (-14%)	109.2 ± 21.1 (-26%)

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

Rotigotine and its metabolites SPM 9141 and SPM 9257 (but not SPM 9206) decreased rat performance on the rotarod test. The results were not consistent. For instance, 5 mg/kg rotigotine caused a significant 42% decrease in rotarod performance in the first test, but had no effect on rat performance in the second test at the same time post dose (30 minutes). A clear dose response relationship was not evident for any of the drugs tested. The inconsistent responses may be in part due to lack of rat training on the rotarod.

2.6.2.4.1.4 Examination of the Influence of SPM 962 on Electroshock-Induced Convulsions in Mice Following Subcutaneous Administration – Proconvulsive Activity

Study – Report No. 12022/99 (GLP)

– 1202299-study-report.pdf

Rotigotine was administered to CD-1 mice (5 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously; the positive control was bemegride (40 mg/kg PO). At 60 minutes postdose, mice were administered a mild electric current (24 volts, 5 mAmperes for 0.8 seconds) which did not cause convulsions in historical controls. The number of number of mice having convulsions was counted.

Rotigotine was positive in this test based on the increased number of mice having convulsions and the increased mortality.

SPM 962 dosage (mg/kg b.w. s.c.)	Percent of animals showing convulsions	Mortality (%)
vehicle control	0%	none
0.1	40%	none
0.5	40%	20
1.0	60%	20
40 mg bemegride/kg b.w. p.o.	80%	60

Figure 21, from page 6 of Report — 12022/99

2.6.2.4.1.5 Examination of the Influence of SPM 962 on Pentetrazol-Induced Convulsions in Mice Following Subcutaneous Administration – Anticonvulsive Activity

Study — Report No. 12083/99 (GLP)

— 1208399-study-report.pdf

Rotigotine was administered to CD-1 mice (5 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously. At 60 minutes postdose, mice were administered 110 mg/kg pentetrazol SC, a proconvulsant dose; 25 mg/kg diazepam PO was positive control

Rotigotine had proconvulsant activity as indicated by an increase in the number of convulsions/10 minutes and deaths were observed starting at 0.1 mg/kg.

Effect of Rotigotine on Pentetrazol-induced convulsions

Dose	Number of Convulsions	Convulsions Per 10 Min.	Deaths	Times of Deaths (min)
0 mg/kg	9	0.4	1/5	10
0.1 mg/kg	16	1.5	4/5	10, 11, 14, 15
0.5 mg/kg	18	2.1	5/5	4, 10, 17, 19, 36
1.0 mg/kg	18	3.3	5/5	6, 9, 12, 13, 14
25 mg/kg Diazepam	0	0.0	0/5	---

2.6.2.4.1.6 Examination of the Influence of SPM 962 on Nociceptive Behavior of Mice Following Subcutaneous Administration – Writhing Activity

Study — Report No. 12024/99 (GLP)

— 1202499-study-report.pdf

Rotigotine was administered to CD-1 mice (5 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously; acetylsalicylic acid (100 mg/kg orally) was the positive control. At 60 minutes postdose, mice were administered acetic acid by intraperitoneal injections. The number of writhing reactions was counted over a period of 15 minutes.

Rotigotine decreased the number of writhing reactions at 0.5 and 1 mg/kg; 0.1 mg/kg had no effect.

Effect of Rotigotine on the Number of Writhing Reactions (% of Control) after Intraperitoneal Acetic Acid Intraperitoneal Injection

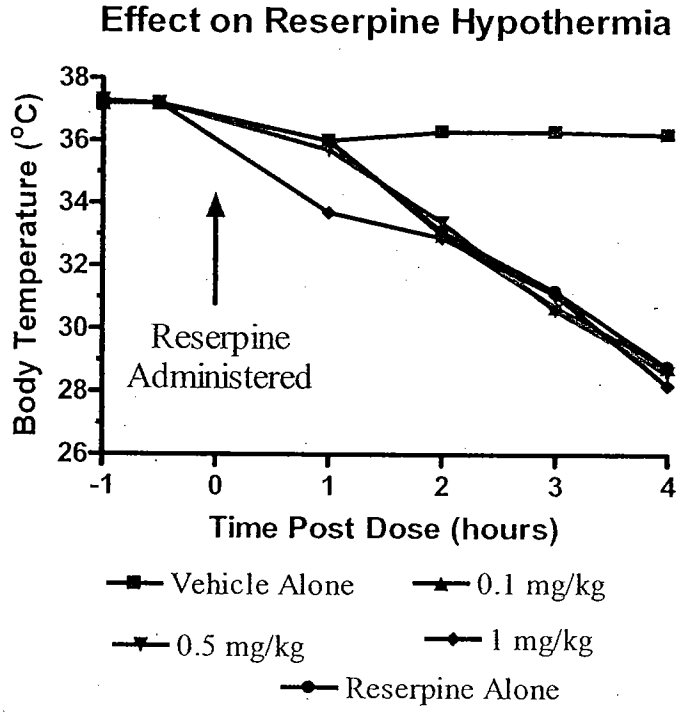
Dose	0 mg/kg	0.1 mg/kg	0.5 mg/kg	1 mg/kg	ASA
Reactions	40.2	37.8 (94%)	**29.0 (72%)**	**17.4 (43%)**	**20.4 (51%)**

Values in **Bold** significantly different from control ($p<0.05$).
ASA = acetylsalicylic acid (positive control)

2.6.2.4.1.7 Examination of the Influence of SPM 962 on Reserpine-reduced Body Temperature in Mice Following Subcutaneous Administration

Study — Report No. 12025/99 (GLP)
— 1202599-study-report.pdf

Rotigotine was administered to CD-1 mice (5 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously. No positive control was used in this study. At 60 minutes postdose, mice were administered reserpine (5 mg/kg) by intraperitoneal injection. Body temperature was measured at 1, 2, 3 and 4 hours after reserpine injection.



Rotigotine had no significant effect on reserpine-induced hypothermia.

2.6.2.4.1.8 Examination of the Influence of SPM 962 on the Hexobarbital Sleeping Time in Mice Following Subcutaneous Administration

Study – Report No. 12021/99 (GLP)

–1202199-study-report.pdf

Rotigotine was administered to CD-1 mice (5 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously. At 60 minutes postdose, mice were administered 45 mg/kg hexobarbital intravenously. Time to recovery of righting reflex was recorded (maximum observation time was 30 minutes)

No effects on recovery were noted.

APPEARS THIS WAY
ON ORIGINAL

2.6.2.4.2 Cardiovascular effects

2.6.2.4.2.1 Examination of the Influence of SPM 962 on Several Cardiovascular Parameters and the Respiration Following Subcutaneous Administration in Anaesthetised Cynomolgus Monkeys

Study — Report No. 12026/99 (GLP)
—1202699-study-report.pdf
Rotigotine was administered to anesthetized (ketamine 50-70 mg/kg/hour by IV drip) cynomolgus monkeys (5 males) at doses of 0, 0.25, 1 or 4 mg/kg by subcutaneous injection. Monkeys were administered ascending doses with a 4 day washout period between doses. Measurements were recorded at 0, 2, 5, 15, 30 and 60 minutes post dosing. Parameters assessed included peripheral arterial blood pressure, heart rate, aortic blood flow velocity, blood flow, left ventricular ejection fraction, ECG (no QT correction made), respiration rate and minute volume. This reviewer performed the QT corrections.

Mean (SEM) ECG parameters following administration of 0.25 mg/kg subcutaneously

Time	Heart Rate (bpm)	QT (msec)	QTcb	QTcf
Predose	89 (8.3)	355 (27.5)	425 (18.3)	400 (20.5)
2 min	87 (8.2)	365 (28.1)	432 (17.0)	408 (19.8)
5 min	88 (8.0)	373 (27.4)	444 (19.2)	419 (21.3)
15 min	89 (6.7)	374 (26.9)	450 (20.0)	423 (22.0)
30 min	89 (5.2)	368 (23.7)	445 (18.9)	417 (20.1)
60 min	87 (4.6)	386 (15.3)	461 (13.2)	434 (13.0)

Mean (SEM) ECG parameters following administration of 1.0 mg/kg subcutaneously

Time	Heart Rate (bpm)	QT (msec)	QTcb	QTcf
Predose	87 (8.1)	384 (25.9)	455 (13.8)	429 (16.5)
2 min	89 (7.0)	388 (23.0)	467 (15.0)	439 (16.6)
5 min	91 (6.1)	388 (19.0)	473 (14.1)	443 (15.0)
15 min	97 (8.5)	369 (15.7)	465 (14.7)	430 (12.7)
30 min	100 (10.2)	369 (17.3)	471 (10.6)	434 (10.8)
60 min	98 (9.2)	371 (16.8)	468 (10.7)	433 (10.9)

Mean (SEM) ECG parameters following administration of 4.0 mg/kg subcutaneously

Time	Heart Rate (bpm)	QT (msec)	QTcb	QTcf
Predose	88 (4.6)	366 (15.6)	443 (25.0)	416 (21.0)
2 min	86 (5.2)	378 (15.6)	452 (25.8)	426 (21.4)
5 min	87 (4.4)	378 (14.8)	456 (24.0)	428 (20.2)
15 min	86 (4.9)	376 (12.2)	450 (21.9)	424 (17.7)
30 min	87 (6.0)	383 (15.4)	461 (23.2)	433 (19.3)
60 min	88 (8.0)	387 (23.4)	463 (20.6)	436 (19.7)

No effects were observed on cardiovascular or respiratory parameters.

2.6.2.4.2.2 Cardiovascular Effects of Dopamine Agonists N-0923, N-0924, N-0437, Apomorphine and Bromocriptine in the Anesthetized Rat

Study [redacted] PH-88001/A (non-GLP)
Ph-88001a-study-report.pdf
Rotigotine was administered to urethane anesthetized male Sprague-Dawley rats (n=4) at asecending doses from 0.01 to 4 mg/kg IV. Blood pressure and heart rate were monitored

Rotigotine caused about a 10% decrease in blood pressure and heart at approximately 0.05 mg/kg and above.

2.6.2.4.2.3 Effect of SPM 962 on Cloned hERG Channels Expressed in Mammalian Cells

Study 020317 [redacted] GLP)
020317 [redacted] study-report.pdf
Rotigotine was dissolved in DMSO and incubated with HEK293 cell stably transfected the hERG cDNA. Effect on the hERG channel was monitored using a patch clamp technique. Three cells were used per concentration of rotigotine (2 cells for the positive control). Terfenadine (60 nM) was the positive control. Final DMSO concentration was 0.1% in all test and control solutions.

Rotigotine inhibited the hERG channel with an IC50 of 0.15 uM.

Effect of Rotigotine and Terfenadine on the hERG channel current (fraction of control)

Rotigotine Concentraion (uM)	Fraction of current ($I_{test}/I_{control}$)	SD	N
0	1.01	0.01	3
0.03	0.84	0.03	3
0.1	0.64	0.01	3
0.3	0.30	0.02	3
1.0	0.13	0.02	3
Terfenadine (60 nM)	0.13	0.06	2

2.6.2.4.2.4 Electrophysiological Examination of Activity of SPM 962 on the hERG-potassium Channel Stably Expressed in CHO Cells

Study E-01-001-013 (non-GLP)
E-01-001-013-study-report.pdf
Rotigotine was dissolved in DMSO and incubated with CHO cells stably transfected the hERG cDNA. Effect on the hERG channel was monitored using a patch clamp technique. Three cells were used per concentration of rotigotine (2 cells for the positive control). Terfenadine (100 nM) was the positive control. DMSO (0.1%) was evaluated in a separate series of studies (n=2).

Rotigotine inhibited the hERG channel with an IC50 of 0.5 uM.

Effect of Rotigotine and Terfenadine on the hERG channel current (fraction of control)

Rotigotine Concentration (uM)	Fraction of current ($I_{test}/I_{control}$)	SD	N
0.01	90.5	0.02	4
0.1	79.6	0.01	5
1	32.6	0.02	4
10	5.9	0.01	4
100	0	---	1
Terfenadine (0.1 uM)	40	---	2

This data was expressed as percent inhibition in Appendix A (page 16) of the study. This reviewer converted the results to fraction of the current to make the study results consistent with the previous hERG channel assay.

2.6.2.4.2.5 SPM 962: Evaluation of Effect on Cardiac Action Potential in Isolated Canine Purkinje Fibres

Study 20020509PECM (GLP)

— -20020509pecm-study-report.pdf

Rotigotine was dissolved in DMSO and incubated with dog purkinje fibers. The effect on the action potential was monitored using a patch clamp technique with stimulation frequencies of 0.33 and 1 Hertz. Cisapride (0.3 uM) was the positive control.

Rotigotine prolonged the action potential duration at 100 nM and above at both stimulation frequencies in a dose dependent fashion. Cisapride prolonged the action potential also (data not presented).

Table 2.1: Effect of SPM 962 on cardiac action potential in isolated canine Purkinje fibre under normal stimulation rate (1 Hz) (mean values)

Treatment		APA (mV)	RP (mV)	Vmax (V/s)	APD_{50} (ms)	APD_{70} (ms)	APD_{90} (ms)
Predose	Mean	126	-92	423	242	279	319
values	SEM	1	0	22	13	12	12
(Tyrode)	N	6	6	6	6	6	6
0.1% DMSO	Mean	-3	1	14	-3	-1	-2
in Tyrode	SEM	3	0	17	2	2	2
	N	6	6	6	6	6	6
SPM 962	Mean	-1	0	15	1	2	2
10 nM	SEM	1	0	12	2	2	2
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
SPM 962	Mean	-2	0	-9	13	20	22
100 nM	SEM	1	0	15	6	5	4
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	*	**
SPM 962	Mean	-2	0	9	11	33	41
1000 nM	SEM	2	0	19	7	7	8
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**
	Threshold	7	1	63	19	15	17

Predose values: control period with Tyrode.
APA: action potential amplitude.
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD_{50} : action potential duration at 50% of repolarisation.
APD_{70} : action potential duration at 70% of repolarisation.
APD_{90} : action potential duration at 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS:$P>0.05$, *$P\leqslant 0.05$, **: $P \leqslant 0.01$, when compared to the vehicle period (0,1% DMSO in Tyrode): analysis of variance with NEWMAN KEULS test if $P \leqslant 0.05$.
Note: values of APA, RP, Vmax, APD_{50} , APD_{70} and APD_{90} were analysed 25 minutes after starting each infusion period.
Threshold : smallest difference being statistically significant ($P \leqslant 0.05$) calculated from Newman-Keuls test.
Electronic signature : table created by — on 09-DEC-2002 at 19:10:46.170 - Study 20020509PECM

Figure 22, from page 30 of Report — 20020509PECM

Table 2.2: Effect of SPM 962 on cardiac action potential in isolated canine Purkinje fibre under low stimulation rate (0.33 Hz) (mean values)

Treatment		APA (mV)	RP (mV)	Vmax (V/s)	APD_{50} (ms)	APD_{70} (ms)	APD_{90} (ms)
Predose values (Tyrode)	Mean	123	-88	430	283	332	376
	SEM	1	1	19	17	17	17
	N	6	6	6	6	6	6
0.1% DMSO in Tyrode	Mean	-2	0	41	-2	2	1
	SEM	3	1	59	3	2	1
	N	6	6	6	6	6	6
SPM 962 10 nM	Mean	-2	0	4	-3	2	5
	SEM	1	1	8	5	3	2
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
SPM 962 100 nM	Mean	-2	0	27	22	34	38
	SEM	1	0	18	7	7	6
	N	6	6	6	6	6	6
	P	NS	NS	NS	*	**	**
SPM 962 1000 nM	Mean	-6	1	-7	18	54	72
	SEM	2	1	20	7	8	10
	N	6	6	6	6	6	6
	P	NS	NS	NS	*	**	**
	Threshold	8	3	130	17	17	22

Predose values: control period with Tyrode.
APA: action potential amplitude.
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD_{50} : action potential duration at 50% of repolarisation.
APD_{70} : action potential duration at 70% of repolarisation.
APD_{90} : action potential duration at 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS:$P>0.05$, *$P\leq 0.05$, **: $P \leq 0.01$, when compared to the vehicle period (0,1% DMSO in Tyrode): analysis of variance with NEWMAN KEULS test if $P \leq 0.05$.
Note: values of APA, RP, Vmax, APD_{50} , APD_{70} and APD_{90} were analysed 30 minutes after starting each infusion period.
Threshold : smallest difference being statistically significant ($P \leq 0.05$) calculated from Newman-Keuls test.
Electronic signature : table created by — on 09-DEC-2002 at 19:17:22.930 - Study 20020509PECM

Figure 23, from page 31 of Report — 20020509PECM

2.6.2.4.2.6 Examination of the Influence of SPM 962 on Electrophysiological Parameters in the Isolated Papillary Muscle of the Guinea Pig

Study — Report No. 12027/99 (GLP)
— 1202799-study-report.pdf

Rotigotine was incubated with papillary muscle at 1, 10, and 100 ng/ml. The effect on the action potential was monitored using a patch clamp technique with stimulation frequencies of 0.5, 1, 2, and 3 Hertz.

100 ng/ml (1 x 10-7 g/ml) rotigotine prolonged the action potential duration and the raised the ERP at all stimulation frequencies.

TABLE 1 - Summary

Protocol	RP (mV)	APA (mV)	APD_{90} (ms)	ERP (ms)	Vmax (V/s)	after depolar-ization
control						
0.5Hz	-90.3±1.2	122.2±1.0	244.7±13.8	288.3±19.1	181.2±18.7	
1 Hz	-89.8±1.3	119.3±1.0	230.2±13.9	268.3±20.6	175.5±22.3	No
2 Hz	-89.0±1.8	115.7±1.0	194.8±12.6	230.0±15.5	168.0±19.5	
3 Hz	-89.5±2.4	115.5±2.0	173.8±9.7	180.0±9.3	160.3±18.8	
SPM 962 10^{-9}g/ml						
0.5Hz	-90.8±1.5	122.5±1.4	240.2±13.0	280.0±16.9	180.0±17.0	
1 Hz	-90.2±1.5	120.0±1.5	226.2±12.9	270.0±20.5	171.5±15.1	No
2 Hz	-88.7±1.6	116.3±1.4	191.5±11.7	223.3±12.8	170.3±15.3	
3 Hz	-86.5±2.8	111.7±4.4	172.2±8.1	181.7±8.7	159.7±20.1	
SPM 962 10^{-8}g/ml						
0.5Hz	-89.5±1.3	118.3±3.4	241.8±15.2	288.3±19.9	150.3±14.7	
1 Hz	-88.2±1.5	116.2±3.5	226.5±14.3	268.3±19.6	156.7±12.8	No
2 Hz	-87.7±1.9	113.2±3.6	193.7±12.0	233.3±13.8	139.2±13.2	
3 Hz	-83.2±4.3	107.3±6.9	172.8±9.7	186.7±8.8	128.0±13.5	
SPM 962 10^{-7}g/ml						
0.5Hz	-88.8±0.6	120.3±2.5	261.3±14.3	311.7±19.2	174.8±12.6	
1 Hz	-87.8±0.7	118.0±2.7	243.5±13.2*	296.7±17.3*	173.7±10.9	No
2 Hz	-88.0±2.0	114.8±3.4	208.3±11.8*	246.7±12.0*	174.8±11.9	
3 Hz	-88.5±2.6	115.0±2.5	186.3±7.8*	193.3±7.2*	155.8±13.9	

mean±S.E. *p<0.05 n=6

Figure 24, from page 19 of Report — -12027-99

2.6.2.4.2.7 Examination of Rotigotine, SPM 10310 and SPM 10311 on L-type Ca^{2+} Inward Current in Isolated Ventricular Myocytes of the Guinea-Pig

Study 16116/02 (GLP)

— 1611602-study-report.pdf

Rotigotine and two other dopamine agonists (ropinirole (SPM 10311) and cabergoline (SPM-10310) were dissolved in DMSO and incubated with freshly isolated guinea pig ventricular myocytes. The effects on the L-type calcium channel were monitored using a patch clamp technique. Four to six replicate cells were used per concentration of rotigotine and the other dopamine agonists (2 cells for the positive control). Nifedipine

(1 uM) was the positive control. DMSO (0.1%) was evaluated in a separate series of studies (n=2).

At 10 uM, Rotigotine decreased the current by 14% (from 68.4% for DMSO to 53.7% for rotigotine). At 100 uM, rotigotine, the current was decreased to 10% of control values.

Doses of up to 10 uM of ropinirole and cabergoline had no significant effect on calcium channel activity.

2.6.2.4.2.8 Electrophysiological Examination of Activity of SPM 962, SPM 10310 and SPM 10311 on the SCN5A-Sodium Channel Expressed in CHO Cells
Study E-01-014-004 (non-GLP)
E-01-014-004-study-report.pdf
Rotigotine and two other dopamine agonists (ropinirole (SPM 10311) and cabergoline (SPM-10310) were dissolved in DMSO and incubated with CHO cells stably transfected the SCN5A sodium channel. Effect on the sodium channel was monitored using a patch clamp technique. Four replicate cells were used per concentration of rotigotine and the other dopamine agonists (2 cells for the positive control). Lidocaine (10 uM) was the positive control. DMSO (0.1%) was evaluated in a separate series of studies (n=2).

The IC50 were:

Rotigotine - 7.8-13.7 uM
Cabergoline- 6.1-7.6 uM
Ropinirole- 192-362 uM

2.6.2.4.3 Pulmonary Effects

See page 35.

2.6.2.4.4 Renal Effects

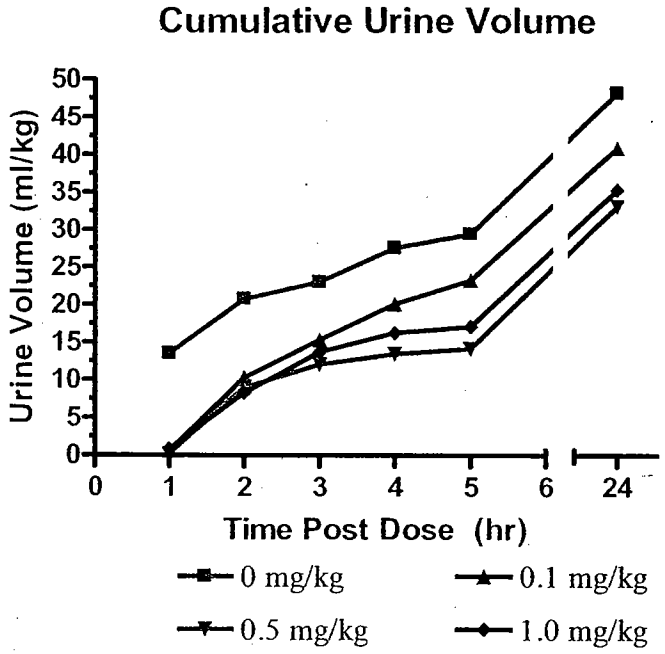
2.6.2.4.4.1 Examination of the Influence of SPM 962 on the Diuresis and Saluresis in Rats Following Subcutaneous Administration
Study — Report No. 12029/99 (GLP)
— 202999-study-report.pdf
Rotigotine was administered to Sprague-Dawley rats (10 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously. Simultaneously, the rats were given an oral water bolus of 20 ml/kg. Urine was collected at 1, 2, 3, 4, 5 and 24 hours post administration. Urine volumes and urine sodium, potassium and chloride concentrations were measured.

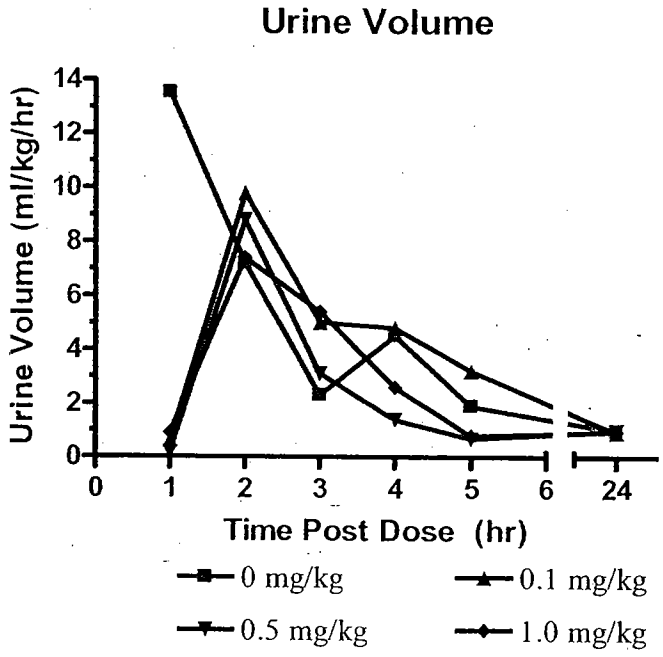
In control rats, there was an initial increase in urine production accompanied by a decrease in the concentration of electrolytes in the urine. Later in the observation period, the rate of urine production decreased while the electrolyte concentration increases as the effects of the fluid overload dissipate. Rotigotine decreased urine production. Most

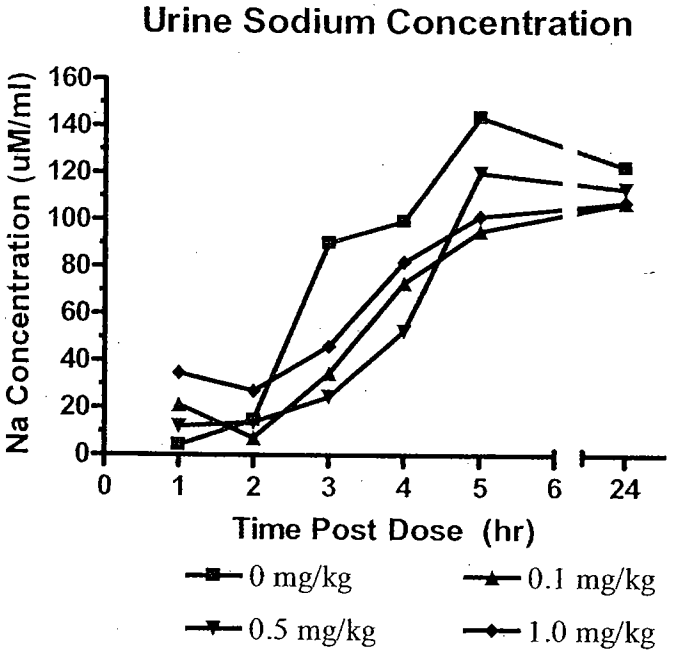
rotigotine treated rats produced less than 1 ml/kg (less than about 0.1 ml total) during the first hour after treatment. Due to the small number of rats producing urine and the small quantities of urine produced, it is difficult to compare electrolyte concentrations, however it appears that the rotigotine treated rats were able to dilute the urine to handle to fluid load (only sodium urine concentration is presented below). The effect was short lasting, possibly reflecting the short half life of rotigotine when administered in a saline vehicle. In any event, the total urine excreted was reduced in rotigotine treated rats.

Number of Rats with Less than 1 ml/kg Urine Production/Time period

	0-1 Hour	1-2 Hour	2-3 Hour	3-4 Hour	4-5 Hour	5-24 Hour
0 mg/kg	1	2	6	2	5	0
0.1 mg/kg	8	0	3	2	3	0
0.5 mg/kg	9	0	1	4	7	0
1.0 mg/kg	8	1	0	3	8	0




Urine Volume
Urine Volume (ml/kg/hr)
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 24
Time Post Dose (hr)
0 mg/kg
0.1 mg/kg
0.5 mg/kg
1.0 mg/kg


Urine Sodium Concentration
Na Concentration (uM/ml)
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 24
Time Post Dose (hr)
0 mg/kg
0.1 mg/kg
0.5 mg/kg
1.0 mg/kg

2.6.2.4.5 Gastrointestinal Effects

2.6.2.4.5.1 Examination of the Influence of SPM 962 on Intestinal Motility Following Subcutaneous Administration (Charcoal Propulsion Test in the Mouse)

Study — Report No. 12028/99 (GLP)
—1202899-study-report.pdf
Rotigotine was administered to CD-1 mice (5 females/dose) at doses of 0 (vehicle 0.9% saline), 0.1, 0.5 and 1 mg/kg subcutaneously. At 60 minutes postdose, mice were administered a charcoal suspension orally. Two hours after charcoal treatment, the mice were sacrificed and the distance the charcoal traveled through the GI tract was measured. No significant effect on intestinal motility was observed. However, there was a trend to decreased transit with individual mice at 0.5 and 1 mg/kg having much lower transit times (0.5 mg/kg mouse #11 transit distance was 63% of intestine length; 1 mg/kg mouse #20 transit distance was 47% of intestine length). All other mice had transit distances of at least 80% of the intestine length.

Effect of Rotigotine on the Charcoal Transit through the GI tract as % of GI tract traversed (% of Control)

Dose	0 mg/kg	0.1 mg/kg	0.5 mg/kg	1 mg/kg
	90.8	88.8 (98%)	84.2 (93%)	82.0 (90%)

2.6.2.4.5.2 Examination of the Influence of SPM 962 for Spasmolytic and Spasmogenic Properties in the Isolated Guinea Pig Ileum

Study — Report No. 12030/99 (GLP)
— 1203099-study-report.pdf
Rotigotine (30 to 70,000 ng/ml; 0.1 to 220 uM) was tested for effects on contractions in the isolated guinea pig ileum.

At the highest concentration tested (70,000 ng/ml), rotigotine induced a contraction equivalent to 23% of the effect of acetylcholine (500 ng/ml). This effect was abolished by papaverine (smooth muscle relaxant/peripheral vasodilator), antazoline (H1 antagonist) and atropine (muscarinic antagonist).

Rotigotine inhibited the contractile actions several stimulants of ileum contraction, most notably acetylcholine and histamine.

Agonist	Agonist concentration	Rotigotine EC50
Acetylcholine	500 ng/ml	350 ng/ml
Histamine	50 ng/ml	140 ng/ml
Barium Chloride	200,000 ng/ml	31,000 ng/ml
5-hydroxytryptamine	15 ng/ml	24,000 ng/ml

2.6.2.5 Pharmacodynamic drug interactions

Pharmacodynamic drug interactions were not evaluated in preclinical models.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

See Page 198.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

2.6.4.3.1 Pilot Study to Evaluate the Pharmacokinetics and the Bioavailability of Several SPM 962 Formulations in Sprague-Dawley Rats

Study Report — Report No. 11640/98
— 1164098-study-report.pdf

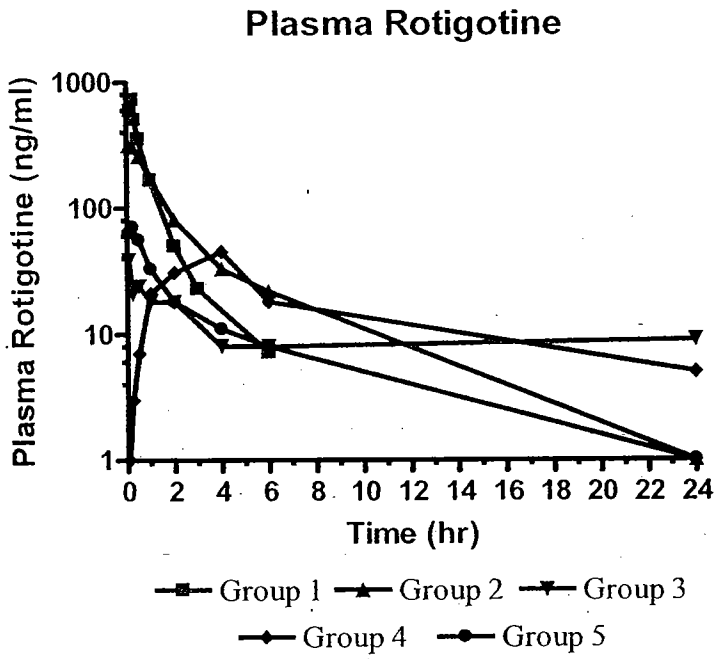
Male Sprague-Dawley rats (4/group) were administered rotigotine according to the following protocol.

Group	Formulation/appearance	Dose	Route	Volume
1	IFL NDO545 / clear solution	3	15 minute IV infusion	6 ml/kg (diluted with saline)
2	IFL NDO545 / clear solution	3	Subcutaneous injection	0.6 mg/kg
3	IFL NDO546 / Clear gel	100	Oral gavage	20 g/kg
4	IFL NDO547 / White oily crystal suspension	3	Subcutaneous injection	0.32 ml/kg
5	IFL NDO548 / White emulsion	3	Intraperitoneal injection	1.2 ml/kg

The sponsor concluded that Group 4 (oily suspension) had the kinetic profile most like the anticipated dermal kinetics since the rotigotine levels were relatively constant over study period. Poor bioavailability was noted with oral administration.

Group	Cmax (ng/ml)	Cmin (ng/ml)	Tmax (hours)	AUC(0-24) (ng-hr/ml)	Bioavailability (percent)
1	730	7	0.25	546*	---
2	340	0.2	0.12	740	132
3	41	9	0.08	222	1.2
4	50	5	5.3	382	68
5	75	0.8	0.12	204	36

*AUC(0-6)



2.6.4.3.2 Follow-up Study to Evaluate the Pharmacokinetics and the Bioavailability of Several SPM 962 Non-patch Formulations in Sprague-Dawley Rats

Study Report — Report No. 11865/98
— 1186598-study-report.pdf

Male Sprague-Dawley rats (5/group) were administered rotigotine subcutaneously in an oily crystal formulation as detailed in the table below.

Group	Concentration in vehicle	Administration volume (ml/kg)	Dose	Days administered (total doses)
1	1%	0.32	3 mg/kg/24 hours	7 (7)
2	1%	1.27	12 mg/kg/48 hours	7 (4)
3	0.5%	1.27	6 mg/kg	1 (single dose)
4	1.5%	0.42	6 mg/kg	1 (single dose)
5	2%	0.32	6 mg/kg	1 (single dose)
6	0.5% (aqueous solution)	0.6 (diluted with saline)	3 mg/kg (5 minute IV infusion)	1 (single dose)

Comparison of Day 1 pharmacokinetic parameters

Group	1	2	3	4	5	6
Dose (mg/kg)	3	12	6	6	6	3
AUC(0-24) ng-hr/ml	303	849	401	522	616	479*
Cmax (ng/ml)	37	47	26	33	42	1030
Tmax (hr)	4	8	4	4	6	0.08
C24 hr (ng/ml)	4	24	9	13	11	4#

*AUC(0-8)
#C8 hr

Comparison of Day 1 and Day 7 pharmacokinetic parameters

Group	1		2	
Dose	3 mg/kg		12 mg/kg	
	Day 1	Day 7	Day 1	Day 7
AUC(0-24) ng-hr/ml	303	294	849	702
AUC (0-30)	361	345	1010	863
Cmax (ng/ml)	37	17	47	39
Tmax (hr)	4	4	8	3
C24 hr (ng/ml)	4	9	24	25

2.6.4.3.3 Pharmacokinetics of Several SPM 962 Non-patch Formulations in Cynomolgus Monkeys

Study Report – Report No. 11932/99
1193299-study-report.pdf

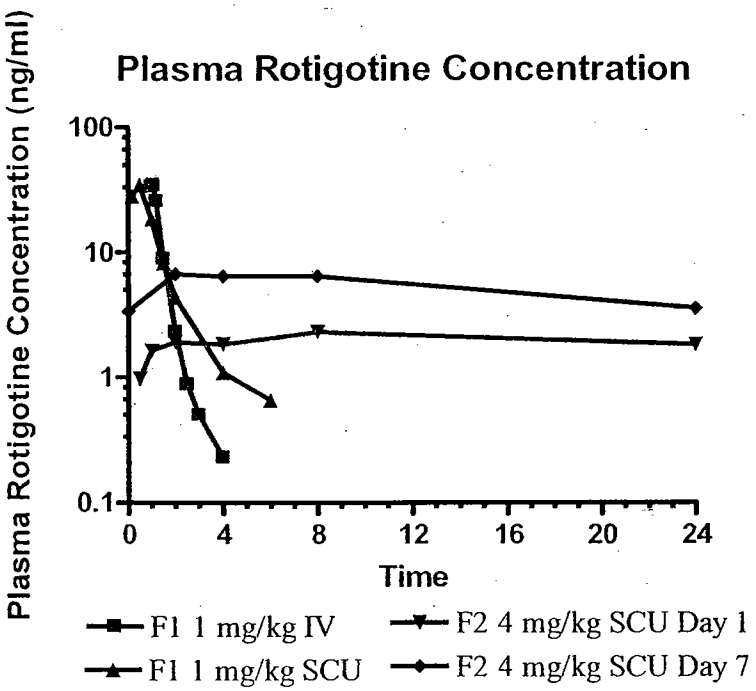
The pharmacokinetics of rotigotine in an aqueous solution (F1) and an oily crystal formulation (F2) in cynomolgus monkeys was examined. Monkeys (1/sex) received single doses of 1 mg/kg F1 by a one hour IV infusion, 1 mg/kg F1 subcutaneous injection, followed by 4 mg/kg/day F2 subcutaneously for 7 days. There were 7 day washouts between regimens. Another group of monkeys were also treated, but due to limited pharmacokinetic sampling in this group (two time points/dose) they will not be discussed. Pharmacokinetics were evaluated.

Rotigotine was rapidly absorbed and eliminated following administration in the aqueous formulation (F1). In contrast, slower absorption and elimination was observed following administration in the oily formulation (F2).

Comparison of pharmacokinetic parameters

Group	Formulation 1		Formulation 2	
Dose	1 mg/kg		4 mg/kg/day SCU	
	Intravenous	Subcutaneous	Day 1	Day 7
AUC(0-24) ng-hr/ml	32*	43&	49	128
Cmax (ng/ml)	37	37	2.6	7.3
Tmax (hr)	1	0.5	12	5
C24 hr (ng/ml)	0.23^	0.66#	1.9	2.9

*AUC(0-4)
&AUC(0-6)
^C4 hours
#C6 hours



2.6.4.3.4 The Disposition of Total Radioactivity in the Cynomolgus Monkey Following Single Subcutaneous and Single Intravenous Administration of [^{14}C] SPM 962

Study Report — 014
— 014-a1-study report.pdf

Cynomolgus monkeys (2/sex) were administered 1 mg/kg rotigotine by 1 hour IV infusion; after a 4 week washout, the same monkeys were administered 1 mg/kg subcutaneously using an "oily vehicle". Serial plasma, urine and fecal samples were taken for up to 168 hours. After another washout period, the monkeys were administered

1 mg/kg by 1 hour IV infusions and sacrificed (1 monkey/timepoint) at 0 (end of infusion), 0.5, 2 and 12 hours post dose for determination of tissue levels of radiolabel.

The high concentrations of radiolabel in the intestinal contents suggests that rotigotine is rapidly cleared from the circulation. The long half life (17-19 hours) following subcutaneous injection is attributable to slow absorption from the oily vehicle as evidenced by the Tmax of 8 hours in subcutaneously injected monkeys. Rotigotine was excreted via the feces and urine. It is uncertain whether the radiolabel in the cage wash and cage debris came from the urine or feces.

Pharmacokinetics of Radiolabel expressed as ng-equivalents

Parameter	1 mg/kg IV infusion		1 mg/kg Subcutaneous injection	
	Males	Females	Males	Females
Cmax (ng eq/ml)	746	665	79	45
Tmax (hours)	---	---	8	8
AUC(0-t) (ng eq-hr/ml)	1626	1459	1856	1386
AUC(0-24) (ng eq-hr/ml)	1626	1459	1263	878
AUC(0-inf) (ng eq-hr/ml)	1786	1571	2188	1706
Half-life (hours)	3.5	3	17.2	18.8

Tissue Concentrations of Total Radioactivity in Male and Female Monkeys at Various Times Following Single Intravenous Administration of [^{14}C] SPM 962 at a Target Dose Level of 1 mg/kg (Phase 3)

Results are expressed as ng equiv/g

Tissue	001M (0 min*)	003F (0.5 h)	002M (2 h)	004F (12 h)
Adrenal				
Bone marrow				
Bone mineral				
Brain				
Eye				
Fat				
Heart				
Kidney				
Large intestine wall				
Large intestine contents				
Liver				
Lung				
Muscle				
Ovary				
Parotid gland				
Prostate				
Skin				
Small intestine wall				
Small intestine contents				
Spleen				
Stomach wall				
Stomach contents				
Testes				
Thyroid				
Uterus				
Plasma				
Whole blood				
Remaining carcass				

n/a not applicable
blq denotes below limit of quantification (, above background)
value obtained from one replicate
* end of infusion

Figure 25, from page 44 of Report — 1014-a1

2 Page(s) Withheld

✓ Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

2.6.4.4 Distribution

See page 48 for data in cynomolgus monkeys.

2.6.4.4.1 The Quantitative Tissue Distribution of Total Radioactivity in the Rat Following Single Intravenous Administration of [^{14}C]SPM 962

Study Report — 1017 (GLP)

— 1017-study report.pdf

Male (n=4) and pregnant gestation day 15 female (n=4) Sprague-Dawley rats as well as pigmented Lister Hooded male rats (n=6) were administered 1.5 mg/kg ^{14}C-rotigotine intravenously. Rats were sacrificed (1/group/timepoint) at 0.0, 0.5, 6, 24, 168 (Lister Hooded rats only) and 336 (List3r Hooded rats only) hours post dose. Radioactivity in tissues was determined.

The results for the male and female albino Sprague-Dawley rats are presented below. The pigmented Lister hooded rat data are not presented since they are similar to the albino rat data except for a high affinity for the eye in pigmented rats. Radiolabel was widely distributed and higher concentrations were observed in the adrenals and the kidney. Rotigotine did cross the placenta in rats.

Table 2 (1 of 2)
Concentrations of Radioactivity in Organs and Tissues at Various Times Post-Dose Following Single Intravenous Administration of [^{14}C] SPM 962 to Male Albino Rats at a Target Dose Level of 1.5 mg/kg

Results expressed as ng equiv/g

Tissue Type	001M (5 min)	002M (0.5H)	003M (6H)	004M (24H)
LOQ				
Adrenal cortex				
Adrenal medulla				
Bone marrow				
Bone				
Brain				
Cerebellum				
Dorsal hippocampus				
Frontal cortex				
Hypothalamus				
Medulla				
Mid brain				
Olfactory bulb				
Posterior cortex				
Thalamus				
Pineal body				
Pituitary gland				
Brown fat				
Caecum contents				
Caecum wall				
Cardiac blood				
Cardiac muscle				
Epididymis				
Fur				
Harderian gland				
Kidney cortex				

LOQ denotes limit of quantification
blq denotes below limit of quantification
* the value for the whole brain was reported because the different regions were not distinguishable from the surrounding tissue

Figure 26, from page 31 of Report → 1017

Table 2 (2 of 2)

Concentrations of Radioactivity in Organs and Tissues at Various Times Post-Dose Following Single Intravenous Administration of [^{14}C] SPM 962 to Male Albino Rats : Target Dose Level of 1.5 mg/kg

Results expressed as ng equiv/g

Tissue Type	001M (5 min)	002M (0.5H)	003M (6H)	004M (24H)
LOQ				
Kidney medulla				
Large intestine contents				
Large intestine wall				
Liver				
Lung				
Nasal mucosa				
Pancreas				
Preputial gland				
Seminal vesicles				
Skeletal muscle				
Small intestine contents				
Small intestine wall				
Spinal cord				
Spleen				
Stomach contents				
Stomach wall				
Submaxillary				
Testes				
Thymus				
Thyroid gland				
Urine				
White fat				
Whole eye				
Whole blood				

LOQ denotes limit of quantification
blq denotes below limit of quantification
ns denotes no sample

Figure 27, from page 32 of Report — 1017

Table 3 (1 of 2)

Concentrations of Radioactivity in Organs and Tissues at Various Times Post-Dose Following Single Intravenous Administration of [^{14}C] SPM 962 to Pregnant Female Albino Rats at a Target Dose Level of 1.5 mg/kg

Results expressed as ng equiv/g

Tissue Type	005F (5 min)	006F (0.5H)	007F (6H)	008F (24H)
LOQ				
Adrenal cortex				
Adrenal medulla				
Bone marrow				
Bone				
Brain				
Cerebellum				
Dorsal hippocampus				
Frontal cortex				
Hypothalamus				
Medulla				
Mid brain				
Olfactory bulb				
Posterior cortex				
Thalamus				
Pineal body				
Pituitary gland				
Brown fat				
Caecum contents				
Caecum wall				
Cardiac blood				
Cardiac muscle				
Chorioallantoic placent				
Foetus				
Foetal liver				
Fur				
Harderian gland				

LOQ denotes limit of quantification
blq denotes below limit of quantification
* the value for the whole brain was reported because the different regions were not distinguishable from the surrounding tissue

Figure 28, from page 33 of Report — 1017

Table 3 (2 of 2)

Concentrations of Radioactivity in Organs and Tissues at Various Times Post-Dose Following Single Intravenous Administration of [^{14}C] SPM 962 to Pregnant Female Albino Rats at a Target Dose Level of 1.5 mg/kg

Results expressed as ng equiv/g

Tissue Type	005F (5 min)	006F (0.5H)	007F (6H)	008F (24H)
LOQ				
Kidney cortex				
Kidney medulla				
Large intestine contents				
Large intestine wall				
Liver				
Lung				
Nasal mucosa				
Ovary				
Pancreas				
Placenta				
Preputial gland				
Skeletal muscle				
Small intestine contents				
Small intestine wall				
Spinal cord				
Spleen				
Stomach contents				
Stomach wall				
Submaxillary				
Thymus				
Thyroid gland				
Urine				
Uterus				
White fat				
Whole eye				
Whole blood				

LOQ denotes limit of quantification
blq denotes below limit of quantification
nd denotes sample is not distinguishable from surrounding tissue
ns denotes no sample

Figure 29, from page 34 of Report → 1017

2.6.4.4.2 Plasma Protein-Binding of SPM 962 in Human, Monkey, Rat and Mouse Plasma Samples

Study Report ~416-03 (non-GLP)
~416-03-study report.pdf
The binding of ^{14}C-rotigotine to plasma proteins was analyzed using equilibrium dialysis. Rotigotine concentrations were between 62.5 and 1000 ng/ml. Protein concentrations were about 45 mg/ml.

Table 2: Concentration Dependence of SPM 962 Protein Binding in Human, Monkey and Rat Plasma Samples and Human Serum Albumin (HSA).

^{14}C-SPM 962 conc. [ng/ml]	Protein Binding [%]			
	HSA (c = 45 mg/ml)	Plasma samples		
		human	monkey	rat
62.5	80.9	92.1	85.2 *	86.7 *
250	81.1	92.2	81.9 *	82.9 *
500	81.6	92.8	86.8	86.8
750	81.9	91.9	84.8	85.7
1000	81.0	91.8	84.9	86.1
mean [%]	81.3	92.2	84.7	85.6
SD	0.4	0.4	1.8	1.6

* data of reassay

Figure 30, from page 12 of Report ~416-03

The binding of rotigotine was determined in duplicate preparations of plasma from five separate patients or animals (monkey, rat). The total number of preparations was 10 samples/species. The mouse results were duplicate preparations from a pooled plasma sample. The results are expressed as mean ± SD (except for the mouse for which the individual result is expressed as mean (individual values)).

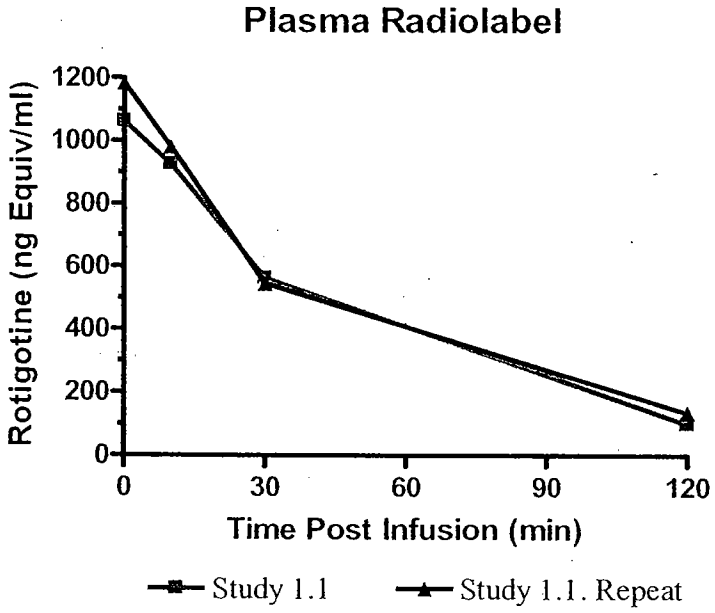
Human = 91.6 ± 1.1%
Cynomolgus Monkey = 87.6 ± 0.9%
Sprague-Dawley Rat = 85.9 ± 3.6%
Mouse = 90.25 (individual values were ~)

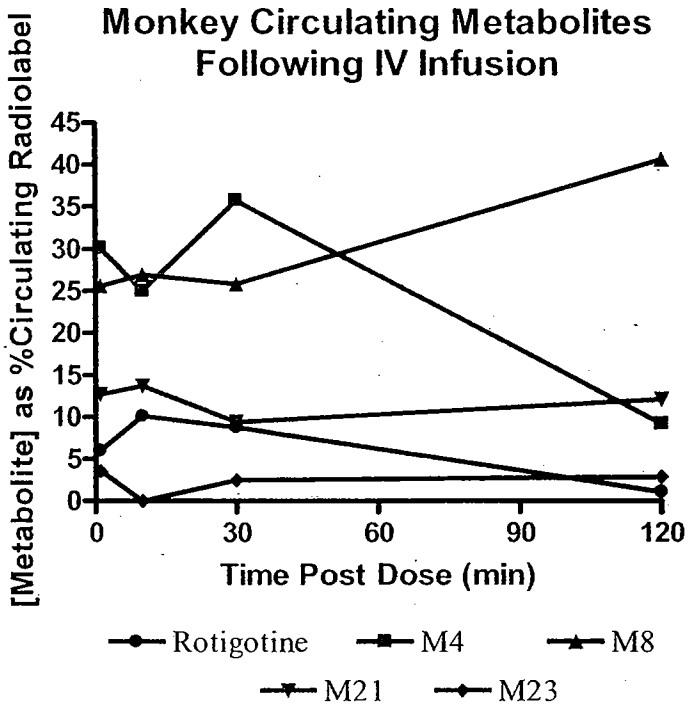
2.6.4.5 Metabolism

2.6.4.5.1 Disposition and Metabolite Identification in the Rat and Cynomolgus Monkey Following Intravenous and Subcutaneous Administration of [^{14}C] SPM 962
Study Report — 1028 (GLP)
— 1028-study report.pdf

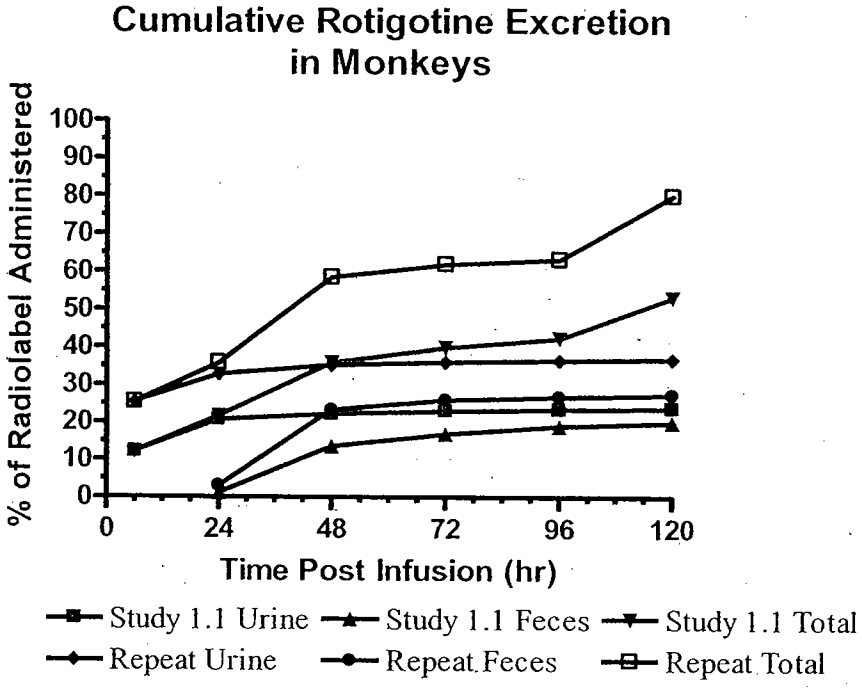
Cynomolgus monkeys (2/sex) received 1 mg/kg rotigotine by 1 hour intravenous infusion. Plasma samples were collected for up to 2 hours post dose while urine and feces samples were collected for up to 120 hours post dose. This study was repeated due to low recovery of excreted radiolabel in the first study (53% of administered dose).

Rotigotine label was rapidly cleared from the circulating plasma following intravenous infusion. Initially, most of the rotigotine label was cleared in the urine (about 27% of administered radiolabel versus 2% in the feces), but after 24 hours, fecal elimination became more important (about 23% of administered radiolabel versus 4% in the urine). The study attributed the difference in total recovery of radiolabel in the two studies to loss of radiolabel urine in the first study. However, the ratio of urine:fecal radiolabel recovery was virtually identical in the two studies (about 1.2:1 urine:feces). If there had been an appreciable loss of radiolabel in the urine, one would expect a change in the ratio of urine:fecal radiolabel. In both studies, about 45% of recovered radioactivity was in the urine while about 38% was in the feces. The remainder of the radioactivity was in the cage washes.

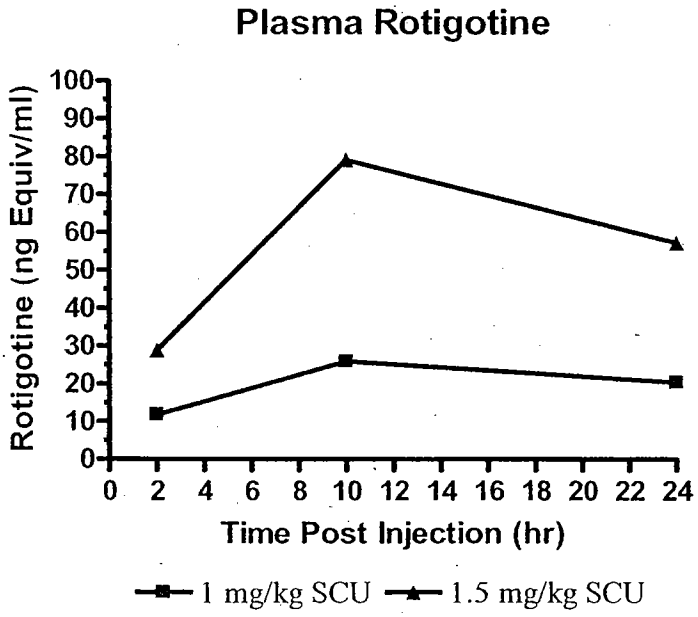




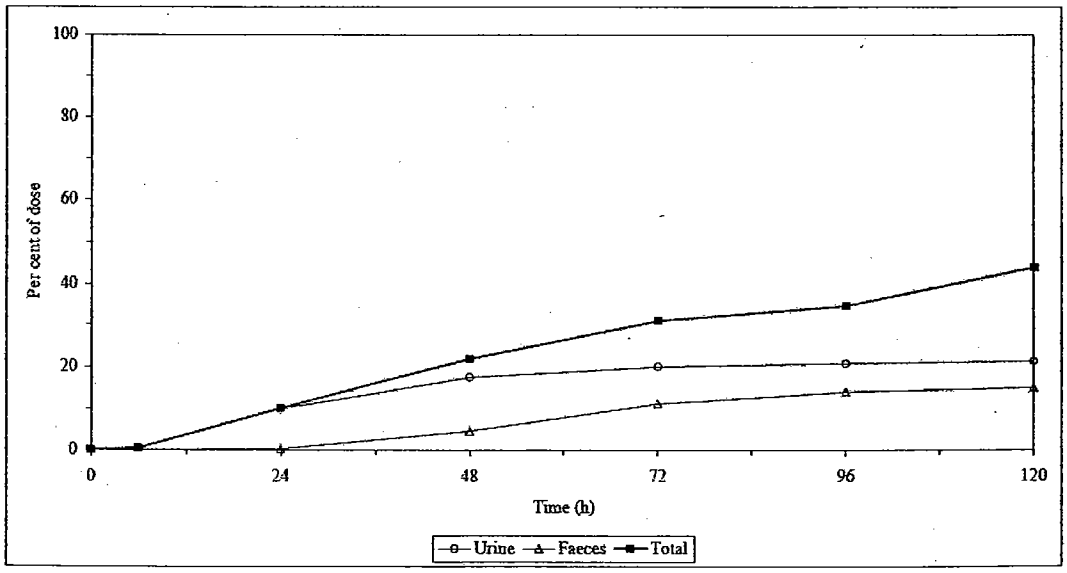
M4=Desthienylethyl despropyl rotigotine sulphate
M8= Desthienylethyl rotigotine sulphate
M21=Despropyl rotigotine sulphate
M23=Rotigotine sulphate



After a 6 week washout, the same monkeys were administered 1 and 1.5 mg/kg by subcutaneous injection in an oily vehicle. Plasma, urine and feces samples were collected up to 120 hours post dose. Following subcutaneous injection, there was a lower peak of radioactivity, but the plasma levels remained relatively stable compared to intravenous injection. Excretion was slower than in the intravenous infusion and the low recovery of radiolabel may be attributed in part to slower absorption. About 48% of recovered radioactivity was in the urine while about 34% was in the feces. These results are similar to the results with intravenous infusion.

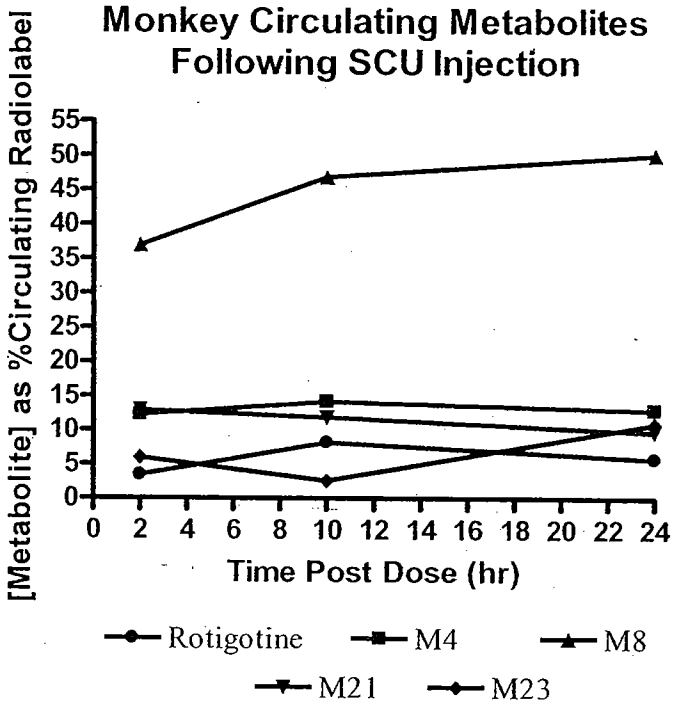


Mean cumulative excretion of radioactivity following a single subcutaneous administration of [^{14}C] SPM 962 to male and female monkeys at a target dose of 1 mg/kg (Phase 1.2)



Note: Total includes cage wash and cage debris

Figure 31, from page 119 of report — 1028

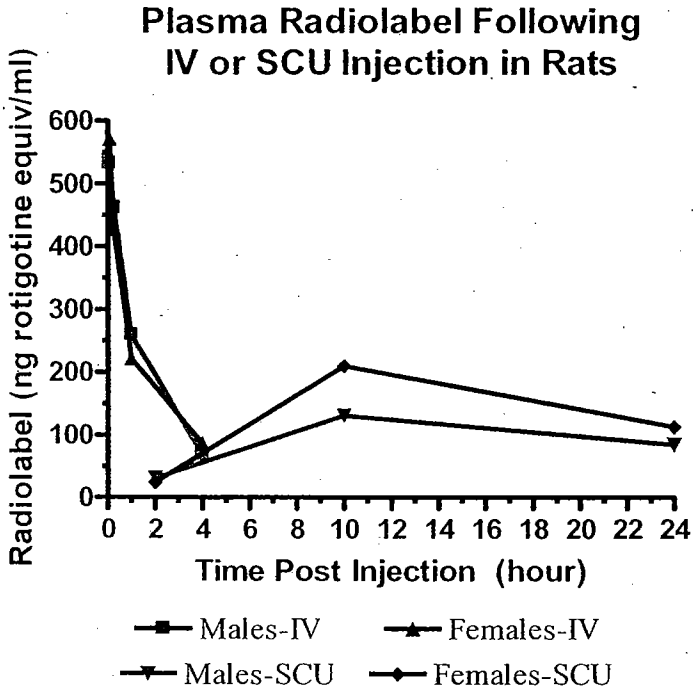


M4=Desthienylethyl despropyl rotigotine sulphate
M8= Desthienylethyl rotigotine sulphate
M21=Despropyl rotigotine sulphate
M23=Rotigotine sulphate

In a separate study, Sprague-Dawley rats (15/sex) were administered 1.5 mg/kg by 5 minute intravenous infusion. Plasma samples were collected for up to 4 hours post dose while urine and feces samples were collected for up to 120 hours post dose. In another study, Sprague-Dawley rats (12/sex) were administered 3 mg/kg by subcutaneous injection in oily vehicle. Plasma, urine and feces samples were collected up to 120 hours post dose.

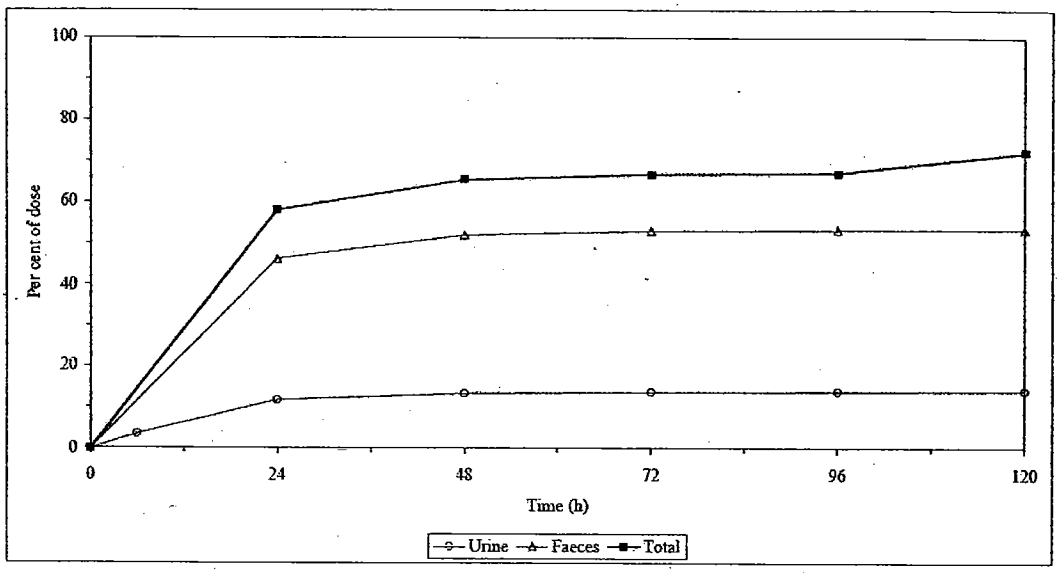
Following intravenous injection, radiolabel was rapidly cleared from the plasma following intravenous infusion. In contrast, subcutaneous injection in oily vehicle resulted in a lower, but more sustained, level of circulating radiolabel. Excretion was primarily via the feces (74%) by both routes of exposure. With intravenous administration, excretion was mostly complete by 24 hours, but more sustained excretion was observed following subcutaneous injection, reflecting slower absorption from the injection site. Rotigotine was rapidly metabolized in the rat following intravenous administration. 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. 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). For the sake of simplicity, only data for the major circulating human metabolites are presented below. Since rotigotine was not detected at all time points, the data are presented as absolute levels of metabolite. 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.



APPEARS THIS WAY
ON ORIGINAL

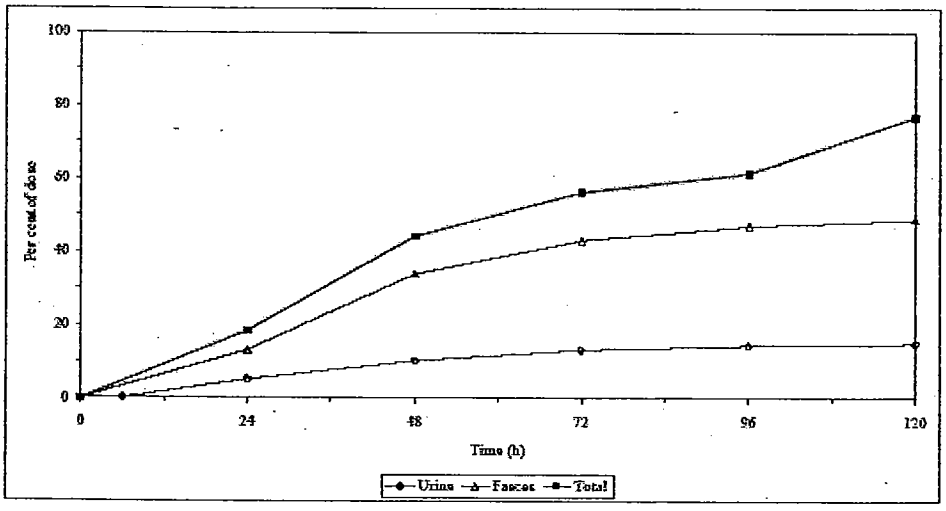
Mean cumulative recovery of radioactivity following a 5 min intravenous infusion of [^{14}C] SPM 962 to male and female rats at a target dose of 1.5 mg/kg (Phase 2.2)



Note: Total includes cage wash and carcass

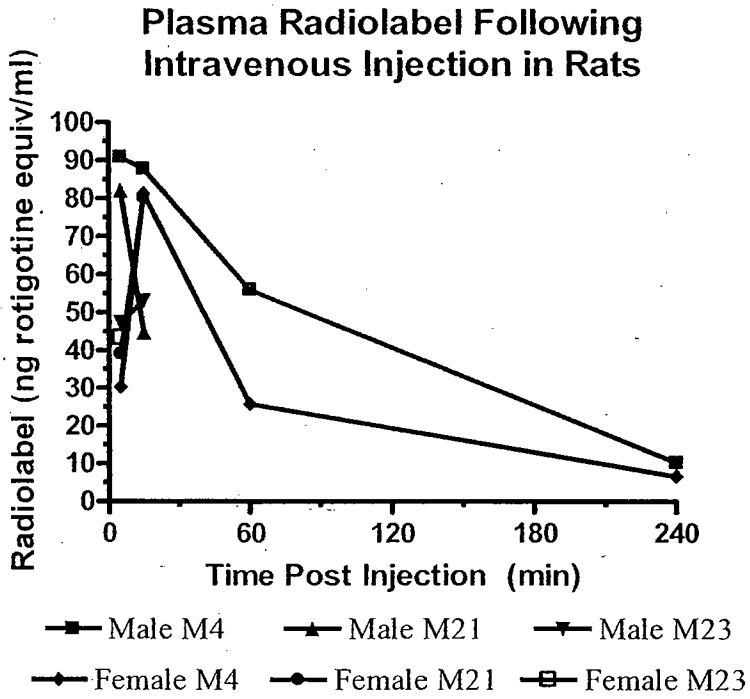
Figure 32, from page 121 of Report — -1028

Mean cumulative recovery of radioactivity following a single subcutaneous administration of [^{14}C] SPM 962 to male and female rats at a target dose of 3 mg/kg (Phase 2.4)



Note: Total includes cage wash and carcass

Figure 33, from page 124 of Report — -1028



M4=Desthienylethyl despropyl rotigotine sulphate
M21=Despropyl rotigotine sulphate
M23=Rotigotine sulphate

Note: M23 was detected at only one time point in female rats.

2.6.4.6 Excretion
See page 59.

2.6.4.7 Pharmacokinetic drug interactions

2.6.4.8 Other Pharmacokinetic Studies

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

See Page 201 of Overall Conclusions and Recommendations

2.6.6.2 Single-dose toxicity

2.6.6.2.1 An Investigation of the Effect of Nine Novel Compounds in the Irwin Dose-Range Study in the Mouse

Study — 9/881429

The rotigotine results of this non-GLP study are presented below.

Intravenous administration of 100 and 50 mg/kg caused the death of all mice treated. Death occurred immediately following drug administration and was preceded by clonic convulsions. Clonic convulsions were similarly provoked by administration of 25 mg/kg causing the death of two mice immediately following injection and a third within 15 minutes.

The convulsions were associated with irregular "gasping" respiration. No other consistent signs were noted.

Mice receiving the lowest dose 10 mg/kg showed evidence of CNS stimulation as shown by increased locomotor activity, alertness, touch response, pilo-erection and vocalisation. Changes in body carriage and gait were also recorded.

No further deaths were recorded in the 7-day post-dose observation period.

Figure 34, from page 8 of Report — 9881429

2.6.6.3 Repeat-dose toxicity

2.6.6.3.1 3-Month MTD/DRF Study of SPM 962 By Subcutaneous Administration to CD-1 Mice

Study no.: — Report No. 12015/99
Volume #, and page #:
Conducting laboratory and location: ———

Date of study initiation: May 4, 1999
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0, 3, 10, 30, 60 (raised to 90 at start of week 8) mg/kg every second day
Species/strain: Mouse, CD-1 (— CD-1(ICR)BR)
Number/sex/group or time point (main study): 10/sex/dose
Route, formulation, volume, and infusion rate: Subcutaneous injections rotated among three areas on the back
Satellite groups used for toxicokinetics/prolactin: 15/sex/dose
Age: 29 days
Weight: 15.5-18 grams

Results:

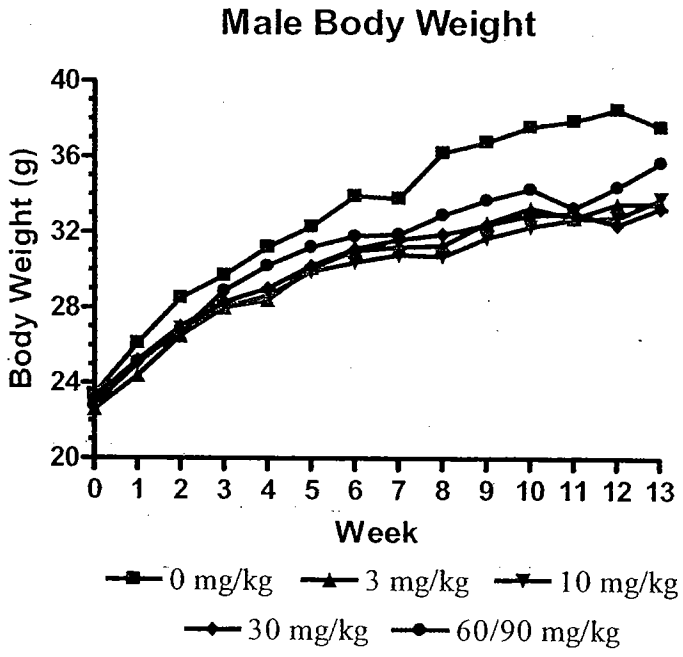
Mortality:
No deaths were observed in rotigotine treated mice. One male control mouse was found dead on Day 81 of the study

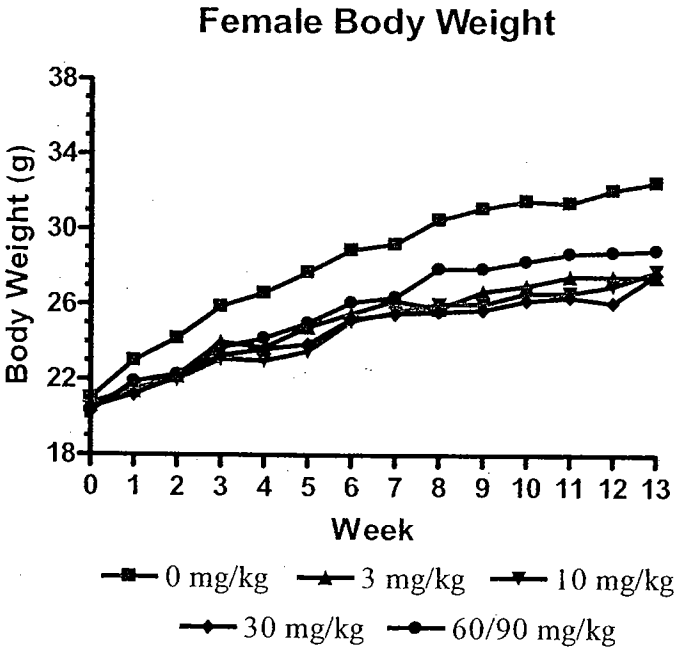
Clinical signs:
Restlessness was observed at 30 and 60/90 mg/kg. Incidence of restlessness increased after the dose was increased to 90 mg/kg.

Body weights:
Decreased body weight was observed at all doses, but all doses appeared equally affected.

Comparison of Week 13 Body Weight data in g (% of control)

Sex	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	60/90 mg/kg
Male	37.6 (100%)	33.5 (89%)	33.8 (90%)	33.3 (89%)	35.7 (95%)
Female	32.5 (100%)	27.5 (85%)	27.8 (86%)	27.6 (85%)	28.9 (89%)


Male Body Weight
Body Weight (g)
40
36
32
28
24
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Week
0 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg
60/90 mg/kg


Female Body Weight
Body Weight (g)
38
34
30
26
22
18
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Week
0 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg
60/90 mg/kg

Food consumption:
Increased relative and absolute food consumption compared to controls was observed at all doses. The effect was most pronounced at 3 mg/kg (about 20% increase in absolute food consumption).

Ophthalmoscopy: Pre, Week 13
No adverse effects were observed.

EKG: Not done

Hematology: Week 13, 5/sex/group
Hemoglobin (Hg), Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), platelets, White Blood Cell Count (WBC) and differential, Reticulocytes

Mean Hematology Parameters (% of control)

	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	60/90 mg/kg
Males					
Hemoglobin (mmol Fe/l)	9.73	9.18 (94%)	9.10 (94%)	7.20 (74%)	**8.32 (86%)**
Erythrocytes (10^{12}/l)	8.88	8.58 (97%)	8.30 (93%)	6.74 (76%)	7.85 (88%)
Hematocrit (%)	40.5	36.2 (89%)	36.0 (89%)	**29.0 (72%)**	**34.0 (84%)**
Thromboplastin Time (Seconds)	8.65	**9.40 (109%)**	**9.62 (111%)**	**10.06 (116%)**	**10.14 (117%)**
Partial Thromboplastin Time (Seconds)	23.95	24.44 (102%)	23.84 (100%)	26.60 (111%)	26.06 (109%)
Females					
Hemoglobin (mmol Fe/l)	9.94	9.46 (95%)	8.82 (89%)	9.64 (97%)	8.16 (82%)
Erythrocytes (10^{12}/l)	8.94	8.84 (99%)	8.10 (91%)	8.82 (99%)	7.72 (86%)
Hematocrit (%)	40.8	38.6 (95%)	35.2 (86%)	38.4 (94%)	33.6 (82%)
Thromboplastin Time (Seconds)	9.00	**10.32 (115%)**	**10.06 (112%)**	9.48 (105%)	9.58 (106%)
Partial Thromboplastin Time (Seconds)	24.00	25.30 (105%)	26.58 (111%)	**28.82 (120%)**	25.38 (106%)

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

Clinical chemistry: Week 13, 5/sex/group
Blood Urea Nitrogen (BUN), Creatinine, Glucose (Glu), Alkaline Phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Total Protein (TP), Albumin, Globulin, Albumin/Globulin ratio (A/G), Bilirubin (Bili), Cholesterol, Calcium, Sodium, Potassium, Chloride

Mean Clinical Chemistry Parameters (% of control)

	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	60/90 mg/kg
Males					
Albumin (g/l serum)	32.88	32.9 (100%)	**29.22 (89%)**	30.06 (91%)	28.4 (86%)
Cholesterol (mmol/l plasma)	3.638	3.326 (91%)	**2.690 (74%)**	**2.444 (67%)**	**2.382 (65%)**
BUN (mmol/l plasma)	9.624	15.398 (160%)	15.966 (166%)	15.890 (165%)	**18.104 (188%)**
Triglycerides (mmol/l plasma)	0.474	0.494 (104%)	**0.356 (75%)**	0.420 (89%)	**0.230 (49%)**
ALT (U/l)	19.6	**39.2 (200%)**	**54.0 (276%)**	**36.4 (186%)**	**44.2 (226%)**
AST (U/l)	51.4	**115.8 (225%)**	**276.4 (538%)**	**120.2 (234%)**	**150.8 (293%)**
LDH (U/l)	201.2	**530.6 (264%)**	**925.4 (460%)**	**460.8 (229%)**	**518.2 (258%)**
Females					
Albumin (g/l serum)	34.52	34.24 (99%)	30.84 (89%)	**30.66 (89%)**	29.34 (85%)
Cholesterol (mmol/l plasma)	2.520	2.268 (90%)	**1.954 (78%)**	**1.926 (76%)**	**2.130 (85%)**
BUN (mmol/l plasma)	8.378	10.190 (122%)	12.716 (152%)	**12.620 (151%)**	**15.860 (189%)**
Triglycerides (mmol/l plasma)	0.514	0.376 (73%)	0.420 (82%)	**0.280 (54%)**	**0.222 (43%)**
ALT (U/l)	24.8	**36.4 (147%)**	**40.0 (161%)**	**43.0 (173%)**	**39.2 (158%)**
AST (U/l)	74.8	**164.2 (220%)**	**177.2 (237%)**	**145.4 (194%)**	**144.2 (193%)**
LDH (U/l)	338.6	615.4 (182%)	**575.8 (170%)**	558.6 (165%)	789.4 (233%)

30 mg/kg male mouse #9 had bilirubin of 8.6 uM (compared to 3.6 uM in controls).

Urinalysis: Not done

Gross pathology:
90 mg/kg- injection site edema in 7/10 male mice after week 11 (after three weeks at 90 mg/kg)
90 mg/kg- inflammation in 2/10 female mice during weeks 12 and or 13, resulting in necrosis in one mouse at the injection site
90 mg/kg- hemorrhagic fluid in peritoneal cavity in 5/10 males and 4/10 females

Organ weights
Adrenal glands, Brain, Heart, Kidney, Liver, Lung, Ovary, Prostate, Testes, Spleen, Thymus, Uterus
Pituitary was not weighed

No effects

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( )

Control and test substance-treated animals revealed the following changes at the subcutaneous injection site: mild to marked chronic granulation tissue with fluid filled blisters, oedemas, mixed cell infiltrations, a moderate fibrosis, neutrophilic granulocytes, macrophages and giant cells with large vacuoles and focal dystrophic mineralization in necrotic areas were noted in control and substance-treated mice.
There was no difference in the incidence and severity of changes after treatment with SPM 962 and its placebo.

Figure 35, from page 255 of Report — 12015/99

No significant systemic histopathology changes were observed.

Toxicokinetics:
SPM 962 drug levels in ng/ml

Dose	Sex	6 Hr		24 Hr		48 Hr	
		Day 3	Day 90	Day 3	Day 90	Day 3	Day 90
3 mg/kg	M	9.33	8.07	1.13	2.18	0.20	0.76
	F	8.36	4.59	1.13	1.29	0.30	0.42
10 mg/kg	M	19.54	15.43	3.61	6.71	1.34	3.30
	F	23.90	13.76	5.40	6.14	1.36	2.08
30 mg/kg	M	42.76	29.88	16.78	17.17	6.89	10.41
	F	44.50	39.32	19.04	20.46	7.56	10.09
60 mg/kg	M	82.80		41.02		18.20	
	F	78.46		40.68		17.30	
90 mg/kg	M		105.14		57.14		35.58
	F		97.86		71.66		38.30

Other: Day 3, Day 90

Table 4: Concentration of Prolactine in Male Mice (ng/ml); (n=5)

		before administration		day 3; 6 h p.a.		week 13; 6 h p.a.	
Group	mg/kg	ng/ml	CV (%)	ng/ml	CV (%)	ng/ml	CV (%)
I	0	8.65	9.1	9.66	17.9	6.32	20.8
II	3	7.71	8.0	4.02	18.0	4.80	10.7
III	10	9.82	19.8	4.60	26.3	4.33	21.6
IV	30	6.95	18.2	4.19	21.8	4.45	6.6
V	90	6.54	12.8	3.74	10.6	3.82	13.1

Table 5: Concentration of Prolactine in Female Mice (ng/ml); (n=5)

		before administration		day 3; 6 h p.a.		week 13; 6 h p.a.	
Group	mg/kg	ng/ml	CV (%)	ng/ml	CV (%)	ng/ml	CV (%)
I	0	13.57	40.5	13.80	28.6	12.17	30.4
II	3	22.34	58.2	7.93	44.6	11.30	42.9
III	10	17.34	27.4	14.84	25.4	16.55	38.1
IV	30	14.35	27.6	11.15	38.7	11.56	53.1
V	90	14.40	30.0	3.70	28.6	15.78	23.7

Figure 36, from page 743 of Report — 12015/99

Key study findings:

1. All dose levels caused a greater than 10% decrease in body weight compared to controls. These changes were not related to altered food consumption since relative and absolute food consumption was increased in treated mice, with the greatest increase observed at 3 mg/kg.
2. Clinical signs included increased restlessness at 90 mg/kg; sporadic increases in restlessness was observed at 30 and 60 mg/kg.
3. Sporadic alterations in hematology and clinical chemistry values were observed. Increases in liver enzyme (ALT, AST) values were observed, however the alterations did not follow a dose response relationship and there were no histopathological effects in the liver of the 30 mg/kg and 60/90 mg/kg mice. It should be noted that the greatest increase in liver enzymes were observed at the two low doses (3 and 10 mg/kg). The decrease in hematocrit observed in high dose mice may be related to the peritoneal cavity hemorrhages observed in this group.
4. Gross pathological lesions were generally confined to the injection site in the high dose group (60/90 mg/kg). In particular, injection site edema was observed in 7/10 male mice after the increase of the high dose to 90 mg/kg. In addition, inflammation was observed in 2/10 female mice resulting in necrosis in one mouse. The only systemic effect noted was hemorrhagic fluid in the peritoneal

cavity in 5/10 male and 4/10 females in the 60/90 mg/kg dose group. No source of the hemorrhagic fluid was identified. Non specific alterations were observed at the injection site of all treatment groups and controls.

5. There were no significant effects on organ weights or histopathology.
6. There are minimal data on the toxicokinetics of SPM 962 in mice. Only 6, 24, and 48 hour data were available. The six hour blood levels were lower on Day 90 than on Day 2. On the other hand the 24- and 48-hour blood levels were increased on Day 90 relative to Day 2.
7. Decreased prolactin levels were observed in males on day 3 and week 13 in all rotigotine treated males. On the other hand, decreased prolactin was only observed in the high dose females (60 mg/kg) on Day 3. No changes in prolactin levels were observed during week 13 in female mice.

APPEARS THIS WAY
ON ORIGINAL

2.6.6.3.2 28-Day Dermal Toxicity Study in Rats

Study no.: 0436RD15.001
Volume #, and page #: 0436rd15001-study-report.pdf
Conducting laboratory and location:
Date of study initiation: September 13, 1996
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 8291646, 8291596

Methods

Doses: 0, 1.7 mg/day (estimated)
Species/strain: Rat, Sprague-Dawley
Number/sex/group or time point (main study): 5/sex/dose
Route, formulation, volume, and infusion rate: Dermal patch
Satellite groups used for toxicokinetics or recovery: 12/sex
Age: 6 weeks
Weight: 151-190 g (males); 127-154 g (females)
Sampling times:
Unique study design or methodology (if any):

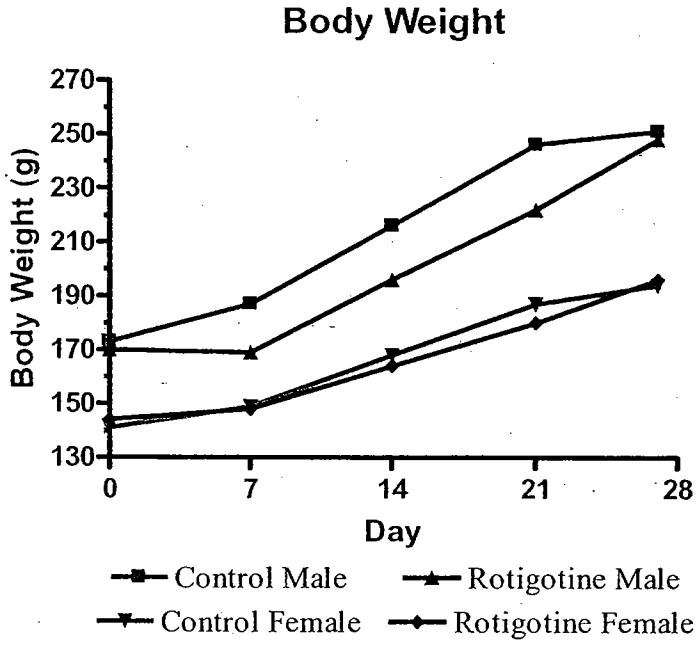
The patch consisted of a 10 cm^2 adhesive overlay with a 5 cm^2 matrix containing 8.4 mg rotigotine or placebo. The patch was applied to shaved skin for 22 hours/day and secured with an ace bandage and — tape. Dose estimate assumes rotigotine absorption at a rate of 0.076 mg/hr. Number of application sites was not specified. Based on average body weights over the study period, the doses are 8.5 and 10 mg/kg/day for males and females, respectively.

Results:

Mortality:
None.

Clinical signs: 2X/day
No compound related effects were observed.
Patch administration was associated with slight to moderate irritation in both control and treated rats.

Body weights: 1X/week
About a 10% decrease in body weight was observed in male rotigotine treated rats on days 7, 14 and 21. Day 27 male body weights were about the same. No effects were observed on female body weights.



Food consumption: 1X/week
No significant effects

Ophthalmoscopy: Pre, Day 28
No effects

EKG: Not done

Hematology: Day 28
Hemoglobin (Hg), Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), platelets, White Blood Cell Count (WBC) and differential, Reticulocytes

No effects

Clinical chemistry: Day 28
Blood Urea Nitrogen (BUN), Creatinine, Glucose (Glu), Alkaline Phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Total Protein (TP), Albumin, Globulin, Albumin/Globulin ratio (A/G), Bilirubin (Bili), Cholesterol, Calcium, Sodium, Potassium, Chloride

No effects

Urinalysis: Not done

Gross pathology:

No effects

Organ weights (specify organs weighed if not in histopath table):
Adrenal glands, Brain, Heart, Kidney, Liver, Ovary, Testes

No effects

Histopathology: Adequate Battery: yes ( ), no (X)— the complete battery of tissues were collected and preserved, but slides were prepared only for the skin and underlying structures.

Peer review: yes ( ), no (X)

No effects

Toxicokinetics: 3 rats/sex/timepoint: Days 1, 18- 1, 6 and 22 hours post application, Days 7, 14- 22 and 24 hours post application.

All Day 1 plasma levels were below the limit of quantification (<0.1 ng/ml) in males. On Day 1, Rotigotine was not detected in females at 1 hour post dose. One out of three females had detectable rotigotine at 6 hours — ng/ml) and three out of three females had detectable rotigotine at 22 hours — ng/ml; mean = 1.9 ng/ml).

Rotigotine concentrations (ng/ml) at various times post administration

	Day 7		Day 14		Day 18		
Time (hr)	22	24	22	24	1	6	22
Males	38.3	5.0	59.1	5.3	26.2	60.3	42.7
Females	46.6	6.9	66.3	8.1	29.8	71.8	36.4

Other:

Key study findings:

1. Decreased body weight was observed in males on days 7, 14 and 21, but not on day 27. No effects were observed on female body weights.
2. No other adverse effects were observed in this study, although only limited histopathological examinations were conducted.
3. Rotigotine was rapidly cleared from the circulating plasma following removal of the patch.

APPEARS THIS WAY
ON ORIGINAL

2.6.6.3.3 6-Month Chronic Toxicity Study of SPM 962 by Subcutaneous Administration to Sprague-Dawley Rats

Study no.: **Report 12482/99**
Location: **1248299-study-report.pdf**
Conducting laboratory and location:

Date of study initiation: **November 2, 1999**
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 99090189, 99100191

Methods

Doses: 0, 0.5, 2.5, 12.5 mg/kg/48 hours
Species/strain: Rat, Sprague-Dawley
Number/sex/group or time point (main study): 20/sex/dose
Route, formulation, volume, and infusion rate: subcutaneous injection
Satellite groups used for 8 week recovery: 5/sex/dose
Satellite groups used for Prolactin Analysis: 6/sex/dose
Age: 30 days (males), 32 days (females)
Weight: 119-150 g (males) 113-140 g (females)
Sampling times:
Unique study design or methodology (if any):
Vehicle is an oily suspension designed to yield sustained plasma levels

Results:

Mortality:
12.5 mg/kg- 1 male (#171) on Day 123 and 1 female (#186) on day 77
male rat #171 had purulent nephritis and abscesses in the lungs and heart.
Female rat #186 had focal ulceration in the stomach; no specific cause of death was evident.

No deaths were observed in the other dose groups.

Clinical signs: 1X/day
No clinical signs were observed in control or 0.5 mg/kg groups.
At 2.5 mg/kg, restlessness and increased water intake were observed starting on day 29 in all animals; clinical signs persisted until day 7 of recovery.
At 12.5 mg/kg, restlessness was observed starting on Day one and increased water intake was observed at 2.5 mg/kg starting on day 29 in all animals clinical signs persisted until day 7 of recovery.

Body weights: 1X/week
Decreased body weight was observed at 2.5 and 12.5 mg/kg. No effects were noted at 0.5 mg/kg.

Dosage (mg/kg)	Body weight: Difference to the control group			
	male animals		female animals	
	TW 1 - 13	TW 14 - 26	TW 1 - 13	TW 14 - 26
2.5	-3% to -7%*	-6% to -9%*	-4% to -9%*	-8% to -12%*
12.5	-6%* to -14%*	-13%* to -18%*	-7%* to -15%*	-14%* to -18%*

* = significantly different from the controls ($p \leq 0.01$)

Figure 37, from page 43 of Report 12482/99

Figure 1 Body weight of male animals
mean values per group

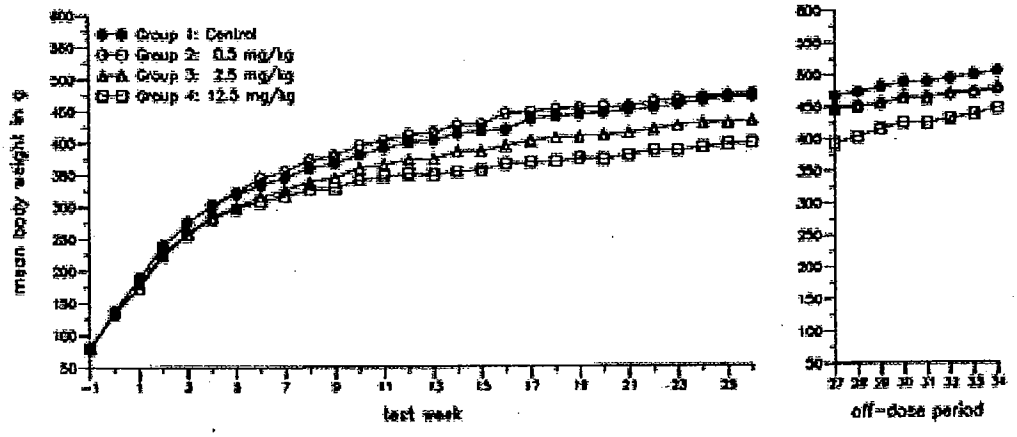


Figure 38, from page 44 of Report 12482/99

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

Figure 2 Body weight of female animals
mean values per group

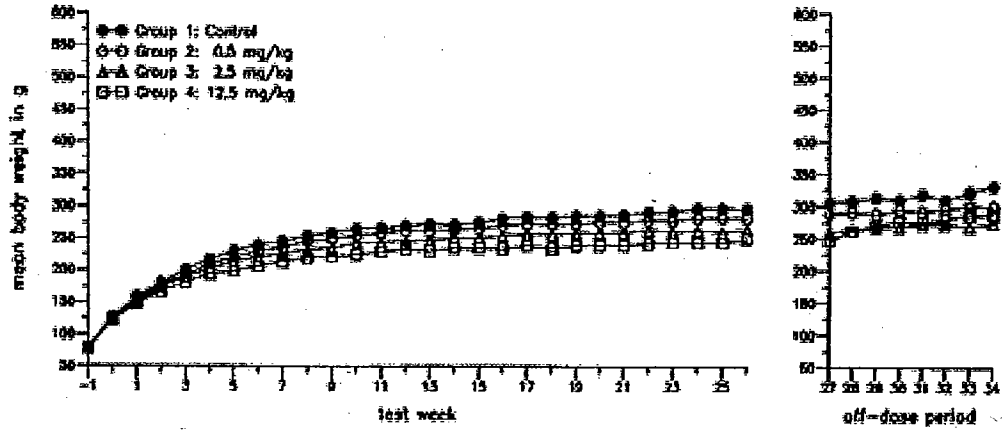


Figure 39, from page 45 of Report — 12482/99

Food consumption: 1X/week
Increased food and water consumption was observed at 12.5 mg/kg. This increase was attributed to spillage due to hyperactivity.

Ophthalmoscopy: Pre, Treatment Week 26, Recovery Week 8
No adverse effects were noted.

EKG: Not done

Hematology: Weeks 6, 13, 26 and 34 (recovery)
Hemoglobin (Hg), Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), platelets, White Blood Cell Count (WBC) and differential, Reticulocytes

No biologically significant effects were observed.

Clinical chemistry: Weeks 6, 13, 26 and 34 (recovery)
Blood Urea Nitrogen (BUN), Creatinine, Glucose (Glu), Alkaline Phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Total Protein (TP), Albumin, Globulin, Albumin/Globulin ratio (A/G), Bilirubin (Bili), Cholesterol, Calcium, Sodium, Potassium, Chloride

Alterations were noted in a variety of clinical chemistry parameters. The changes were reversible upon discontinuation of treatment (week 34 data not presented).

Parameter	Week	Sex	0 mg/kg	0.5 mg/kg	2.5 mg/kg	12.5 mg/kg
Cholesterol mmol/l plasma (% of control)	13	M	1.813	1.880 (104%)	1.706 (94%)	1.482 (82%)
		F	2.364	2.150 (91%)	**1.120 (47%)**	**0.542 (23%)**
	26	M	2.357	2.453 (104%)	2.051 (87%)	1.863 (79%)
		F	2.844	2.394 (84%)	**0.973 (34%)**	**0.532 (19%)**
Triglycerides mmol/l plasma (% of control)	13	M	0.501	0.420 (84%)	**0.288 (57%)**	**0.309 (62%)**
		F	0.327	0.286 (87%)	**0.204 (62%)**	**0.196 (60%)**
	26	M	0.460	0.435 (95%)	0.356 (77%)	0.438 (95%)
		F	0.526	0.472 (90%)	**0.312 (59%)**	**0.307 (58%)**
BUN mmol/l plasma (% of control)	6	M	5.387	5.447 (101%)	6.360 (118%)	6.269 (116%)
		F	5.978	6.097 (102%)	5.950 (99%)	7.054 (118%)
	13	M	5.610	5.885 (105%)	5.868 (105%)	6.221 (111%)
		F	5.424	5.977 (110%)	6.498 (120%)	**7.086 (131%)**
	26	M	4.907	5.173 (105%)	5.206 (106%)	5.675 (116%)
		F	5.295	5.577 (105%)	5.789 (109%)	**6.614 (125%)**
Calcium mmol/l plasma[3] (% of control)	13	M	2.660	2.641 (99%)	2.607 (98%)	**2.514 (95%)**
		F	2.722	2.702 (99%)	**2.599 (95%)**	**2.576 (95%)**
	26	M	2.550	2.626 (103%)	2.631 (103%)	2.516 (99%)

		F	2.569	2.636 (103%)	2.682 (104%)	2.520 (98%)
Alkaline Phosphatase U/l plasma (% of control)	6	M	204.4	209.2 (102%)	202.9 (99%)	236.4 (116%)
		F	157.2	145.6 (93%)	154.8 (98%)	217.4 (138%)
	13	M	134.1	148.6 (111%)	136.2 (102%)	169.6 (126%)
		F	69.1	77.6 (112%)	96.0 (139%)	**129.0 (187%)**
	26	M	102.0	106.5 (104%)	95.5 (94%)	120.2 (118%)
		F	45.6	55.9 (123%)	59.9 (131%)	**85.7 (188%)**
AST U/l plasma (% of control)	13	M	48.1	55.1 (115%)	59.1 (123%)	61.4 (128%)
		F	49.0	48.5 (99%)	**82.1 (168%)**	**77.9 (159%)**
	26	M	49.0	50.4 (103%)	55.2 (113%)	57.9 (118%)
		F	48.5	53.4 (109%)	**76.1 (155%)**	**81.9 (167%)**

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

Notable individual alterations included:

12.5 mg/kg- Male rat 159 had elevated ALT (110 vs 24 in control) at week 6
Female rat 184 had elevated ALT (66 vs 26 in control) at week 26
Male rats 153 and 160 had elevated CPK (103 and 439 vs 43 in controls) at week 13
Female rat 176 had elevated CPK (111 vs 39 in controls) at 13 weeks

2.5 mg/kg- Male rat 107 had elevated CPK (201 vs 43 in controls) at 13 weeks
Female rat 134 had elevated CPK (156 vs 39 in controls) at 13 weeks

0.5 mg/kg- Male rat 56 had elevated ALT at weeks 13 and 26 (week 13: 131 vs 27 in control; week 26: 61 vs 29 in control)

Serum Prolactin Concentrations: Weeks Pre, 1, 6, 13, 26
For prolactin serum measurements, 3 rats/sex/timepoint were sampled at either 2 hours post dose or 4 hours post dose. Samples were frozen and later thawed for analysis by radioimmunoassay. Statistical comparisons were not made due to the small number of samples per timepoint.

Mean Prolactin Levels in ng/ml (SEM) in Male Rats

Dose	Day -1	Day -1	Day 3	Day 43	Day 91	Day 181
Time post dose	2	4	2	4	2	4
0 mg/kg	24.1 (7.1)	8.1 (1.8)	16.2 (1.8)	26.7 (12.6)	39.2 (1.8)	13.4 (11.0)
0.5 mg/kg	17.1 (4.0)	15.8 (4.8)	0.4 (0.4)	6.2 (2.5)	4.4 (0.4)	1.6 (0.3)
2.5 mg/kg	30.0 (2.9)	19.4 (2.8)	0.5 (0.4)	2.0 (0.3)	3.6 (1.8)	0.0 (0.0)
12.5 mg/kg	12.3 (7.1)	20.0 (4.2)	0.0 (0.0)	2.2 (0.1)	1.8 (0.1)	0.0 (0.0)

Mean Prolactin Levels in ng/ml (SEM) in Female Rats

Dose	Day -1	Day -1	Day 3	Day 43	Day 91	Day 181
Time post dose	2	4	2	4	2	4
0 mg/kg	46.6 (5.1)	133.9 (44.1)	52.9 (18.8)	240.1 (161.6)	50.6 (41.0)	16.3 (1.7)
0.5 mg/kg	19.2 (9.9)	15.4 (0.5)	11.1 (6.7)	13.9 (9.4)	20.1 (15.1)	35.3 (26.8)
2.5 mg/kg	92.2 (84)	11.8 (0.6)	1.3 (1.3)	14.0 (8.1)	7.9 (3.8)	5.6 (1.7)
12.5 mg/kg	138.9 (84.5)	40.0 (22.5)	0.4 (0.7)	4.1 (1.2)	1.8 (0.2)	2.9 (0.2)

Urinalysis: Weeks 6, 13, 26 and 34 (recovery)

Parameter	Week	Sex	0 mg/kg	0.5 mg/kg	2.5 mg/kg	12.5 mg/kg
Sodium Excretion Mmol/l urine (% of control)	6	M	70.48	72.49 (103%)	102.35 (145%)	95.75 (136%)
		F	78.67	99.82 (127%)	110.81 (141%)	**181.10 (230%)**
	13	M	27.95	33.74 (121%)	60.27 (216%)	**66.99 (240%)**
		F	53.35	88.12 (165%)	84.90 (159%)	**133.31 (250%)**
	26	M	32.14	46.07 (143%)	**73.82 (230%)**	**81.47 (153%)**
		F	44.99	74.99 (167%)	80.64 (179%)	110.58 (246%)
Chloride Excretion Mmol/l urine (% of control)	6	M	84.68	76.57 (90%)	101.10 (119%)	78.43 (93%)
		F	72.96	79.20 (108%)	99.61 (137%)	**144.55 (198%)**
	13	M	58.28	52.73 (90%)	68.42 (117%)	86.92 (149%)
		F	65.33	83.93 (128%)	86.77 (133%)	**123.10 (188%)**
	26	M	65.64	54.79 (83%)	61.14 (93%)	96.57 (147%)
		F	51.87	66.24 (128%)	74.79 (144%)	**121.43 (234%)**

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

Urine volume and specific gravity were unaffected by treatment.
No effects were observed at recovery.

Gross pathology:
Increased ovary size was noted in 15/20 females at 2.5 mg/kg and 20/20 females at 12.5 mg/kg. No effects were observed at recovery.

Organ weights:
Adrenal glands, Brain, Heart, Kidney, Liver, Lung, Ovary, Pituitary, Prostate, Testes, Spleen, Thymus, Uterus

Relative Organ Weights in g/kg body weight (percent of control)

Organ	Sex	0 mg/kg	0.5 mg/kg	2.5 mg/kg	12.5 mg/kg
Adrenal	M	0.178	0.140 (79%)	0.166 (93%)	0.167 (94%)
	F	0.287	0.296 (103%)	**0.361 (126%)**	**0.424 (148%)**
Brain	M	4.83	4.64 (96%)	5.12 (106%)	**5.36 (111%)**
	F	7.07	7.21 (102%)	7.70 (109%)	**7.93 (112%)**
Ovaries	F	0.546	0.637 (117%)	**1.522 (278%)**	**2.072 (379%)**

Values in **Bold** significantly different from control.

No effects observed at recovery

Histopathology: Adequate Battery: yes (X), no ( ) explain: High and control dose only
Peer review: yes ( ), no (X)
The injection sites were characterized by fluid filled blisters with fibrosis around the blister. A granulomatous response was observed in about half of the injections sites.

Organ	Sex	0 mg/kg	0.5 mg/kg	2.5 mg/kg	12.5 mg/kg
Eye, Retinal Degeneration	M	0/20	---	---	3/20
	F	0/20	---	---	1/19
Lung, Hemorrhage	M	0/20	---	---	5/20
	F	1/20	---	---	2/19
Lung, Foamy Macrophages	M	2/20	---	---	3/20
	F	0/20	---	---	7/20
Urinary Bladder, Proteinaceous content	M	7/20	---	---	17/20
	F	0/20	---	---	0/20
Ovary, Increased Enlarged Corpora Lutea	F	1/20	0/20	16/20	19/19

12.5 mg/kg male Rat 161 had marked degeneration of the germinating epithelium and moderate Leydig cell hyperplasia.
Severity of enlarged corpora lutea increased with dose.
No significant effects were noted in the uterus.

No effects noted on recovery animals.
Toxicokinetics: Days 3, 43, 91 and 181; 2, 4, 8, 24, 32 and 48 hours post dose

Descriptive data were presented as combined male and female values. Inspection of male and female individual data suggests that male and female values were generally comparable.

Mean AUC(2-48) in ng-hr/ml

	Day 3	Day 43	Day 91	Day 181
0.5 mg/kg	30	42	38	39
2.5 mg/kg	130	154	165	216
12.5 mg/kg	648	866	1,052	1,117

Mean Css in ng/ml

	Day 3	Day 43	Day 91	Day 181
0.5 mg/kg	0.65	0.91	0.83	0.85
2.5 mg/kg	2.83	3.35	3.59	4.70
12.5 mg/kg	14.08	18.83	22.86	24.28

Mean Cmax in ng/ml

	Day 3	Day 43	Day 91	Day 181
0.5 mg/kg	1.91	2.28	2.51	2.76
2.5 mg/kg	10.95	5.58	6.98	10.68
12.5 mg/kg	41.40	25.07	48.40	36.02

Other:

APPEARS THIS WAY
ON ORIGINAL

2.6.6.3.4 Drug Interaction Study Between SPM 962 and a Combination of L-Dopa and Carbidopa in CD Rats

Study no.: Report 16083/02
Location: 1608302-study-report.pdf
Conducting laboratory and location:

Date of study initiation: May 21, 2003
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 20107016, 20107058, 20107057

Methods

Doses: see table below
Species/strain: Rat, CD / CD
Number/sex/group or time point (main study): see table below
Route, formulation, volume, and infusion rate: see table below
Satellite groups used for toxicokinetics or recovery: see table below
Age: 8 weeks
Weight: 246-312 g (males) 202-249 g (females)
Sampling times:
Unique study design or methodology (if any):
Vehicle is an oily suspension designed to yield sustained plasma levels

APPEARS THIS WAY
ON ORIGINAL

Treatment schedule

Group	SPM 962 dose (administration: every second day from days 22 to 112)		L-dopa and Carbidopa dose (administration: daily from days 1 to 112)	No. of animals and sex
	[mg/kg b.w./48h] s.c.	Volume [mL/kg b.w./48h] (concentration used)	[mg/kg b.w./day] at 2.4 mL/kg b.w. p.o.	MS+(SA_1+SA_2)+ RP
1	0 (Control)	1 SPM 962 placebo (0%)	0 (Vehicle for L-dopa and Carbidopa)	15 + 5 m 15 + 5 f
2	0 (Placebo)	1 SPM 962 placebo (0%)	120/30	15 + (9 + 3) + 5 m 15 + (9 + 3) + 5 f
3	1	0.2 (0.5%)	120/30	15 m 15 f
4	3	0.6 (0.5%)	120/30	15 m 15 f
5	10	1 (1%)	120/30	15 + (9 + 3) + 5 m 15 + (9 + 3) + 5 f
6	10	1 (1%)	0 (Vehicle for L-dopa and Carbidopa)	15 + (9 + 3) + 5 m 15 + (9 + 3) + 5 f

MS: main study
RP: recovery period
SA_1: satellite animals for determination of the L-dopa and Carbidopa plasma levels
SA_2: satellite animals for determination of the SPM 962 plasma levels
m: male
f: female

Figure 40, from page 37 of Report — 1608302

Results:

Mortality:

Group 5 (HD rotigotine + Sinemet)- 1 male (test day 182) and 5 females (test day 27, 27, 76, 57, 113) died or were sacrificed moribund. The deaths on Day 27 occurred after the third rotigotine injection.

No other deaths were observed.

Clinical signs: 1X/day

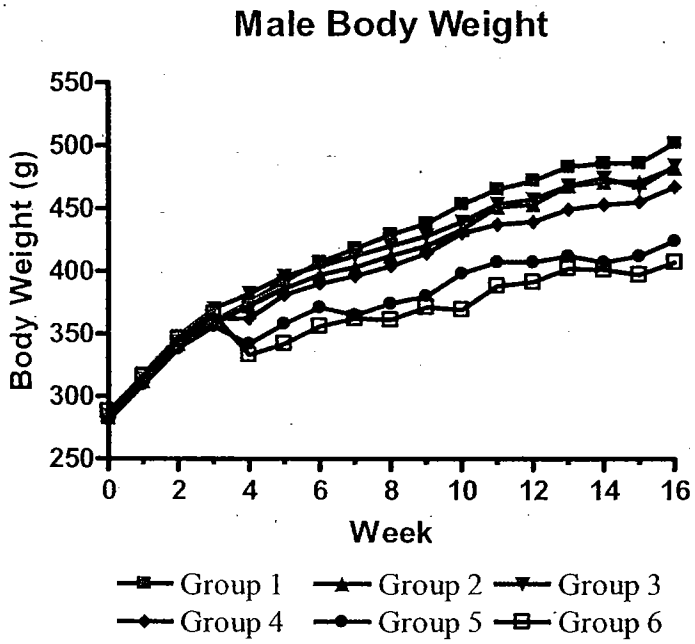
No clinical signs were observed during the first four weeks of the study in any group. During Weeks 5 and 6 (Day 29) piloerection was observed in all treated animals. Rough fur was observed in all group 6 rats (males and females).

Wet bedding material and restlessness were observed in groups 3-6.

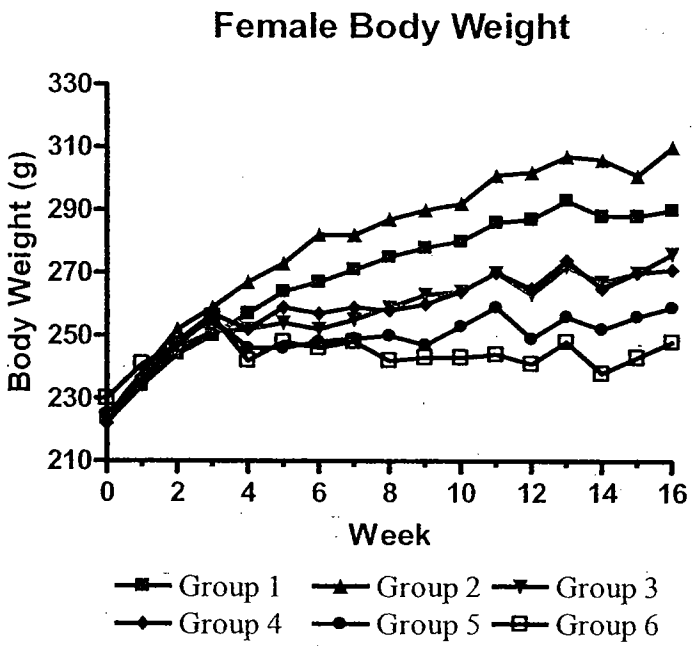
Body weights: 1X/week

Comparison of Week 16 Body Weights in g (% of control)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Males	502 (100%)	482 (96%)	484 (96%)	467 (93%)	424 (84%)	407 (81%)
Females	290 (100%)	310 (107%)	276 (95%)	271 (93%)	259 (89%)	248 (86%)



APPEARS THIS WAY
ON ORIGINAL



Food consumption: 1X/week
No significant effects

Ophthalmoscopy: Pre, Treatment Week 16, Recovery Week 4
No effects

EKG: Not done

Hematology: Weeks 16, Recovery Week 4
Hemoglobin (Hg), Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), platelets, White Blood Cell Count (WBC) and differential, Reticulocytes

No effects

Clinical chemistry: Weeks 16, Recovery Week 4
Blood Urea Nitrogen (BUN), Creatinine, Glucose (Glu), Alkaline Phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Total Protein (TP), Albumin, Globulin, Albumin/Globulin ratio (A/G), Bilirubin (Bili), Cholesterol, Calcium, Sodium, Potassium, Chloride, triglycerides

Male Week 16 Mean Clinical Chemistry Parameters (% of control)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Triglycerides (mmol/L)	0.527	0.486	0.411	0.419	0.451	0.382
BUN (mmol/L)	7.6	9.6	9.4	8.1	9.1	8.5
AST (U/L)	78	72	96	102	177	105
CPK (U/L)	150	109	118	119	122	105

Female Week 16 Mean Clinical Chemistry Parameters (% of control)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Triglycerides (mmol/L)	0.585	0.399	0.409	0.291	0.238	0.236
BUN (mmol/L)	9.0	9.1	9.9	11.5	**13.4**	11.1
ALT (U/L)	48	36	39	46	55	88
AST (U/L)	87	97	138	**211**	**239**	**219**
CPK (U/L)	114	102	169	221	287	492
LDH (U/L)	47	53	61	116	**178**	150

Group 5 Male rat #143 had elevated BUN (15.0 mmol/L versus 7.6 mmol/L in controls), ALT (161 U/l versus 43 U/l in controls), AST (859 U/L versus 59 in controls) and LDH (315 U/L versus 56 in controls)

Group 6 female rat #210 had increased ALT (228 U/L versus 48 U/L in controls)

No effects were observed at recovery.

Urinalysis: Weeks 6, 15 and 20 (recovery)
No significant effects

Gross pathology:
Subcutaneous blisters are the injection site in all animals.
No other significant effects.

Organ weights:
Adrenal glands, Brain, Heart, Kidney, Liver, Lung, Ovary, Pituitary, Prostate, Testes, Spleen, Thymus, Uterus

Male Organ Weight Changes in g/kg BW (% of control)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Adrenal	0.259	0.247	0.195	0.211	0.211	0.230
Pituitary	0.030	0.027	0.051	0.025	0.027	0.029
Liver	33.60	36.42	34.95	38.70	39.85	33.31
Kidney	7.593	7.608	7.965	8.068	7.557	7.328

Female Organ Weight Changes in g/kg BW (% of control)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Adrenal	0.476	0.371	0.431	0.442	0.251	0.516
Pituitary	0.064	0.055	0.057	0.045	0.044	0.053
Liver	35.11	37.17	40.66	42.83	44.00	30.28
Kidney	7.502	7.596	8.002	7.639	7.901	7.886
Ovary	0.488	0.481	0.560	0.888	1.244	1.357
Uterus	3.512	3.702	4.358	3.846	6.043	8.103

Histopathology: Adequate Battery: yes (X), no ( ) explain
Peer review: yes ( ), no (X)
Groups 1, 2, 5 and 6 were the only groups examined histopathologically.

Group 5 rat #143 had mild purulent pyelonephritis, but no liver histpathological alterations.

Male Histopathology Changes

	Group 1	Group 2	Group 5	Group 6
Liver, Peripheral Fatty Infiltration	0/15	0/15	0/15	6/15
Liver, Hepatocyte atrophy	0/15	0/15	0/15	1/15
Seminal vesicle, purulent vesculitis	0/15	0/15	4/15	0/15
Testes, Leydig Cell Hyperplasia	0/15	1/15	1/15	2/15

Additional cases of testicular Leydig cell hyperplasia was observed in single rats in Groups 3 and 4.
One Group 5 recovery rat had testicular Leydig cell hyperplasia
Injection sites had blisters, granulation tissue and fibrosis.

Toxicokinetics: Days 3, 43, 91 and 181; 2, 4, 8, 24, 32 and 48 hours post dose

Toxicokinetic parameters for Levodopa (calculated from mean data, n=3/sex/time)

Group	Day	Regimen	Males			Females		
			C_{max} [ng/mL]	t_{max} [h]	AUC_{last} [h ng/mL]	C_{max} [ng/mL]	t_{max} [h]	AUC_{last} [h ng/mL]
2	14	S	11580	0.25[a)]	53623	8573	1	32700
	22	S	9550	2	36254	11500	1	39435
	112	S	7170	0.5	21351	5273	1	16085
5	14	S	11053	1	46704	10340	1	34493
	22	$S+I_{sd}$	10720	0.25[a)]	19426	4113	0.25[a)]	6944
	112	$S+I_{rd}$	16880	0.25[a)]	24288	11253	0.25[a)]	15713

a) first sampling time

Toxicokinetic parameters for Carbidopa (calculated from mean data, n=3/sex/time)

Group	Day	Regimen	Males			Females		
			C_{max} [ng/mL]	t_{max} [h]	AUC_{last} [h ng/mL]	C_{max} [ng/mL]	t_{max} [h]	AUC_{last} [h ng/mL]
2	14	S	199	1	1030	395	1	1206
	22	S	153	1	499	408	1	951
	112	S	168	0.5	616	316	1	912
5	14	S	313	1	938	344	1	1199
	22	$S+I_{sd}$	228	0.25[a)]	263	130	0.5	231
	112	$S+I_{rd}$	657	0.5	871	572	0.5	653

a) first sampling time

Figure 41, from page 1402 of Report — 16083/02

Toxicokinetic parameters for SPM 962 (mean ± SD, n=3, median for t_{max})

Group	Day	Regimen	Males			Females		
			C_{max} [ng/mL]	t_{max} [h]	AUC_{24h} [h ng/mL]	C_{max} [ng/mL]	t_{max} [h]	AUC_{24h} [h ng/mL]
5	22	$S+I_{sd}$	11.2 ± 3.0	24	207 ± 61	10.8 ± 1.7	24	194 ± 29
	112	$S+I_{rd}$	22.4 ± 2.1	4	437 ± 30	17.7 ± 2.5	4	321 ± 18
6	22	I_{sd}	10.2 ± 5.9	24	178 ± 88	10.8 ± 0.3	24	189 ± 25
	112	I_{rd}	30.6 ± 14.2	4	428 ± 144	21.1 ± 13	4	395 ± 169

Figure 42, from page 1403 of Report — 12083/02

Key Study Findings:

1. Mortality was observed when 10 mg/kg rotigotine was administered with Sinemet.
2. Restlessness was observed in all rotigotine treated groups, but not in rats administered Sinemet alone.
3. Decreased body weight was observed in rats administered 10 mg/kg rotigotine with and without Sinemet.
4. No effects were observed on hematology parameters.
5. Increased AST was observed in rats administered 10 mg/kg rotigotine.
6. A dose dependent decrease in triglyceride levels was observed in rotigotine treated rats.
7. No significant histological changes were observed.
8. No interactions were observed on the toxicokinetics of rotigotine or Sinemet.

APPEARS THIS WAY
ON ORIGINAL

2.6.6.3.5 12-Month Chronic Toxicity Study of SPM 962 by Subcutaneous Administration to Cynomolgus Monkeys

Study no.: Report 12483/99
Location: -1248399-study-report.pdf
Conducting laboratory and location:

Date of study initiation: November 10, 1999
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 99100192, 20003073, 20006035, 99100193, 20003082, 20006037

Methods

Doses: See table below
Species/strain: Monkey, Cynomolgus
Number/sex/group or time point (main study): 4/sex/dose
Route, formulation, volume, and infusion rate: subcutaneous injection rotated among seven injection sites.
Satellite groups used for recovery: 2/sex/dose
Age: 41-56 month (male), 41-55 month (female)
Weight: 2.3-5.5 kg (males); 2.4-3.4 kg (female)
Sampling times:
Unique study design or methodology (if any): step-wise increase (and decrease) in HD. Original HD (4 mg/kg) was administered only for first 3 months.

Group	SPM 962 dose in mg/kg b.w./day s.c.			SPM 962 dose volume in ml/kg b.w./ day s.c.	Number of animals / Sex	Monkey No.
1	0 (control)			0.20 - 0.80# (SPM 962 placebo)	4 + 2 ♂ 4 + 2 ♀	1 - 6 7 - 12
2	0.25 (low dose)			0.05 (0.5%)	4 + 2 ♂ 4 + 2 ♀	13 - 18 19 - 24
3	1 (intermediate dose)			0.05 (2.0%)	4 + 2 ♂ 4 + 2 ♀	25 - 30 31 - 36
4			high dose:			
	TW 1-14	TD 1-98	4	0.20 (2.0%)		
	TW 15	TD 99-105	5	0.25 (2.0%)		
	TW 16-20	TD 106-140	6	0.30 (2.0%)		
	TW 21-22	TD 141-154	8	0.40 (2.0%)		
	TW 23-28	TD 155-196	10	0.50 (2.0%)	4 + 2 ♂ 4 + 2 ♀	37 - 42 43 - 48
	TW 29-30	TD 197-210	13	0.65 (2.0%)		
	TW 31	TD 211-217	16	0.80 (2.0%)		
	TW 32-49	TD 218-343	13	0.65 (2.0%)		
	TW 50-52	TD 344-364	10	0.50 (2.0%)		

Figure 43, from page 27 of Report — -12483/99

Results:

Mortality:
16 mg/kg- female 44 died 3 days after the increase of the high dose from 13 to 16 mg/kg (test day 215); symptoms included ataxia, salivation, tremor, convulsions and abdominal position
13 mg/kg- males 42 and 40 died on test days 320 and 342; symptoms included salivation, sedation, ataxia, tremor, convulsions, abdominal position and/or gnawing at the rods of the cage.

Clinical signs: Daily
No clinical signs were observed at 0.25 or 1 mg/kg
During the first week, restlessness was observed in high dose (4 mg/kg) rats (males and females)
When the high dose was increased to 13-16 mg/kg/day, increased activity was observed in both males and females.

Body weights: 1X/week
No effects were observed on female body weight or 0.25 and 1 mg/kg male body weights. The sponsor reported a slight decrease in body weight in high dose males from week 14 onwards was observed (which corresponded with the increase in rotigotine dose), but the effect appears to be relatively small.

Figure 1 Body weight of male animals

mean values per group

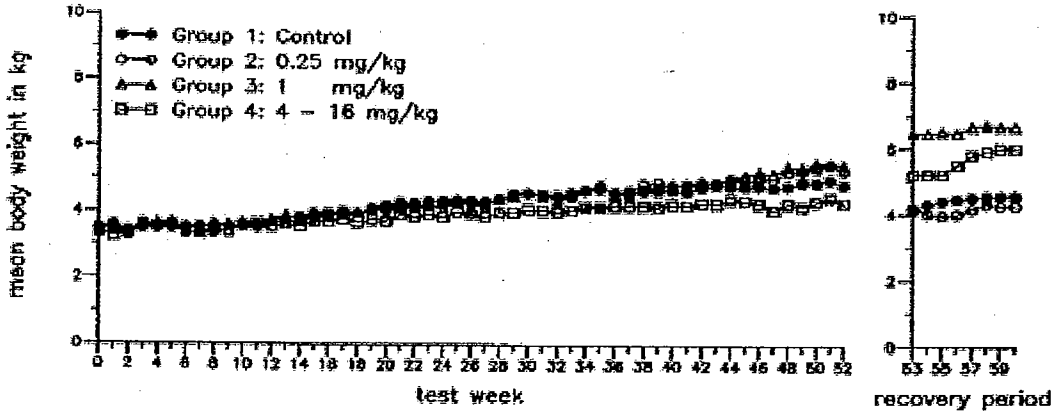


Figure 44, from page 48 of Report — 12483/99

Figure 2 Body weight of female animals

mean values per group

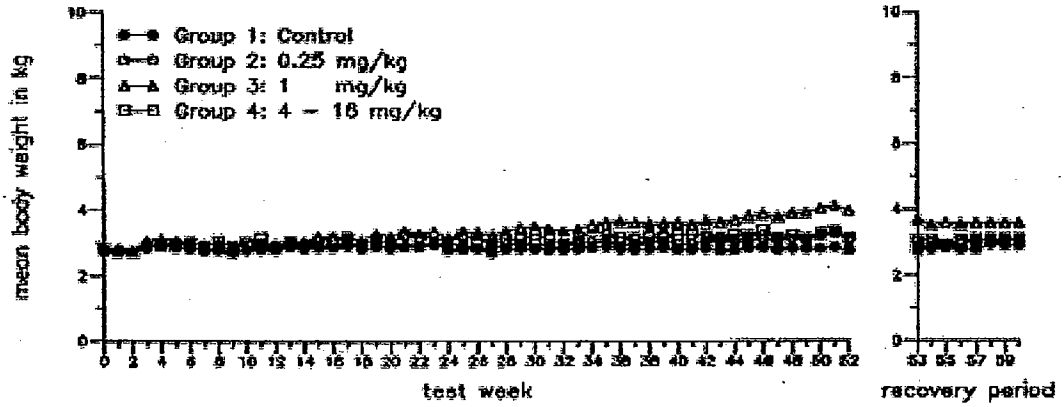


Figure 45, from page 49 of Report — 12483/99

Body weights (in kg) in male and female monkeys

	Males				Females			
Week	Con	LD	MD	HD	Con	LD	MD	HD
0	3.50	3.30	3.45	3.35	2.82	2.72	2.83	2.80
4	3.57	3.48	3.65	3.55	2.93	2.95	3.12	2.93
8	3.58	3.35	3.63	3.42	2.83	2.77	3.02	3.07
12	3.6	3.52	3.78	3.52	2.87	2.78	2.95	3.87
16	3.95	3.87	3.92	3.70	2.85	2.95	3.20	3.08
20	4.22	4.05	4.13	3.73	2.83	2.98	3.20	3.02
24	4.35	4.23	4.10	3.85	2.77	2.95	3.15	3.05
28	4.43	4.38	4.40	3.97	2.80	2.93	3.35	3.08
32	4.52	4.45	4.37	4.02	2.80	2.87	3.35	2.98
36	4.60	4.63	4.55	4.20	2.82	2.92	3.65	3.16
40	4.68	4.83	4.88	4.25	2.80	2.97	3.60	3.24
44	4.80	5.03	5.07	4.40	2.78	3.02	3.70	3.26
48	4.83	5.25	5.42	4.28	2.83	3.13	3.90	3.24
52	4.87	5.30	5.47	4.33	2.77	3.07	3.98	3.16
BW Gain	1.37	2.00	2.02	0.98	-0.05	0.35	1.15	0.36

Food consumption: 1X/week
No effects were observed on food consumption.

Ophthalmoscopy:

EKG: Pre (2X), Day 1, Weeks 6, 13, 26, 39, 52 (pre-dose and 1 hour post dose)
No effects

Blood Pressure: Pre (2X), Day 1, Weeks 6, 13, 26, 39, 52 (pre-dose and 1 hour post dose)
No effects

Hematology:
Hemoglobin (Hg), Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), platelets, White Blood Cell Count (WBC) and differential, Reticulocytes

Increased reticulocytes in high dose females starting at 4 mg/kg; high dose monkey 41 had consistently high ESR (which increased at higher doses), which lowered after cessation of exposure.

Clinical chemistry:
Blood Urea Nitrogen (BUN), Creatinine, Glucose (Glu), Alkaline Phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Total Protein (TP), Albumin, Globulin, Albumin/Globulin ratio (A/G), Bilirubin (Bili), Cholesterol, Calcium, Sodium, Potassium, Chloride

1 mg/kg male monkey 28 had increased ALT at week 26 (75 vs 17.2 in controls); 4-16 mg/kg monkey 40 had increased ALT at week 26 (56 vs 17.2 in controls); these monkeys also had elevated AST levels (102 and 72, respectively vs 23.5 in controls) and LDH (1631 and 678, respectively, vs 330 in controls). Some increase in creatinine phosphokinase level starting at 1 mg/kg in males at week 52

Serum Prolactin Levels:

Sex	Week	0 mg/kg	0.25 mg/kg	1 mg/kg	4-16 mg/kg
Male	48	3.25	2.45	3.20	0.49
	52	4.34	2.62	2.24	2.48
Female	48	5.36	3.18	3.09	0.44
	52	2.84	1.10	1.71	1.12

No effects on cytochrome P450 induction in the liver

Urinalysis:
No effects

Gross pathology:
No effects

Organ weights (specify organs weighed if not in histopath table):

Adrenal glands, Brain, Heart, Kidney, Liver, Lung, Ovary, Pituitary, Prostate, Testes, Spleen, Thymus, Uterus
No effects

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )
Injection site findings included chronic granulation tissue with fluid-filled blisters, subcutaneous edema, mixed cell infiltrates and fibrosis in both control and rotigotine treated monkeys.

No other significant effects were observed.

Toxicokinetics: Days 3, 90, 180, 270, 358; 0, 2, 4, 6, 8, 24 hours post dose

Tab. 4 Systemic 24-hour Exposure (AUD(0-24h))

Day	AUD(0-24h) [h*ng/mL]						
	Group 2 0.25 mg/kg/day		Group 3 1 mg/kg/day		Dose	Group 4 4 - 16 mg/kg/day*	
	M1	F1	M2	F2	[mg/kg]	M3	F3
3	15.58	6.95	43.88	30.28	4	631.79	186.08
90	36.43	30.17	136.73	129.03	4	432.51	880.40
108	NA	NA	NA	NA	6	547.70	245.22
152/153	NA	NA	NA	NA	8	712.00	326.29
160	NA	NA	NA	NA	10	1170.30	326.29
180	2.97	9.43	47.55	34.16	10	1133.20	391.33
202	NA	NA	NA	NA	13	1129.50	452.19
217	NA	NA	NA	NA	16	1505.30	913.00
270	13.54	13.62	45.86	48.18	13	2000.60	676.60
358	258.37	8.66	60.06	34.13	10	1306.04	562.40
median	15.58	9.43	47.55	34.16			
min	2.97	6.95	43.88	30.28			
max	258.37	30.17	136.73	129.03			

NA not applicable
* dose was adjusted during the study
results without outlier: M1/day 358: 7.72
F3/day 90: 183.87

Figure 46, from page 1553 of Report — -12483/99

Other:

Key study findings:

1. High dose ranged from 4 to 16 mg/kg. 13-16 mg/kg caused excessive CNS effects resulting in the death of 2 monkeys at 13 mg/kg and one monkey at 16 mg/kg. Increased respiratory rate observed in male monkeys that died.
2. Decreased body weight was observed at 4-16 mg/kg in males.
3. Sporadic increases in ALT and AST at 1 and 4-16 mg/kg.
4. Increased reticulocytes was observed at 4-16 mg/kg in females.
5. Increased erythrocyte sedimentation rate was observed at 4-16 mg/kg in both sexes.
6. No significant histopathological findings were observed, even in premature deaths. There was extensive autolysis in premature decedents that made it difficult to assess cause of deaths.

2.6.6.3.6 Drug Interaction Study Between SPM 962 and a Combination Of L-Dopa and Carbidopa in Cynomolgus Monkeys

Study no.: Report 16084/02
Location: 1608402-study-report.pdf
Conducting laboratory and location: [redacted]

Date of study initiation: June 18, 2003
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 20107030, 20302037, 20302038, 20302039, 20302040

Methods

Doses: See table below
Species/strain: Monkey, Cynomolgus
Number/sex/group or time point (main study): 4/sex/dose
Route, formulation,: subcutaneous injection rotated among seven injection sites.
Age: 41-56 month (male), 41-55 month (female)
Weight: 2.3-5.5 kg (males); 2.4-3.4 kg (female)

Treatment schedule

Group	SPM 962 dose (administration: daily from days 22 to 112)		L-dopa and Carbidopa dose (administration: daily from days 1 to 112)	No. of animals and sex
	[mg/kg b.w./day]	Volume [mL/kg b.w./day] (concentration used)	[mg/kg b.w./day] at 1.6 mL/kg b.w.	MS + RP
1	0 (Control)	0.8 SPM 962 placebo (0%)	0 (Vehicle for L-dopa and Carbidopa)	4 + 2 m 4 + 2 f
2	0 (Control)	0.8 SPM 962 placebo (0%)	80/20	4 + 2 m 4 + 2 f
3	0.25	0.05 (0.5%)	80/20	4 m 4 f
4	1	0.2 (0.5%)	80/20	4 m 4 f
5	4	0.8 (0.5%)	80/20	4 + 2 m 4 + 2 f
6	4	0.8 (0.5%)	0 (Vehicle for L-dopa and Carbidopa)	4 + 2 m 4 + 2 f

MS: main study
RP: recovery period and kinetics
m: male
f: female

Figure 47, from page 36 of Report — 16084/02

Results:

Mortality:
No mortality was observed

Clinical signs: Daily
Restlessness was observed in all monkeys treated with levodopa/carbidopa. The incidence increased with increasing rotigotine dose.
Restlessness was not generally observed in monkeys treated with rotigotine alone (only one incident).

Body weights: 1X/week
No effects were observed.

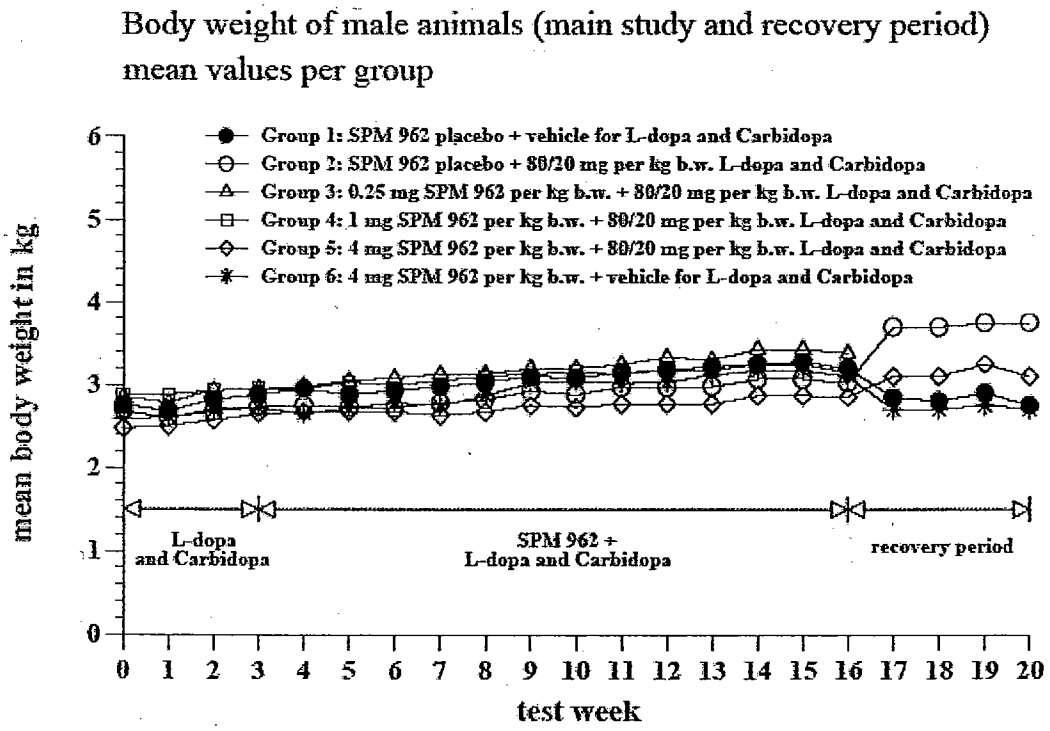


Figure 48, from page 62 of Report — 16084/02

Body weight of female animals (main study and recovery period)
mean values per group

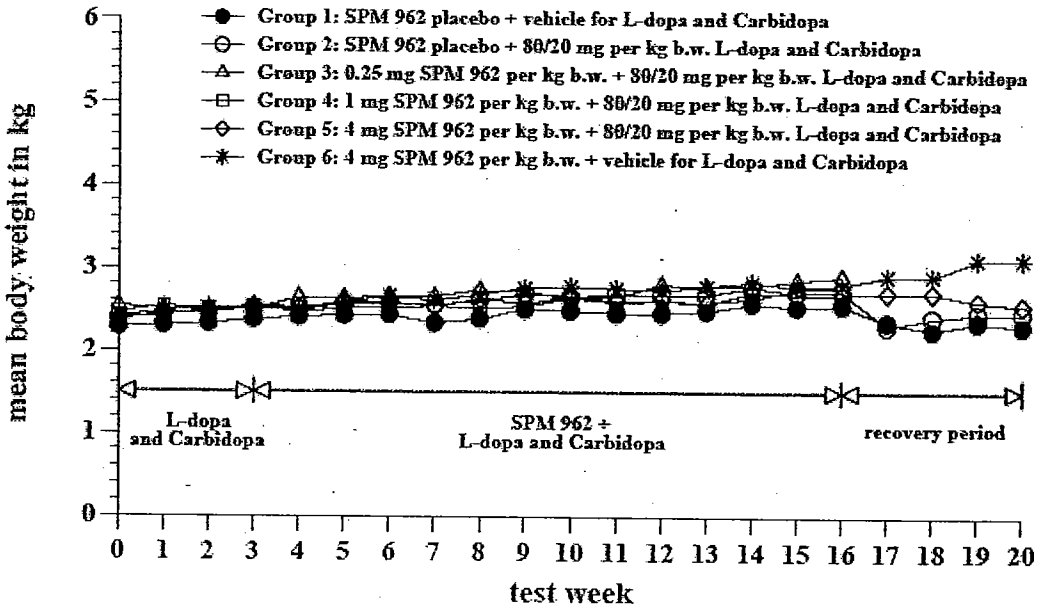


Figure 49, from page 63 of Report — 16084/02

Food consumption: 1X/week
No effects were observed on food consumption.

Ophthalmoscopy: Pre, Week 16, 20 (recovery)
No effects were observed.

EKG: Pre (2X), Day 1, 20, 4, 71, 113, 138 (recovery) (pre-dose and 1 hour post dose), Bazett, Framingham and Fridericia corrections for QT intervals.
No effects

Blood Pressure: Pre (2X), Day 1, 20, 4, 71, 113, 138 (recovery) (pre-dose and 1 hour post dose)
No effects

Hematology: Pre, Day 21, 113, 138 (recovery)
Hemoglobin (Hg), Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), platelets, White Blood Cell Count (WBC) and differential, Reticulocytes

No effects

Clinical chemistry: Pre, Day 21, 113, 138 (recovery)

Blood Urea Nitrogen (BUN), Creatinine, Glucose (Glu), Alkaline Phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Total Protein (TP), Albumin, Globulin, Albumin/Globulin ratio (A/G), Bilirubin (Bili), Cholesterol, Calcium, Sodium, Potassium, Chloride

Male Group 5 monkey #45 had elevated AST (206 vs 28 u/l in controls) and LDH (1111 versus 314 u/l in controls) at Day 113; values returned to normal at recovery

Male Group 5 monkeys #45 and 46 had elevated CPK (3595 and 1391 u/l versus 129 u/l in controls).

Female Group 5 monkey #52 had elevated CPK (1035 u/l versus 116 u/l in controls).

Urinalysis: Pre, Day 21, 113, 138 (recovery)
No effects, volume not measured.

Gross pathology:
No effects

Organ weights (specify organs weighed if not in histopath table):
Adrenal glands, Brain, Heart, Kidney, Liver, Lung, Ovary, Pituitary, Prostate, Testes, Spleen, Thymus, Uterus

No effects

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )
Injection site findings included chronic granulation tissue with fluid-filled blisters, subcutaneous edema, mixed cell infiltrates and fibrosis in both control and rotigotine treated monkeys.

No systemic effects

Toxicokinetics: Days 14, 22, 112

Toxicokinetic parameters (range, n=2/sex/group)

Group	Day	Regime	Levodopa			Carbidopa		
			C_{max} [ng/mL]	t_{max} [h]	AUC_{last} [h · ng/mL]	C_{max} [ng/mL]	t_{max} [h]	AUC_{last} [h · ng/mL]
2	14	S	12200-26300	2	34640-45301	200-442	2-4	933-2119
	22	S	23400-32600	1-2	36823-54513	358-1060	1-2	1424-4409
	112	S	7460-23100	2	31925-42716	134-618	1-4	651-2697
5	14	S	11900-15200	1-2	29274-53470	312-658	0.5-2	1224-2228
	22	$S+I_{sd}$	10700-25900	0.5-2	28065-59138	166-454	0.5-1	741-2072
	112	$S+I_{rd}$	14400-30400	1-2	36151-63984	290-701	1-4	1637-2975

Figure 50, from page 75 of Report — 6084/02

APPEARS THIS WAY
ON ORIGINAL

Table 9 SPM 962 plasma concentrations at 4 hours post-dose and descriptive statistics after subcutaneous administration of SPM 962 at 4 mg/kg/day starting on day 22 (group 5 and 6)

Group	Day		SPM 962 plasma conc. [ng/mL]			
5			#45m	#46m	#51f	#52f
	22	(I_{sd})				
	112	(I_{rd})				
6			#57m	#58m	#63f	#64f
	22	($S+I_{sd}$)				
	112	($S+I_{rd}$)				
5	R (rd/sd)		88	1.3	5.8	4.9
6	R (rd/sd)		1.7	1.5	3.1	2.2

Group	Day		Desc. stat. of SPM 962 plasma conc.			
5			Median	Mean	SD	CV
	22	(I_{sd})	2.09	2.12	0.51	24%
	112	(I_{rd})	10.0	48.6	81.7	168%
6			Median	Mean	SD	CV
	22	($S+I_{sd}$)	2.61	2.75	0.73	27%
	112	($S+I_{rd}$)	6.10	5.68	1.42	25%
5	R (rd/sd)		5.4	25	42	168%
6	R (rd/sd)		2.0	2.1	0.7	33%

Figure 51, from page 1272 of Report 16084/02

Other:

Key study findings:

1. Dose dependent increase in restlessness was observed in Sinemet treated monkeys treated with rotigotine.
2. No other significant adverse effects were observed.
3. No significant effects on levodopa/carbidopa pharmacokinetics were observed.

2.6.6.4 Genetic toxicology

2.6.6.4.1 Ames/Salmonella-E. coli Reverse Mutation Assay on N-0923

Study no.: 301-DIS-002-95

Conducting laboratory and location:
Date of study initiation: August 25, 1995
GLP compliance: No. Test and control article dosing solutions were not analyzed to determine stability, homogeneity or accuracy of preparation. Otherwise GLP was followed (page 13 of study).
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 7311-60

Methods

Strains/species/cell line: Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537; Escherichia coli WP2 uvrA

Doses used in definitive study: see below

Basis of dose selection: Reduced bacterial lawn in prescreen using both plate incorporation and liquid preincubation methods. Tested only in the absence of S9 metabolic activation; no counts of viable colonies.

Negative controls: Vehicle (DMSO);

Positive controls:

Incubation and sampling times: Preincubation and plate incorporation methods were used. Triplicate samples were incubated for 48 hours. Colonies were counted using an automated colony counter. Metabolic activation system was by addition of 6% (v/v) aroclor 1254 induced male rat liver microsomes.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
Study was not conducted according to GLP standards. The results were within historical control range. Low doses need to be used due to toxicity. When excessive toxicity prevented at least four doses being available for evaluation, the assay was repeated at lower doses.

Study outcome:

No significant increase in revertants was observed.

Table 2. Summary Data - Liquid Pre-incubation Assay

CONTROLS								
			AVERAGE REVERTANTS/PLATE					
SOLVENT CONTROLS		S9	TA1535	TA1537	TA98	TA100	TA102	UVR A
DMSO (100 UL)		(-)	5 (3)	8 (5)	23 (6)	53 (11)	194 (23)	5 (2)
DMSO (100 UL)		(+)	9 (4)	6 (1)	27 (2)	73 (5)	322 (33)	5 (3)
POSITIVE CONTROLS (UG/PL)								
SODIUM AZIDE	10.0	(-)	1072*(72)	--- (---)	--- (---)	1155*(34)	--- (---)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	1657*(42)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	620*(31)	--- (---)	--- (---)	--- (---)
MITOMYCIN C	2.50	(-)	--- (---)	--- (---)	--- (---)	--- (---)	648*(60)	--- (---)
ENNG	2.00	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	385*(143)
2-ANTHRAMINE	2.50	(+)	63*(24)	174*(32)	2136*(73)	1376*(214)	--- (---)	--- (---)
2-AMINOFLUORENE	30.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	712*(112)	--- (---)
2-ANTHRAMINE	80.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	665*(34)
TEST ARTICLE: N-0923								
			AVERAGE REVERTANTS/PLATE					
DOSE LEVEL (UG/PL)		S9	TA1535	TA1537	TA98	TA100	TA102	UVR A
1.67		(-)	6 (4)	8 (4)	26 (6)	56 (5)	179 (15)	5 (2)
5.00		(-)	3 (1)	5 (1)	23 (5)	63 (6)	200 (25)	4 (4)
16.7		(-)	2 (1)a/b	5 (1)a	22 (4)	49 (14)a	194 (17)	4 (3)
50.0		(-)	4 (4)c	3 (4)b	10 (4)b	24 (6)b/c	150 (32)a/b	2 (2)a/b
100		(-)	4 (2)c	0 (0)c	10 (3)c	30 (10)c	80 (10)b/c	4 (2)c
167		(-)	0 (0)c	0 (0)c	0 (0)c	16 (2)c	0 (0)c	1 (2)c
1.67		(+)	6 (2)	11 (5)	36 (3)	78 (15)	266 (25)	6 (6)
5.00		(+)	5 (1)	7 (6)	31 (4)	75 (6)	305 (15)	4 (2)
16.7		(+)	4 (2)	6 (2)	32 (8)	80 (6)	283 (21)	6 (4)
50.0		(+)	8 (5)	6 (2)	26 (7)	67 (9)	245 (13)	5 (2)
100		(+)	6 (2)a/b	9 (1)a	28 (3)	72 (3)a	226 (15)	5 (3)
167		(+)	7 (1)b/c	5 (1)b	16 (1)b	35 (16)b	143 (37)a/b	6 (4)a/b

*Positive Response: ≥2X Solvent (TA1535, TA1537, TA98, TA100, TA102, UVR A).
Data Reported as: Mean (Standard Deviation).
Test article freely soluble at all doses ±S9.
a/b/c =slight/moderate/severe toxicity.

Figure 52, from page 18 of Report — 301-DIS-002-95

APPEARS THIS WAY
ON ORIGINAL

Table 3. Summary Data - Plate Incorporation Assay

CONTROLS

SOLVENT CONTROLS		S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA98	TA100	TA102	UVR A
DMSO (100 UL)		(-)	5 (2)	8 (1)	25 (6)	68 (1)	247 (37)	5 (3)
DMSO (100 UL)		(+)	6 (1)	11 (4)	29 (9)	77 (2)	331 (27)	7 (2)
POSITIVE CONTROLS	(UG/PL)							
SODIUM AZIDE	10.0	(-)	1043*(39)	--- (---)	--- (---)	1116*(77)	--- (---)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	1611*(34)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	555*(61)	--- (---)	--- (---)	--- (---)
MITOMYCIN C	2.50	(-)	--- (---)	--- (---)	--- (---)	--- (---)	1200*(104)	--- (---)
ENNG	2.00	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	279*(56)
2-ANTHRAMINE	2.50	(+)	98*(16)	144*(46)	2348*(202)	1318*(77)	--- (---)	--- (---)
2-AMINOFLUORENE	30.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	763*(37)	--- (---)
2-ANTHRAMINE	80.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	633*(40)

TEST ARTICLE: N-0923

DOSE LEVEL (UG/PL)	S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA98	TA100	TA102	UVR A
0.167	(-)	9 (3)	9 (2)	24 (5)	84 (23)	210 (52)	8 (2)
0.500	(-)	6 (3)	7 (5)	29 (11)	93 (13)	279 (12)	4 (0)
1.67	(-)	5 (3)	4 (1)	28 (10)	141*(15)	268 (12)	2 (2)
5.00	(-)	6 (2)	7 (3)	23 (2)	75 (15)	262 (25)	5 (2)
16.7	(-)	10 (5)	6 (1)	29 (10)	82 (8)	207 (8)	4 (3)
33.3	(-)	6 (1)	6 (6)	27 (6)	73 (13)	176 (18)	2 (0)
0.500	(+)	9 (7)	9 (6)	35 (6)	118 (19)	264 (52)	5 (3)
1.67	(+)	3 (1)	6 (1)	32 (6)	112 (3)	326 (33)	3 (1)
5.00	(+)	3 (3)	10 (7)	36 (8)	116 (7)	330 (38)	8 (3)
16.7	(+)	8 (7)	8 (1)	33 (8)	105 (6)	316 (53)	4 (2)
33.3	(+)	7 (1)	7 (2)	41 (3)	91 (9)	280 (10)	4 (3)
50.0	(+)	9 (3)	9 (3)	29 (3)	91 (17)	196 (49)	4 (2)

*Positive Response: ≥2X Solvent (TA1535, TA1537, TA98, TA100, TA102, UVR A).
Data Reported as: Mean (Standard Deviation).
Test article freely soluble at all doses ±S9.
Apparently normal growth all strains/doses ±S9.

Figure 53, from page 19 of Report 301-DIS-002-95

APPEARS THIS WAY
ON ORIGINAL

Table 4. Summary Data - Liquid Pre-incubation Retest

		CONTROLS	
		AVERAGE REVERTANTS/PLATE	
SOLVENT CONTROLS	S9	TA1535	TA100
DMSO (100 UL)	(-)	7 (1)	69 (6)
POSITIVE CONTROLS (UG/PL)			
SODIUM AZIDE	10.0 (-)	1323*(82)	1437*(22)
		TEST ARTICLE: N-0923	
		AVERAGE REVERTANTS/PLATE	
DOSE LEVEL (UG/PL)	S9	TA1535	TA100
0.167	(-)	7 (1)	47 (6)
0.500	(-)	8 (4)	56 (7)
1.67	(-)	5 (1)	55 (10)
5.00	(-)	5 (1)	49 (27)
16.7	(-)	7 (4)a/b	45 (4)a/b
33.3	(-)	3 (2)b	26 (7)b/c

*Positive Response: ≥2X Solvent (TA1535, TA100).
Data Reported as: Mean (Standard Deviation).
Test article freely soluble at all doses.
a/b/c =slight/moderate/severe toxicity.

Figure 54, from page 20 of Report —301-DIS-002-95

APPEARS THIS WAY
ON ORIGINAL

Table 5. Summary Data - Plate Incorporation Retest

CONTROLS

SOLVENT CONTROLS		S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA98	TA100	TA102	UVR A
DMSO (100 UL)		(-)	4 (1)	6 (3)	29 (2)	75 (12)	244 (55)	5 (1)
DMSO (100 UL)		(+)	9 (2)	7 (2)	27 (3)	102 (9)	321 (63)	5 (1)
POSITIVE CONTROLS (UG/PL)								
SODIUM AZIDE	10.0	(-)	1050*(67)	--- (---)	--- (---)	1072*(108)	--- (---)	--- (---)
9-AMINOACRIDINE	150	(-)	--- (---)	1824*(70)	--- (---)	--- (---)	--- (---)	--- (---)
2-NITROFLUORENE	5.00	(-)	--- (---)	--- (---)	582*(123)	--- (---)	--- (---)	--- (---)
MITOMYCIN C	2.50	(-)	--- (---)	--- (---)	--- (---)	--- (---)	1326*(61)	--- (---)
ENNG	2.00	(-)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	149*(38)
2-ANTHRAMINE	2.50	(+)	64*(16)	213*(60)	1791*(129)	1796*(12)	--- (---)	--- (---)
2-AMINOFLUORENE	30.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	1126*(341)	--- (---)
2-ANTHRAMINE	30.0	(+)	--- (---)	--- (---)	--- (---)	--- (---)	--- (---)	500*(39)

TEST ARTICLE: N-0923

DOSE LEVEL (UG/PL)	S9	AVERAGE REVERTANTS/PLATE TA1535	TA1537	TA98	TA100	TA102	UVR A
1.67	(-)	11*(4)	9 (6)	22 (6)	81 (17)	282 (66)	5 (3)
5.00	(-)	8 (5)	7 (2)	31 (6)	86 (1)	280 (14)	3 (1)
16.7	(-)	5 (1)	7 (2)	27 (3)	93 (10)	317 (35)	5 (3)
50.0	(-)	5 (1)	10 (4)	34 (5)	100 (10)	304 (24)	5 (1)
100	(-)	6 (3)	7 (1)a/b	25 (9)	66 (22)	242 (6)a	6 (4)
167	(-)	7 (2)a/b	6 (2)a/b	20 (1)a/b	46 (16)a/b	188 (47)a/b	6 (2)a/b
1.67	(+)	6 (6)	11 (2)	37 (6)	92 (6)	341 (21)	6 (2)
5.00	(+)	7 (3)	12 (2)	24 (5)	98 (24)	340 (25)	5 (3)
16.7	(+)	9 (4)	5 (1)	35 (7)	105 (2)	352 (44)	5 (4)
50.0	(+)	7 (3)	8 (5)a	25 (8)a	78 (9)	360 (7)	3 (1)
167	(+)	6 (2)a/b	8 (4)b	16 (2)a/b	36 (14)a/b	207 (58)a/b	5 (2)a/b
333	(+)	6 (6)b/c	4 (1)c	16 (2)c	19 (4)b/c	82 (40)b/c	4 (2)b/c

*Positive Response: ≥2X Solvent (TA1535, TA1537, TA98, TA100, TA102, UVR A).
Data Reported as: Mean (Standard Deviation).
Test article freely soluble at all doses ±S9.
a/b/c =slight/moderate/severe toxicity.

Figure 55, from page 21 of Report – 301-DIS-002-95

APPEARS THIS WAY
ON ORIGINAL

MUTATION ASSAY AT THE TK LOCUS
IN L5178Y MOUSE LYMPHOMA CELLS
WITHOUT METABOLIC ACTIVATION
(3 HOUR SHORT TREATMENT)

TABLE 9

ASSAY 1

Starting date : 4/2/1999
Completion date: 16/2/1999

TIME 2 days after treatment

CULTURE A		MUTATION											VIABILITY at T2			Mutation Frequency	
		Number of positive wells											Number of negative wells				
		SMALL COLONIES					LARGE COLONIES					TOTAL Wells					
COMPOUND	DOSE	Plate				TOTAL	Plate				TOTAL		Plate		TOTAL	PE	x10 $^{-6}$ cells	Treated vs. Solvent
	µg/ml	1	2	3	4		1	2	3	4		+	-	1	2		%		
Solvent Control	0															104.6	130.9	
TEST COMPOUND	14.5															96.5	176.8	1.4
	17.4															110.1	186	1.4
	20.8															96.5	152.7	1.2
	25															88.0	241.7	1.8
	30																	
MMS	*10*															90.7	825.1	6.3

CULTURE B		MUTATION											VIABILITY at T2			Mutation Frequency	
		Number of positive wells											Number of negative wells				
		SMALL COLONIES					LARGE COLONIES					TOTAL Wells					
COMPOUND	DOSE	Plate				TOTAL	Plate				TOTAL		Plate		TOTAL	PE	x10 $^{-6}$ cells	Treated vs. Solvent
	µg/ml	1	2	3	4		1	2	3	4		+	-	1	2		%		
Solvent Control	0															101.2	138.7	
TEST COMPOUND	14.5															110.1	158.3	1.1
	17.4															88.0	202.3	1.5
	20.8															95.0	170	1.2
	25															95.0	209.3	1.5
	30																	
MMS	*10*															89.3	753.3	5.4

* Mutation frequency not performed due to a strong toxicity (RTG<10%)
PE: Plating efficiency

Figure 56, from page 26 of Report — R 990310

MUTATION ASSAY AT THE TK LOCUS
IN L5178Y MOUSE LYMPHOMA CELLS
WITH METABOLIC ACTIVATION
(3 HOUR TREATMENT)

TABLE 11

ASSAY 1

Starting date : 3/2/1999
Completion date: 15/2/1999

TIME 2 days after treatment

CULTURE A		MUTATION										VIABILITY at T2			Mutation Frequency	
		Number of positive wells										Number of negative wells				
		SMALL COLONIES					LARGE COLONIES				TOTAL Wells					
COMPOUND	DOSE	Plate				TOTAL	Plate				TOTAL					
	µg/ml	1	2	3	4		1	2	3	4		+	-	Plate 1	Plate 2	TOTAL	PE %	x10 -6 cells	Treated vs. Solvent
Solvent Control	0																106.4	133.6	
TEST COMPOUND	6.4																104.6	188.2	1.4
	7.7																90.7	243.3	1.8
	9.6																101.2	324.6	2.4
	12																89.3	511.6	3.8
	15																		
CPA	2																96.5	1159	8.7

CULTURE B		MUTATION										VIABILITY at T2			Mutation Frequency	
		Number of positive wells										Number of negative wells				
		SMALL COLONIES					LARGE COLONIES				TOTAL Wells					
COMPOUND	DOSE	Plate				TOTAL	Plate				TOTAL					
	µg/ml	1	2	3	4		1	2	3	4		+	-	Plate 1	Plate 2	TOTAL	PE %	x10 -6 cells	Treated vs. Solvent
Solvent Control	0																92.1	135.9	
TEST COMPOUND	6.4																88.0	157.6	1.2
	7.7																104.6	195.6	1.4
	9.6																96.5	300.5	2.2
	12																85.4	539	4.0
	15																		
CPA	2																99.6	1044	7.7

* Mutation frequency not performed due to a strong toxicity (RTG<10%)
PE: Plating efficiency

Figure 57, from page 28 of Report ~ R 990310

MUTATION ASSAY AT THE TK LOCUS
IN L5178Y MOUSE LYMPHOMA CELLS
WITHOUT METABOLIC ACTIVATION
(24 HOUR CONTINUOUS TREATMENT)

TABLE 14

ASSAY 2

Starting date : 4/3/1999
Completion date: 16/3/1999

TIME 2 days after treatment

CULTURE A		MUTATION										VIABILITY at T2			Mutation Frequency	
		Number of positive wells										Number of negative wells				
		SMALL COLONIES					LARGE COLONIES				TOTAL Wells				x10 $^{-6}$	Treated
COMPOUND	DOSE	Plate				TOTAL	Plate				TOTAL			PE	cells	vs.
	µg/ml	1	2	3	4		1	2	3	4		+	-	1 2	TOTAL	%		Solvent
Solvent Control	0																132.9	
TEST COMPOUND	8.9																102	0.8
	13.3																210.6	1.5
	20																242.9	1.8
	30																253.3	1.9
	45																233	1.8
MMS	2																819.3	6.2

CULTURE B		MUTATION										VIABILITY at T2			Mutation Frequency	
		Number of positive wells										Number of negative wells				
		SMALL COLONIES					LARGE COLONIES				TOTAL Wells				x10 $^{-6}$	Treated
COMPOUND	DOSE	Plate				TOTAL	Plate				TOTAL			PE	cells	vs.
	µg/ml	1	2	3	4		1	2	3	4		+	-	1 2	TOTAL	%		Solvent
Solvent Control	0															108.2	137.8	
TEST COMPOUND	8.9															06.4	207.4	1.5
	13.3															132.6	191.6	1.4
	20															92.1	264.3	1.9
	30															79.3	278.3	2.0
	45															95.0	223.8	1.6
MMS	2															92.1	812.5	5.9

PE: Plating efficiency

Figure 58, from page 31 of Report — R 990310

MUTATION ASSAY AT THE TK LOCUS
IN L5178Y MOUSE LYMPHOMA CELLS
WITH METABOLIC ACTIVATION
(3 HOUR TREATMENT)

TABLE 16

ASSAY 2

Starting date : 17/3/1999
Completion date: 29/3/1999

TIME 2 days after treatment

CULTURE A		MUTATION											VIABILITY at T2			Mutation Frequency	
		Number of positive wells											Number of negative wells				
		SMALL COLONIES					LARGE COLONIES					TOTAL Wells					
COMPOUND	DOSE	Plate				TOTAL	Plate				TOTAL		Plate		TOTAL	PE	x10 $^{-6}$ cells	Treated vs. Solvent
	µg/ml	1	2	3	4		1	2	3	4		+	-	1	2	%		
Solvent Control	0															127.4	162.2	
TEST COMPOUND	3.5															89.3	218.3	1.3
	4.4															88.0	267.2	1.6
	5.4															82.9	256.6	1.6
	6.8															88.0	319.2	2.0
	8.5															88.0	463.2	2.9
CPA	2															85.4	883.4	5.4

CULTURE B		MUTATION											VIABILITY at T2			Mutation Frequency	
		Number of positive wells											Number of negative wells				
		SMALL COLONIES					LARGE COLONIES					TOTAL Wells					
COMPOUND	DOSE	Plate				TOTAL	Plate				TOTAL		Plate		TOTAL	PE	x10 $^{-6}$ cells	Treated vs. Solvent
	µg/ml	1	2	3	4		1	2	3	4		+	-	1	2	%		
Solvent Control	0															12.0	154	
TEST COMPOUND	3.5															77.0	189	1.2
	4.4															74.8	247.9	1.6
	5.4															78.1	193	1.3
	6.8															99.6	291.1	1.9
	8.5															82.9	596.1	3.9
CPA	2															99.6	583.8	3.8

PE: Plating efficiency

Figure 59, from page 33 of Report — R 990310

HISTORICAL CONTROLS
4/3/97 to 1/2/98 (8 assays)

WITHOUT METABOLIC ACTIVATION

S9 - 3 h treatment	MUTATION FREQUENCY x 10^{-6} CELLS Mean ± sd *extreme deviations*
NEGATIVE CONTROLS Solvent	116.4 ± 26.1 *81.7 - 167.6*
POSITIVE CONTROLS MMS 10 µg/ml	708 ± 155.3 *443.8 - 947.7*

S9 - 24 h treatment (5 assays)	MUTATION FREQUENCY x 10^{-6} CELLS Mean ± sd *extreme deviations*
NEGATIVE CONTROLS Solvent	126 ± 39.4 *75.8 - 191.3*
POSITIVE CONTROLS MMS 2 µg/ml	902.8 ± 198.6 *544.9 - 1253*

WITH METABOLIC ACTIVATION

S9 +	MUTATION FREQUENCY x 10^{-6} CELLS Mean ± sd *extreme deviations*
NEGATIVE CONTROLS Solvent	122.2 ± 23.7 *84.2 - 187.5*
POSITIVE CONTROLS CPA 2 µg/ml	947.3 ± 262.1 *581.5 - 1412.8*

Figure 60, from page 21 of Report — R 990310

2.6.6.4.2 Mutation Assay at the TK Locus in L5178Y Mouse Lymphoma Cells Using a Microtiter Cloning Technique (Trifluorothymidine Resistance) with N-0923 (SPM 962) Free Base

Study no.: — R 990310

Conducting laboratory and location: [redacted]
Date of study initiation: January 18, 1999
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: WE6765

Methods

Strains/species/cell line: L5178Y TK+- Mouse Lymphoma Cells

Doses used in definitive study:

Basis of dose selection: Cytotoxicity at 62.5 ug/ml in the absence of metabolic activation and 15.6 ug/ml in the presence of metabolic activation

Negative controls: Vehicle (DMSO, ethanol)

Positive controls: Methyl methane sulfonate (without metabolic activation); cyclophosphamide (with metabolic activation)

Incubation and sampling times: Cells were preincubated for 3 hours (with and without metabolic activation) and 24 hours (without metabolic activation). Aroclor 1254 induced male rat hepatic microsomes were used as the activating system.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The positive and negative controls were within the historical control range. The doses were adequate based on cytotoxicity at the high doses. All assays were done in duplicate. One deficiency in this study is that mutation frequencies were not calculated at doses that induced more then 90% cytotoxicity.

Study outcome:
Rotigotine was clearly positive in two assays (using two different solvents, DMSO, ethanol) in the presence of metabolic activation. The incidence of mutations increased with dose and was observed at doses that did not cause cytotoxicity. The proportions of small colonies were increased suggesting that rotigotine is clastogenic in this system.

Rotigotine was also positive in the absence of metabolic activation after 24 hours incubation with cells. A dose dependent increase in mutation frequency was observed which reached two-fold at 30 ug/ml. This reviewer considers rotigotine to be positive under these assay conditions.

In contrast to the results in the presence of metabolic activation, no significant increase in mutation frequency (defined as a two fold increase in mutation frequency) was observed in the absence of metabolic activation after the 3 hour pretreatment. However, there was a general trend toward increased mutation frequency at the higher doses. In addition, six of the assay conditions were higher than the historical control range (upper limit = 167.6 mutations/10^6 cells). The difference in the results between the 3 and 24 hour incubations may be attributed in part to differences in vehicle (DMSO in the 3 hour preincubation and ethanol in the 24 hours preincubation). Higher concentrations were evaluated in the 24 hour study than in the 3 hour study. This reviewer considers rotigotine to be equivocal under these conditions (three hours in the absence of metabolic activation).

APPEARS THIS WAY
ON ORIGINAL

2.6.6.4.3 In Vivo Micronucleus Test with N-0923 in Mouse Bone Marrow Erythropoietic Cells

Study no.: 0309FD15.001

Conducting laboratory and location: [redacted]
Date of study initiation: April 16, 1996
GLP compliance: No, analyses were not performed to verify the homogeneity, stability, or accuracy of preparation of the test and control article dosing solutions (page 13 of study);
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 7442-8

Methods

Strains/species/cell line: Mouse, CD-1, 5/sex/dose/timepoint

Doses used in definitive study: 0, 1, 5, 15 mg/kg IV

Basis of dose selection: lack of toxicity (general or bone marrow) at 15 mg/kg (highest dose in dose range finding study). High dose limited by solubility considerations.

Negative controls: deionized water

Positive controls: triethylenemelamine, 0.5 mg/kg, 24 hour timepoint only

Incubation and sampling times: Mice harvested at 24, 48 and 72 hours post dose

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The methods used in the conduct of the study were adequate. The positive and negative controls were within historical control ranges. This reviewer is concerned about the appropriateness of intravenous injections as a route of administration for this drug. Rotigotine is administered as a transdermal patch in the clinic which would result in sustained serum levels. The intravenous administration would result in a relatively short exposure. This study will need to be repeated with a more appropriate route of administration.

Study outcome:
No significant effects were observed on PCE/NCE ratio. Three mice died (two at 15 mg/kg and one at 5 mg/kg). A slight statistically significant increase in micronuclei was observed in females only at the 48 hour endpoint only. The increase was small and similar to the levels in the other groups. Increases were not observed at other time points or in males. This reviewer does not regard the increase as biologically significant.

Table 6. MPCE Frequencies (by-sex) for N-0923 in the Definitive MNT[a]

Compound	Dose (mg/kg)	Time (hr)	Sex	Total MPCEs (range)	MPCEs (%) $\bar{x} \pm$ 1SD
di-H_2O	0.00	24	M	1 (0-1)	0.010 ± 0.022
N-0923	1.00	24	M	4 (0-2)	0.040 ± 0.042
N-0923	5.00	24	M	4 (0-2)	0.040 ± 0.042
N-0923	15.0	24	M	3 (0-2)	0.030 ± 0.045
TEM	0.500	24	M	224 (19-63)	2.240 ± 0.822**
di-H_2O	0.00	24	F	4 (0-2)	0.040 ± 0.042
N-0923	1.00	24	F	1 (0-1)	0.010 ± 0.022
N-0923	5.00	24	F	4 (0-2)	0.040 ± 0.042
N-0923	15.0	24	F	5 (0-2)	0.050 ± 0.050
TEM	0.500	24	F	195 (21-59)	1.950 ± 0.731**
di-H_2O	0.00	48	M	5 (0-3)	0.050 ± 0.061
N-0923	1.00	48	M	5 (0-2)	0.050 ± 0.035
N-0923	5.00	48	M	5 (0-2)	0.050 ± 0.035
N-0923	15.0	48	M	4 (0-3)	0.040 ± 0.065
di-H_2O	0.00	48	F	2 (0-1)	0.020 ± 0.027
N-0923	1.00	48	F	4 (0-2)	0.040 ± 0.042
N-0923	5.00	48	F	2 (0-1)	0.020 ± 0.027
N-0923	15.0[b]	48	F	5 (1-2)	0.063 ± 0.025*
di-H_2O	0.00	72	M	1 (0-1)	0.010 ± 0.022
N-0923	1.00	72	M	5 (0-3)	0.050 ± 0.061
N-0923	5.00	72	M	4 (0-2)	0.040 ± 0.042
N-0923	15.0[b]	72	M	3 (0-2)	0.038 ± 0.048
di-H_2O	0.00	72	F	4 (0-1)	0.040 ± 0.022
N-0923	1.00	72	F	5 (0-3)	0.050 ± 0.071
N-0923	5.00[b]	72	F	3 (0-2)	0.038 ± 0.048
N-0923	15.0	72	F	2 (0-1)	0.020 ± 0.027

[a]As described in text, and except as noted, by-sex data are for 10,000 PCEs/group (2000 PCEs/mouse; five/group).
[b]Only 8000 PCEs scored per group due to the death of one animal in each group.
*,**Statistically significant increase ($p < 0.05$ and $p < 0.01$, respectively).

Figure 61, from page 23 of Report 0309FD15.001

Table 8. PCE/NCE Ratios (by-sex) for N-0923 in the Definitive MNT[a]

Compound	Dose (mg/kg)	Time (hr)	Sex	PCE/NCE Ratio range	PCE/NCE Ratio $\bar{x} \pm 1SD$
di-H_2O	0.00	24	M	0.855 - 1.174	0.986 ± 0.144
N-0923	1.00	24	M	1.053 - 1.326	1.144 ± 0.108
N-0923	5.00	24	M	0.751 - 1.481	1.072 ± 0.269
N-0923	15.0	24	M	0.815 - 1.041	0.980 ± 0.094
TEM	0.50	24	M	0.493 - 0.718	0.582 ± 0.083**
di-H_2O	0.00	24	F	0.730 - 1.481	1.063 ± 0.290
N-0923	1.00	24	F	0.996 - 1.618	1.227 ± 0.237
N-0923	5.00	24	F	0.887 - 1.062	0.975 ± 0.074
N-0923	15.0	24	F	0.898 - 1.404	1.088 ± 0.226
TEM	0.50	24	F	0.346 - 0.529	0.447 ± 0.074**
di-H_2O	0.00	48	M	1.041 - 1.564	1.257 ± 0.248
N-0923	1.00	48	M	0.923 - 1.364	1.123 ± 0.180
N-0923	5.00	48	M	0.876 - 1.481	1.119 ± 0.260
N-0923	15.0	48	M	0.919 - 1.392	1.126 ± 0.184
di-H_2O	0.00	48	F	0.890 - 1.404	1.139 ± 0.197
N-0923	1.00	48	F	0.792 - 1.488	1.131 ± 0.299
N-0923	5.00	48	F	0.894 - 1.513	1.291 ± 0.242
N-0923	15.0[b]	48	F	1.004 - 1.381	1.152 ± 0.171
di-H_2O	0.00	72	M	1.198 - 1.387	1.274 ± 0.099
N-0923	1.00	72	M	0.736 - 1.538	1.035 ± 0.358
N-0923	5.00	72	M	1.020 - 1.488	1.277 ± 0.176
N-0923	15.0[b]	72	M	1.070 - 1.294	1.183 ± 0.092
di-H_2O	0.00	72	F	1.174 - 1.370	1.274 ± 0.071
N-0923	1.00	72	F	0.818 - 1.232	1.082 ± 0.165
N-0923	5.00[b]	72	F	0.815 - 1.088	0.962 ± 0.115**
N-0923	15.0	72	F	0.783 - 1.475	1.178 ± 0.300

[a]As described in text, and except as noted, by-sex data are for 5000 erythrocytes per dose group (1000/mouse; five/group).
[b]Only 4000 erythrocytes scored per group due to the death of one animal in each group.
**Statistically significant decrease ($p < 0.01$).

Figure 62, from page 25 of Report 0309FD15.001

Table 4. Pharmacotoxic Signs Observed for N-0923 in the Definitive MNT

	Observation Time (after dosing)[a]							
	0 hrs		24 hrs		48 hrs		72 hrs	
Dose Level	♂	♀	♂	♀	♂	♀	♂	♀
0.00 mg/kg								
Normal	15	15	15	15	10	10	5	5
1.00 mg/kg								
Normal	15	15	15	15	10	10	5	5
5.00 mg/kg								
Abnormal gait	0	3						
Circling	0	1						
Found dead					0	1		
Hopping	0	2						
Quivering	0	2						
Sudden intermittent movement	15	0						
Normal	0	10	15	15	10	9	5	4
15.0 mg/kg								
Abnormal gait	4	5						
Found dead	1	1						
Hopping	11	10						
Labored respiration	1	0						
Prostration	1	0						
Quivering	9	6						
Rolling	3	0						
Twitching	3	0						
Normal	0	1	14	14	9	9	4	4
TEM (0.500 mg/kg)								
Normal	5	5	5	5				

[a]Animals were observed immediately, and approximately 24, 48 and 72 hours after dosing (five/sex/sacrifice time, if applicable). See also page 25.

Figure 63, from page 21 of Report 0309FD15.001

2.6.6.4.4 Measurement of Unscheduled DNA Synthesis (UDS) in Rat Hepatocytes Using an In Vivo Procedure with N-0923 (SPM 962)

Study no.: R 990704

Conducting laboratory and location: [redacted]
Date of study initiation:
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: WE10868

Methods

Strains/species/cell line: Rat, Fischer, Males only

Doses used in definitive study: 0, 6.25, 12.5 mg/kg IV

Basis of dose selection: lethality at 20 mg/kg IV

Negative controls: vehicle (distilled water)

Positive controls: dimethylhydrazine, 2-acetamidofluorene PO

Incubation and sampling times: 2-4 hours, 12-16 hours

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The methods used in the conduct of the study were adequate. The positive and negative controls were within historical control ranges. This reviewer is concerned about the appropriateness of intravenous injections as a route of administration for this drug. Rotigotine is administered as a transdermal patch in the clinic which would result in sustained serum levels. The intravenous administration would result in a relatively short exposure.

Study outcome:
Rotigotine was negative in this assay.

***In Vivo* UNSCHEDULED DNA SYNTHESIS TEST IN RAT HEPATOCYTES**

RECAPITULATIVE TABLE

Animal species: Fischer rats
Sex: male
Route: Intravenous
Number of animals per group: 3

ASSAY I: 2-4 HOUR EXPRESSION TIME

DOSE	Net nuclear grain count NNG		% cells in repair NNG≥5		Net nuclear grain count of cells in repair NNG≥5		% cells in S-phase
	Mean	± sd	Mean	± sd	Mean	± sd	Mean
SOLVENT CONTROL 0 Distilled water	-1.63	4.43	5.59	2.99	8.66	0.98	0.9
LOW DOSE 6.25 mg/kg	-2.04	3.94	2.79	2.11	6.8	0.28	0.4
HIGH DOSE 12.50 mg/kg	-2.42	4.18	4.44	1.73	7.37	1.73	0.6
POSITIVE CONTROL Dimethylhydrazine 10 mg/kg	15.94	6.86	87.38	3.76	19.2	7.55	1.3

ASSAY II: 12-16 HOUR EXPRESSION TIME

DOSE	Net nuclear grain count NNG		% cells in repair NNG≥5		Net nuclear grain count of cells in repair NNG≥5		% cells in S-phase
	Mean	± sd	Mean	± sd	Mean	± sd	Mean
SOLVENT CONTROL 0 Distilled water	-0.85	3.35	3.59	1.59	8.08	1.90	2.8
LOW DOSE 6.25 mg/kg	-0.36	2.90	4.15	2.65	7.38	0.00	0
HIGH DOSE 12.50 mg/kg	-0.29	3.01	3.09	2.52	6.39	0.90	0
POSITIVE CONTROL 2-acetamidofluorene 25 mg/kg	7.76	4.90	67.13	6.07	10.29	3.62	0.1

Figure 64, from page 19 of Report —R 990704

2.6.6.5 Carcinogenicity

2.6.6.5.1 104-Week Carcinogenicity Study of SPM 962 by Subcutaneous Administration in CD-1 Mice

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:

1. Rotigotine is a dopamine agonist which is administered via a dermal patch. Due to concerns about the feasibility of conducting a two year study using a dermal patch, it was decided that the sponsor could use subcutaneous injections every other day in an oily vehicle.
2. Although the available toxicokinetic data are inadequate for calculating AUC levels, the plasma levels exceed the plasma levels in the clinic (1 ng/ml).
3. No significant effects were observed on mortality at any dose. An adequate number of mice survived to termination to permit an adequate assessment of lifetime exposure.
4. At the high dose (30 mg/kg/48 hours), female body weight was decrease by 8-9% indicating that this dose was the approximate MTD.
5. At the high dose (30 mg/kg/48 hours), male body weight was decreased by about 4-6%, suggesting that higher doses could have been used.
6. No dose limiting toxicity was observed. Histopathological observations were confined to local irritation at the injection sites.

Evaluation of tumor findings:
No significant increase in tumors incidence was observed in either male or female mice. Data were reviewed by the Executive CAC (see page 214)

Study no.: 12484/99
Volume #, and page #:
Conducting laboratory and location:

Date of study initiation: December 6, 1999
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:
CAC concurrence: Yes (see page 212)

Methods

Doses: 0 (saline), 0 (vehicle), 3, 10, 30 mg/kg/48 hours

Group	SPM 962 dose [mg/kg b.w.]	SPM 962 dose volume [mL/kg b.w.] (concentration used)	Number and sex of animals M + F	Animal number	
				Main study	Satellite animals
1	0 (negative control)	3.0 (NaCl solution)	50 + 10 50 + 10	1 - 50 51 - 100	501 - 510 511 - 520
2	0 (vehicle control)	3.0 (SPM 962 placebo)	50 + 10 50 + 10	101 - 150 151 - 200	521 - 530 531 - 540
3	3 (low dose)	0.6 (0.5%)	50 + 10 50 + 10	201 - 250 251 - 300	541 - 550 551 - 560
4	10 (intermediate dose)	1.0 (1.0%)	50 + 10 50 + 10	301 - 350 351 - 400	561 - 570 571 - 580
5	30 (high dose)	3.0 (1.0%)	50 + 10 50 + 10	401 - 450 451 - 500	581 - 590 591 - 600

Figure 65, from page 27 of Report 12484/99

Basis of dose selection (MTD, MFD, AUC etc.): MTD
Species/strain: Mouse, CD-1 / .CD-1(ICR)BR
Number/sex/group (main study): 50/sex/dose
Route, formulation, volume: subcutaneous injection (3 regions on the back);
Frequency of dosing: every other day
Satellite groups used for toxicokinetics and prolactin: 10/sex/dose
Age: 5-6 weeks
Animal housing: 1 mouse/cage
Restriction paradigm for dietary restriction studies: NA
Drug stability/homogeneity:
Dual controls employed: yes (saline, vehicle)
Interim sacrifices: None
Deviations from original study protocol:

Results

Mortality:

No effects on mortality were observed.

Group	Survival rate [%] at test week 104	
	Males	Females
1	36	24
2	30	42[##]
3	36	46
4	38	28
5	34	32

* significantly different from the vehicle control (group 2) at $p \leq 0.05$ (Fisher test)
** significantly different from the vehicle control (group 2) at $p \leq 0.01$ (Fisher test)
[##] significantly different from the negative control (group 1) at $p \leq 0.01$ (Fisher test)

Figure 66, from page 46 of Report 12484/99

Figure 1 Mortality of male animals

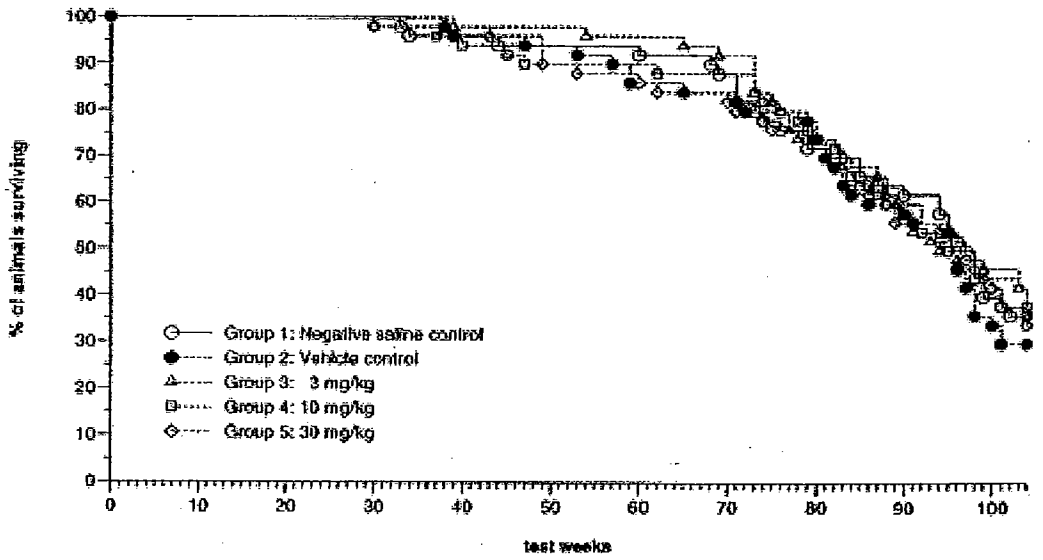


Figure 67, from page 47 of Report 12484/99

Figure 2 Mortality of female animals

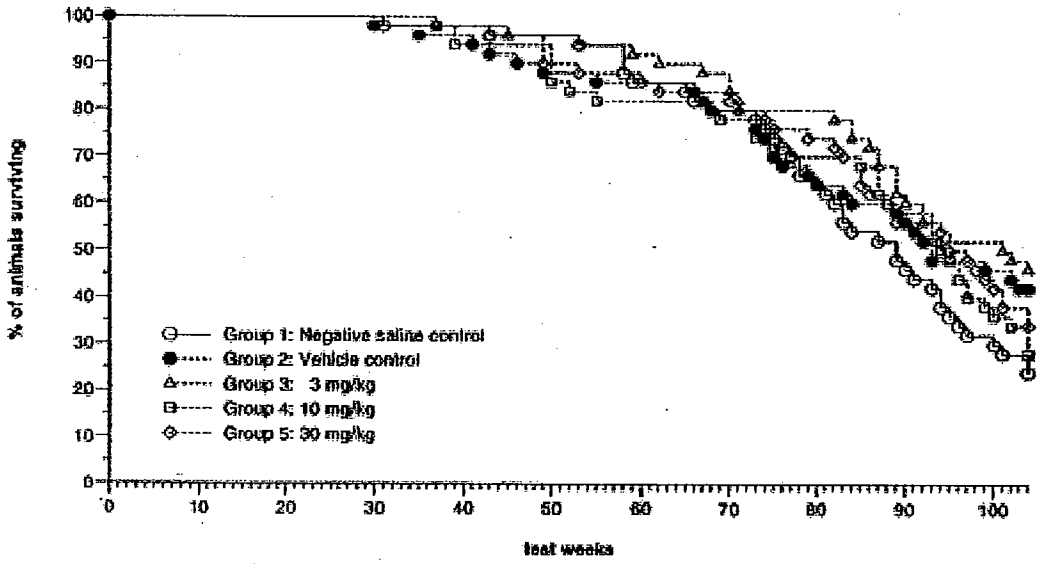


Figure 68, from page 48 of Report 12484/99

APPEARS THIS WAY
ON ORIGINAL

Clinical signs:
30 mg/kg- increased restlessness was observed in all mice for 2 to 6 hours post injection from week two through the end of the study.

Body weights:
Decreased body weights were observed at 10 mg/kg and 30 mg/kg. Although the changes were not statistically significant, they were consistent throughout the study. Body weight changes did not exceed 10%, suggesting that the doses did not exceed the MTD.

Comparison of Male Body Weights in g (% of Vehicle Control)

Week	Saline Control	Vehicle Control	3 mg/kg	10 mg/kg	30 mg/kg
0	26.2 (101%)	26.0 (100%)	26.4 (102%)	26.6 (102%)	26.4 (102%)
13	33.2 (99%)	33.4 (100%)	33.2 (99%)	32.2 (96%)	32.1 (96%)
27	34.7 (99%)	35.0 (100%)	35 (100%)	34.1 (97%)	33.7 (96%)
39	35.2 (100%)	35.3 (100%)	34.7 (98%)	33.9 (96%)	33.6 (95%)
53	35.6 (100%)	35.6 (100%)	35.3 (99%)	34.7 (97%)	34.2 (96%)
65	35.6 (99%)	35.9 (100%)	35.1 (98%)	34.5 (96%)	33.9 (94%)
79	36.5 (100%)	36.5 (100%)	36.1 (99%)	35.9 (98%)	34.4 (94%)
91	35.3 (100%)	35.3 (100%)	35.2 (100%)	34.6 (98%)	33.8 (96%)
103	36.4 (99%)	36.8 (100%)	36.0 (98%)	35.2 (96%)	34.8 (95%)

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

Comparison of Female Body Weights in g (% of Vehicle Control)

Week	Saline Control	Vehicle Control	3 mg/kg	10 mg/kg	30 mg/kg
0	21.2 (97%)	21.8 (100%)	21.6 (99%)	21.5 (99%)	21.7 (100%)
13	27.1 (99%)	27.5 (100%)	26.7 (97%)	26.4 (96%)	26.2 (95%)
27	29.2 (100%)	29.3 (100%)	28.1 (96%)	27.8 (95%)	27.5 (94%)
39	30.0 (101%)	29.8 (100%)	29.3 (98%)	28.4 (95%)	27.9 (94%)
53	30.9 (101%)	30.6 (100%)	29.9 (98%)	29.3 (95%)	27.8 (91%)
65	31.1 (100%)	31.0 (100%)	30.4 (98%)	29.8 (96%)	28.4 (92%)
79	31.6 (99%)	32.0 (100%)	30.9 (97%)	29.7 (93%)	29.5 (92%)
91	32.7 (103%)	31.6 (100%)	30.9 (98%)	29.8 (94%)	29.2 (92%)
103	33.8 (104%)	32.4 (100%)	30.8 (95%)	31.3 (97%)	29.9 (92%)

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

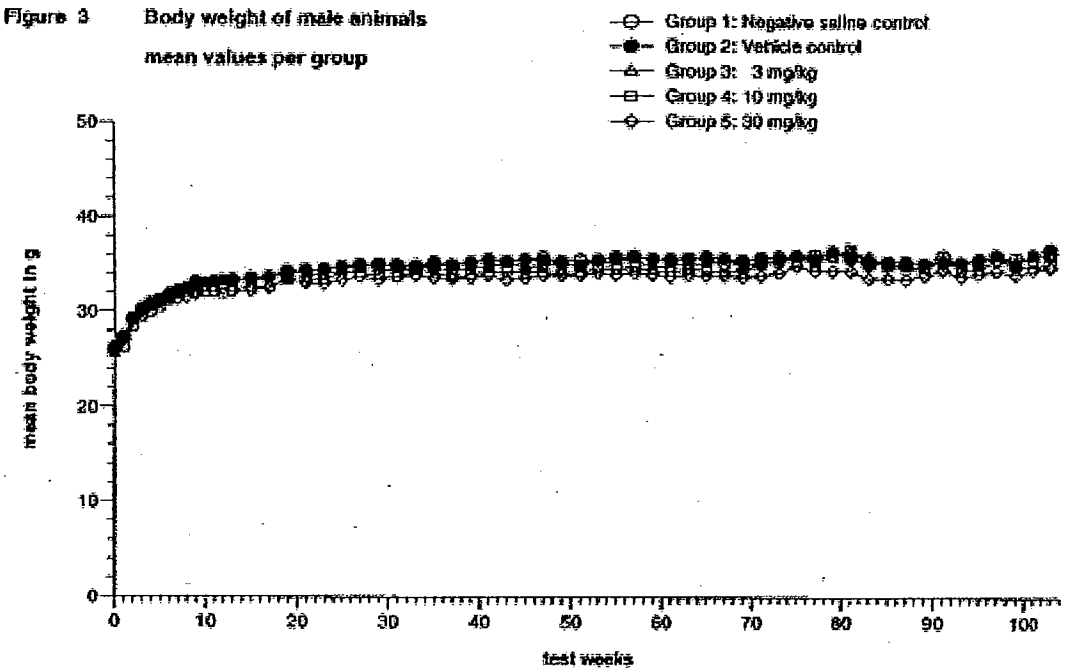


Figure 69, from page 56 of Report 12484/99

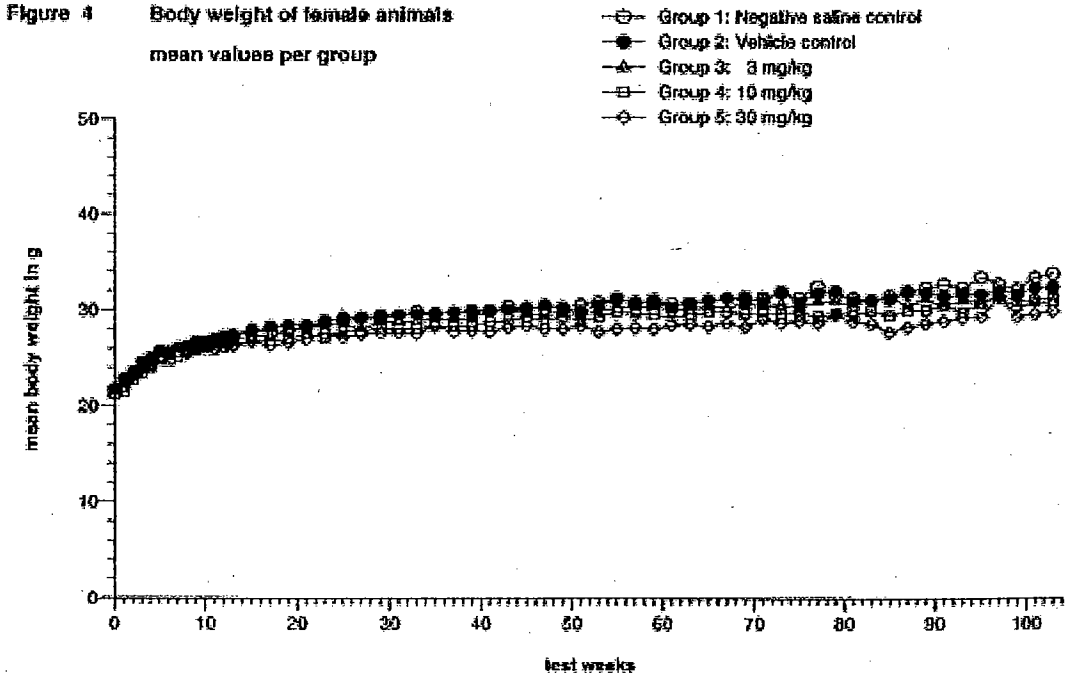


Figure 70, from page 57 of Report 12484/99

Food consumption:
No effects on food consumption were observed.

Gross pathology:
See discussion of injection site findings under non-neoplastic findings below. No other significant pathology was observed.

Histopathology:

Non-neoplastic:

Incidence of Retinal Atrophy

Sex		Saline	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
Male	Unilateral	0/50	1/50	4/50	5/50	0/50
	Bilateral	9/50	0/50	6/50	2/50	8/50
	Combined	9/50	1/50	10/50	7/50	8/50
Female	Unilateral	0/50	4/50	8/50	3/49	1/47
	Bilateral	8/50	2/50	5/50	3/49	12/47
	Combined	8/50	6/50	13/50	6/49	13/47

Incidence of Cardiac Fibrosis

Sex		Saline	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
Male	Ventricle	27/50	11/50	18/50	11/50	10/50
	Septum	15/50	3/50	5/44	4/46	1/50
Female	Ventricle	5/50	7/50	8/50	6/50	10/50
	Septum	2/50	1/50	1/49	1/49	6/50

The vehicle control group and all animals dosed with rotigotine had chronic inflammatory reactions in the area of the injection sites. Findings included fluid filled blisters, granulation tissue, edema and fibrosis. Findings were more severe in animals that received the highest volume (vehicle control and 30 mg/kg) than in animals receiving lower volumes (3 and 10 mg/kg).

Neoplastic:

No significant increase was observed.

Summary Tumor Incidence

PROJECT ID: 12484 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	36	(72)	30	(60)	24	(48)	29	(58)	27	(54)
Total Animals with Tumors	26	(52)	22	(44)	22	(44)	22	(44)	22	(44)
Total Animals w/ Multiple Tumors	8	(16)	7	(14)	2	(4)	5	(10)	5	(10)
Total Benign #	11	(30)	14	(46)	7	(29)	12	(41)	16	(59)
Total Malignant #	25	(69)	16	(53)	17	(70)	17	(58)	11	(40)
Total Malignant with Metastasis##	13	(52)	8	(50)	11	(64)	13	(76)	6	(54)

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 71, from page 1099 of Report — 12484/99

APPEARS THIS WAY ON ORIGINAL

Summary Tumor Incidence

PROJECT ID: 12484 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	49	(98)	44	(88)	38	(76)	44	(88)	27	(54)
Total Animals with Tumors	31	(62)	30	(60)	28	(56)	30	(60)	21	(42)
Total Animals w/ Multiple Tumors	13	(26)	10	(20)	9	(18)	11	(22)	5	(10)
Total Benign #	16	(32)	20	(45)	17	(44)	17	(38)	6*	(22)
Total Malignant #	33	(67)	24	(54)	21	(55)	27	(61)	21	(77)
Total Malignant with Metastasis##	22	(66)	13	(54)	12	(57)	16	(59)	18	(85)

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 72, from page 1103 of Report — 12484/99

The following table is taken form Dr. Roswitha Kelly's statistical review (pages 23 to 25)

Table 1: Pair-Wise and Trend Tests in Tumors among Male Mice*

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	HIGH	Pair-wise P-Value (Exact Method)	Pair-wise P-Value (Asymptotic Method)	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic Method)
1	Adrenals	15	ADENOMA SUBCAPSULAR CELL unila	1	0	0	0	1	0.4286	0.1311	0.2195	0.0411
1	Adrenals	22	CORTICAL ADENOMA unilat	1	1	0	0	0	1.0000	0.8188	1.0000	0.8236
1	Adrenals	49	PHAEOCHROMOCYTOMA	0	0	0	0	1	0.5455	0.1896	0.2500	0.0544
10	Harderian glands	12	ADENOMA PAPILLARY unilat	0	1	0	0	0	1.0000	0.8705	1.0000	0.8396
10	Harderian glands	7	ADENOMA	2	2	2	0	0	1.0000	0.8955	0.9696	0.9359
12	Injection site (I)	27	FIBROSARCOMA	0	1	0	0	0	1.0000	0.8705	1.0000	0.8396
12	Injection site (I)	43	MASTOCYTOMA	0	1	0	0	0	1.0000	0.8159	1.0000	0.8168
13	Injection site (III)	42	MALIGNANT MAST CELL TUMOR	0	1	0	0	0	1.0000	0.8493	1.0000	0.8135
16	Liver	30	HAEMANGIOMA	0	0	0	0	1	0.4348	0.1342	0.2500	0.0549
16	Liver	32	HAEMAGIOSARCOMA	2	1	0	0	0	1.0000	0.8159	1.0000	0.8168
16	Liver	33	HEPATOCELLULAR ADENOMA	2	3	2	2	4	0.4768	0.3332	0.2235	0.2164
16	Liver	34	HEPATOCELLULAR	3	2	6	5	2	0.6303	0.4434	0.7615	0.7653

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	HIGH	Pair-wise P-Value (Exact Method)	Pair-wise P-Value (Asymptotic Method)	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic Method)
			CARCINOMA									
17	Lower Jaw	27	FIBROSARCOMA	0	0	0	0	1	0.5455	0.1896	0.2500	0.0544
18	Lungs with bronchi	19	CARCINOMA BRONCHIOLO ALVEOLAR	6	0	1	1	1	0.5455	0.1896	0.3009	0.3089
18	Lungs with bronchi	8	ADENOMA BRONCHIOLO ALVEOLAR	2	4	1	6	7	0.2656	0.1750	0.0509	0.0472
2	Back side (skin)	27	FIBROSARCOMA	0	1	0	0	1	0.7431	0.4982	0.4375	0.3145
21	Mononuclear phagocytic tissue	35	HISTIOCYTIC SARCOMA	0	1	1	0	0	1.0000	0.8727	0.9559	0.8839
27	Prostate	30	HAEMANGIOMA	0	0	1	0	0	n/a	n/a	0.7073	0.7386
27	Prostate	7	ADENOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
28	Rectum	30	HAEMANGIOMA	0	0	1	0	0	n/a	n/a	0.6750	0.7506
3	Bone marrow (sternum)	47	OSTEOSARCOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
31	Spleen	32	HAEMANGIOSACROMA	1	0	0	0	0	n/a	n/a	n/a	n/a
32	Stomach (fundus r forestomach)	21	CARCINOMA UNDIFFERENTIATED	0	0	1	0	0	n/a	n/a	0.6750	0.7506
33	Testes	10	ADENOMA LEYDIG CELL bilat	1	0	0	0	0	n/a	n/a	n/a	n/a
33	Testes	11	ADENOMA LEYDIG CELL unilat	1	2	0	2	1	0.8532	0.6897	0.5211	0.5501
33	Testes	14	ADENOMA RETE TESTIS unilat	0	0	0	1	0	n/a	n/a	0.5139	0.5507
33	Testes	53	SCHWANNOMA	0	1	0	0	0	1.000	0.8188	1.0000	0.8236
33	Testes	54	SEX CORD STROMAL TUMOR	0	0	0	1	0	n/a	n/a	0.5139	0.5507
36	Urinary bladder	31	HAEMANGIOMA CARVERNOUS	0	0	0	0	1	0.6667	0.2509	0.4000	0.1291
36	Urinary bladder	57	TRANSITIONAL CELL CARCINOMA	0	1	0	0	0	1.0000	0.8159	1.0000	0.8168
4	Brain (cerebrum)	17	ASTROCYTOMA	0	0	1	0	0	n/a	n/a	0.7813	0.7341
4	Brain (cerebrum)	46	OLIGODENDROGLIOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
5	Coagulation gland seminal ves	54	SEX CORD STROMAL TUMOR	0	0	0	1	0	n/a	n/a	0.5139	0.5507
7	Dorsal region skin	27	FIBROSARCOMA	0	0	0	0	1	0.4286	0.1311	0.2195	0.0411
8	Epididymides	53	SCHWANNOMA	0	1	0	0	0	1.000	0.8705	1.0000	0.8396
9	Haematopoietic tissue	39	LYMPHOMA LYMPHOBLASTIC TYPE	0	0	1	1	0	n/a	n/a	0.6557	0.7198
9	Haematopoietic tissue	40	LYMPHOMA LYMPHOCYTIC TYPE	6	1	4	3	2	0.5089	0.2930	0.5877	0.5988
9	Haematopoietic tissue	41	LYMPHOMA PLEOMORPHIC TYPE	5	4	2	6	3	0.8305	0.7212	0.5669	0.5833
9	Haematopoietic tissue	45	MYELOID LEUCAEMIA	0	1	0	0	0	1.000	0.9264	1.0000	0.9028

* Tumor incidences from the saline controls are included for completeness only. Pair-wise comparisons are for vehicle control and high dose groups. Trend tests are for vehicle control, low, medium, and high dose groups.

The following table is taken form Dr. Roswitha Kelly's statistical review (pages 19 to 21)

Table 2: Pair-wise and Trend Tests for Tumor Incidences for Female Mice*

Organ Code	Organ Name	Tumor Code	Tumor Name	CTRL	CTRZ	LOW	MED	HIGH	Pair-wise P-Value (Exact Method)	Pair-wise P-Value (Asymptotic Method)	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic Method)
1	Adrenals	49	PHAEOCHROMOCYTOMA	0	2	0	1	0	1.0000	0.9167	0.8981	0.8754
10	Harderian glands	7	ADENOMA	0	1	0	0	0	1.0000	0.8177	1.0000	0.8056
11	Hindleg foreleg	2	ADENOACANTHOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
11	Hindleg foreleg	47	OSTEOSARCOMA	0	0	0	0	1	0.4324	0.1331	0.2162	0.0381
13	Injection site (III)	41	LYMPHOMA PLEOMORPHIC TYPE	0	1	0	0	0	1.0000	0.8649	1.0000	0.8229
14	Lacrimal glands	16	ADENOMA unilat	1	0	0	0	0	n/a	n/a	n/a	n/a
15	Leg	27	FIBROSARCOMA	0	0	0	0	1	0.5385	0.1861	0.2059	0.0380
16	Liver	30	HAEMANGIOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
16	Liver	32	HAEMAGIOSARCOMA	1	3	2	0	0	1.0000	0.9516	0.9954	0.9635
16	Liver	33	HEPATOCELLULAR ADENOMA	0	1	1	0	1	0.8077	0.5551	0.4826	0.4793
16	Liver	34	HEPATOCELLULAR CARCINOMA	0	2	0	1	1	0.8501	0.6871	0.4674	0.4810
18	Lungs with bronchi	19	CARCINOMA BRONCHIOLO ALVEOLAR	4	2	0	4	1	0.8784	0.7209	0.5703	0.5863
18	Lungs with bronchi	8	ADENOMA BRONCHIOLO ALVEOLAR	4	0	2	1	0	n/a	n/a	0.7413	0.8026
19	Lymph node (mesenteric)	30	HAEMANGIOMA	1	0	1	1	0	n/a	n/a	0.6316	0.6967
2	Back side (skin)	52	SARCOMA NOS	0	0	1	0	0	n/a	n/a	0.7813	0.7491
20	Mammary gland	25	FIBROADENOMA	0	1	0	0	0	1.0000	0.8649	1.0000	0.8229
20	Mammary gland	27	FIBROSARCOMA	0	0	0	1	0	n/a	n/a	0.6471	0.5620
20	Mammary gland	3	ADENOCARCINOMA	1	0	1	0	0	n/a	n/a	0.7600	0.8096
20	Mammary gland	6	ADENOCARCINOMA pr tumor not pr	1	0	0	0	0	n/a	n/a	n/a	n/a
21	Mononuclear phagocytic tissue	35	HISTIOCYTIC SARCOMA	4	1	3	4	3	0.2745	0.1381	0.2203	0.2150
22	Nasal cavity I (incl Nasoph)	47	OSTEOSARCOMA	0	0	0	1	0	n/a	n/a	0.6471	0.5620
23	Ovaries	23	CYSTADENOMA unilat	0	1	0	0	0	1.0000	0.8177	1.0000	0.8056
23	Ovaries	28	GRANULOSA CELL TUMOR bilat	0	0	3	0	0	n/a	n/a	0.8589	0.8694
23	Ovaries	29	GRANULOSA CELL TUMOR unilat	0	2	1	1	0	1.0000	0.8990	0.9383	0.9107
23	Ovaries	38	LUTEOMA unilat	0	1	0	1	0	1.0000	0.8673	0.8286	0.7980
23	Ovaries	55	SEX CORD STROMAL TUMOR mixed	0	1	0	0	0	1.0000	0.8177	1.0000	0.8056
23	Ovaries	58	TUBULOSTROMAL ADENOMA	0	0	1	1	1	0.4324	0.1331	0.2179	0.2269
24	Oviducts	36	LEIOMYOMA	0	1	0	0	0	1.0000	0.8177	1.0000	0.8056
24	Oviducts	48	PAPILLARY ADENOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
25	Pancreas	1	ACINAR CELL ADENOMA	0	1	0	0	0	1.000	0.8673	1.0000	0.8689
25	Pancreas	4	ADENOCARCINOMA ACINAR CELL	0	0	0	0	1	0.4323	0.1331	0.2162	0.0381
25	Pancreas	9	ADENOMA ISLET CELL	1	0	0	0	0	n/a	n/a	n/a	n/a
26	Pituitary	13	ADENOMA PARS DISTALIS	1	2	0	0	0	1.000	0.9275	1.0000	0.9160
3	Bone marrow (sternum)	35	HISTIOCYTIC SARCOMA	0	0	0	0	1	0.5238	0.1788	0.3143	0.0836

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	HIGH	Pair-wise P-Value (Exact Method)	Pair-wise P-Value (Asymptotic Method)	Trend P-Value (Exact Method)	Trend P-Value (Asymptotic Method)
30	Shoulder (left and right)	5	ADENOCARCINOMA MAMMA	0	0	0	1	0	n/a	n/a	0.6471	0.5620
31	Spleen	32	HAEMAGIOSARCOMA	2	2	0	0	0	1.0000	0.9167	1.0000	0.9003
34	Thymus	30	HAEMANGIOMA	0	0	0	2	0	n/a	n/a	0.5437	0.5882
34	Thymus	56	THYMOMA	0	2	0	1	1	0.9064	0.7608	0.6905	0.6559
35	Thyroids	18	CARCINOMA FOLLICUL CELL unilat	0	1	0	0	0	1.000	0.8649	1.0000	0.8229
37	Uterus (incl cervix)	24	EDNOMETRIAL STROMAL SARCOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
37	Uterus (incl cervix)	26	FIBROMA	0	0	0	2	1	0.5385	0.1861	0.2035	0.1827
37	Uterus (incl cervix)	27	FIBROSARCOMA	0	0	0	2	0	n/a	n/a	0.4217	0.4781
37	Uterus (incl cervix)	3	ADENOCARCINOMA	0	0	1	0	1	0.5333	0.1835	0.2939	0.2175
37	Uterus (incl cervix)	32	HAEMAGIOSARCOMA	1	0	2	0	0	n/a	n/a	0.8450	0.8339
37	Uterus (incl cervix)	36	LEIOMYOMA	0	2	1	0	1	0.8288	0.6467	0.6209	0.5842
37	Uterus (incl cervix)	37	LEIOMYOSARCOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
37	Uterus (incl cervix)	50	POLYP ENDOMETRIAL STROMAL	1	1	3	0	1	0.7380	0.4929	0.7150	0.7177
37	Uterus (incl cervix)	51	POLYP GLANDULAR	3	2	6	7	0	1.000	0.9474	0.9426	0.9348
37	Uterus (incl cervix)	53	SCHWANNOMA	0	1	0	1	0	1.000	0.8177	0.6498	0.7158
37	Uterus (incl cervix)	7	ADENOMA	2	0	0	0	0	n/a	n/a	n/a	n/a
38	Vagina	20	CARCINOMA SQUAMOUS CELL	0	0	1	0	0	n/a	n/a	0.7162	0.7252
38	Vagina	26	FIBROMA	0	0	0	1	0	n/a	n/a	0.6471	0.5620
6	Colon	44	MUCINOUS ADENOCARCINOMA	1	0	0	0	0	n/a	n/a	n/a	n/a
9	Haematopoietic tissue	39	LYMPHOMA LYMPHOBLASTIC TYPE	3	1	0	1	1	0.7380	0.4929	0.3951	0.3326
9	Haematopoietic tissue	40	LYMPHOMA LYMPHOCYTIC TYPE	6	3	4	5	4	0.4268	0.2860	0.3308	0.3328
9	Haematopoietic tissue	41	LYMPHOMA PLEOMORPHIC TYPE	6	6	4	4	6	0.5409	0.4319	0.2669	0.2712

* Pair-wise comparisons between vehicle control and high dose; Trend for vehicle control, low, medium, and high dose.
CTR1=saline control, CTR2=vehicle control.

Prolactin:

Mean (SEM) Serum Prolactin Levels in ng/ml

	Males			Females		
	TW26	TW52	TW104	TW26	TW52	TW104
Saline	9.0 (0.42)	9.0 (0.34)	1.3 (0.13)	17.2 (3.23)	28.7 (6.03)	4.0 (0.55)
Vehicle	9.7 (0.61)	9.9 (1.83)	1.6 (0.13)	31.3 (6.55)	21.7 (2.55)	7.2 (2.49)
3 mg/kg	8.8 (0.40)	8.5 (0.58)	1.2 (0.12)	17.0 (1.58)	18.3 (5.88)	4.1 (0.48)
10 mg/kg	8.8 (0.15)	8.9 (0.93)	1.1 (0.22)	20.4 (2.56)	22.1 (2.97)	4.1 (1.04)
30 mg/kg	8.0 (0.09)	9.3 (0.35)	0.9 (0.14)	16.1 (2.43)	16.1 (1.81)	4.7 (0.74)

Toxicokinetics: Weeks 26, 52 and 104; three pooled plasma samples/sex/dose; 3 mice were used per pooled sample (9 total mice/sex/dose).

Since there were only three samples/sex/timepoint, the median, minimum and maximum data are in essence the complete set of data for each time point.
Note: At 26 weeks, one 3 mg/kg male and two 3 mg/kg females had plasma levels less than the level of quantification (0.2 ng/ml).

This reviewer does not consider it appropriate to calculate AUC(0-48) values with only three time points/dose.

Week	Time [h]	Descriptive statistics [ng/mL] Males med	min	max	Females med	min	max
26	6						
	24						
	48						
52	6						
	24						
	48						
104	6						
	24						
	48						

At week 26, 48 hours results. Males n = 2, Females n = 1

Figure 73, 3 mg/kg data, from page 3191 of Report — -12484/99

Week	Time [h]	Descriptive statistics [ng/mL] Males med	min	max	Females med	min	max
26	6						
	24						
	48						
52	6						
	24						
	48						
104	6						
	24						
	48						

Figure 74, 10 mg/kg data, from page 3192 of Repor' — 12484/99

Week	Time [h]	Descriptive statistics [ng/mL] Males med	min	max	Females med	min	max
26	6						
	24						
	48						
52	6						
	24						
	48						
104	6						
	24						
	48						

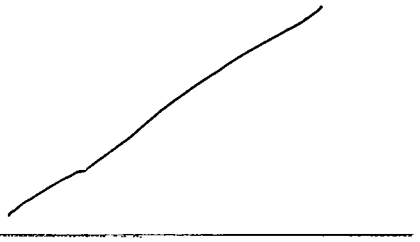


Figure 75, 30 mg/kg data, from page 3193 of Report — 12484/99

APPEARS THIS WAY
ON ORIGINAL

2.6.6.5.2 104-Week Carcinogenicity Study of SPM 962 by Subcutaneous Administration to Sprague-Dawley Rats

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:

1. Rotigotine is a dopamine agonist which is administered via a dermal patch. Due to concerns about the feasibility of conducting a two year study using a dermal patch, it was decided that the sponsor could use subcutaneous injections every other day in an oily vehicle.
2. The plasma levels exceeded the plasma levels in the clinic (1 ng/ml).
3. No significant effects were observed on mortality at any dose. An adequate number of mice survived to termination to permit an adequate assessment of lifetime exposure.
4. At the high dose (3 mg/kg/48 hours), female body weight was decrease by 8-17% indicating that this dose was the may have slightly exceeded the MTD. No effects on body weight were noted at the mid dose (1 mg/kg/48 hours).
5. At the high dose (3 mg/kg/48 hours), male body weight was decreased by about 7-9%, suggesting that an MTD was achieved in males.
6. No dose limiting histopathology changes were observed.
7. Mammary gland structure (only ductules formed) was altered in the mid and high dose females.
8. Vaginal squamous epithelium with keratin was observed at all doses.
9. Local irritation at the injection sites in vehicle and rotigotine treated rats. Severity of the irritation corresponded to the volume of vehicle injected.
10. Decreased serum prolactin levels were observed in rotigotine treated rats.

Evaluation of tumor findings:

1. A dose related increase in the incidence of Leydig cell hyperplasia and adenomas was observed in rotigotine treated rats at all doses. This is an expected finding since dopamine agonists (such as ropinirole and pramipexole) are known to increase the incidence of Leydig cell hyperplasia and adenoma.
2. An increased incidence of squamous cell carcinoma and adenocarcinoma was observed in the uterus. The number of tumors is small (0, 0, 2, 5 and 5 combined tumors in the saline, vehicle, low, mid and high dose groups, respectively) but these are rare tumors. Historical control data in this strain obtained from the Charles River Laboratories web site (http://www.criver.com/index.html) indicate that these are very rare tumors. In 24 carcinogenicity studies involving 1,729 rats, only 1 adenocarcinoma and 1 squamous cell carcinoma were observed (0.06% incidence, each). The Sponsor considers these tumors be secondary to the effects of a dopamine agonist on prolactin levels. The decreased prolactin levels would result in increased estrogen/progesterone ratio, but they did not provide data on estrogen/progesterone levels. This reviewer is less certain of this assumption. Increases in uterine tumors were not observed in carcinogenicity studies using two other dopamine agonists (ropinirole and pramipexole).
3. No effects on total tumor incidence were observed.
4. Data were reviewed by Executive CAC (see page 214)

Study no.: — 12485/99
Volume #, and page #: —248599-study-report.pdf
Conducting laboratory and location: [redacted]

Date of study initiation: January 13, 2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:
CAC concurrence: Yes (see page 212)

Methods

Doses: 0, 0, 0.3, 1, 3 mg/kg/48 hours
Basis of dose selection (MTD, MFD, AUC etc.): MTD
Species/strain: Rat, Sprague-Dawley / C .CD BR
Number/sex/group (main study): 50/sex/dose
Route, formulation, volume: subcutaneous injection into one of 3 injection sites (neck, just behind shoulder, caudal part of the back region)
Frequency of dosing: Every other day
Satellite groups used for toxicokinetics and prolactin levels: 10/sex/dose
Age: 5-6 weeks at initiation of dosing
Animal housing: individually housed
Restriction paradigm for dietary restriction studies:
Drug stability/homogeneity: Drug substance was stable
Dual controls employed: Yes, saline (group 1), vehicle (group 2)
Interim sacrifices: none
Deviations from original study protocol:

Results

Mortality:

No effect on mortality was observed.

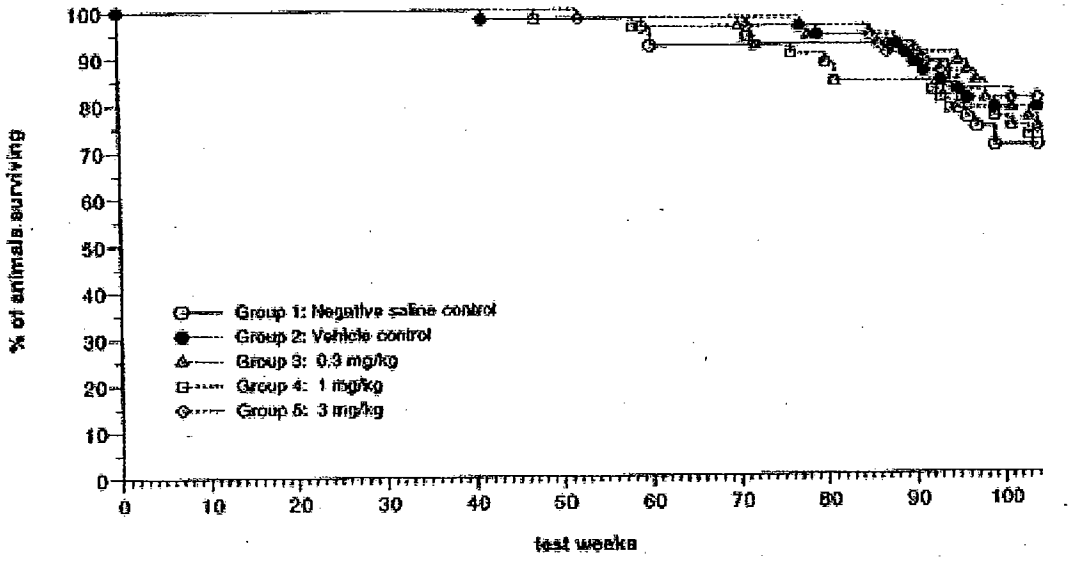


Figure 76, Male mortality, from page 51 of Report — 12485/99

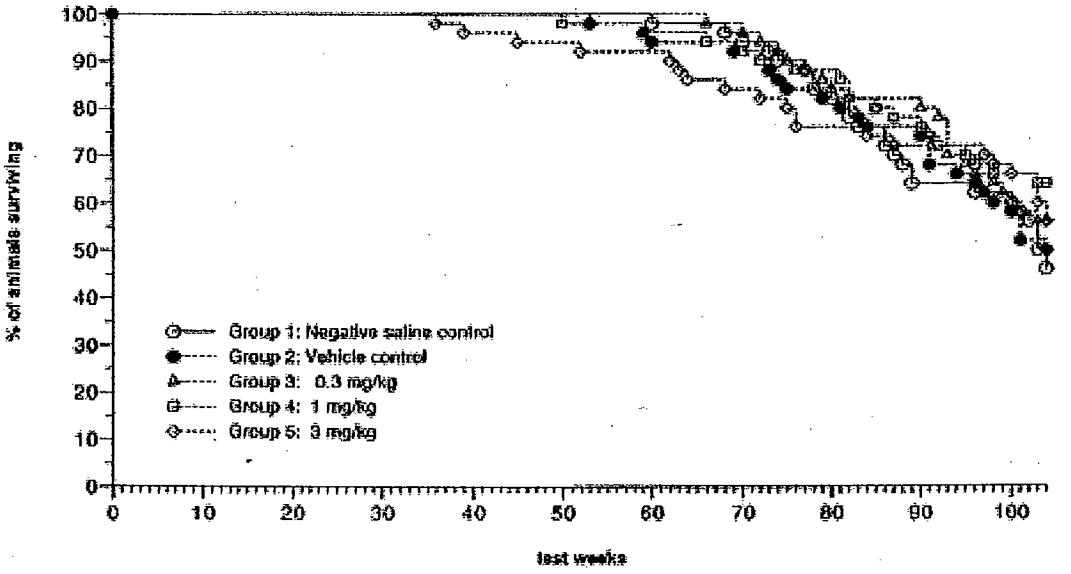


Figure 77, Female mortality, from page 52 of Report — 12485/99

Survival (in percent) in test week 104

	Group 1	Group 2	Group 3	Group 4	Group 5
Male	70	78	74	72	80
Female	46	50	56	64	56

Clinical signs:
Indurations were observed at the injection sites of vehicle control and high dose rats from week 27 onwards.

Increased restlessness was observed 2-6 hours post injection in high dose 3 mg/kg rats (males and females) from week 3 to the end of the study.

Body weights: 1X/week (weeks 1-13), 1X/2 weeks (weeks 15-103)

A dose dependent decrease in body weight was observed in both males and females, Male high dose body weight was reduced 7-9% compared to vehicle control. Female high dose body weight was reduced 8-17% compared to vehicle control. No effects were noted at the low dose, while the effect on the mid dose was intermediate.

Comparison of Male Body Weights in g (% of Vehicle Control)

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
0	194 (102%)	191 (100%)	188 (98%)	189 (99%)	189 (99%)
13	431 (100%)	432 (100%)	431 (100%)	422 (98%)	**400 (93%)**
27	508 (98%)	517 (100%)	514 (99%)	498 (96%)	**468 (91%)**
39	543 (100%)	545 (100%)	540 (99%)	527 (97%)	**495 (91%)**
53	566 (99%)	570 (100%)	563 (99%)	552 (97%)	**532 (93%)**
65	588 (99%)	592 (100%)	585 (99%)	574 (97%)	**553 (93%)**
79	612 (100%)	615 (100%)	618 (100%)	591 (96%)	**565 (92%)**
91	585 (98%)	599 (100%)	607 (101%)	587 (98%)	**558 (93%)**
103	583 (100%)	581 (100%)	600 (103%)	599 (103%)	559 (96%)

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

Comparison of Female Body Weights in g (% of Vehicle Control)

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
0	144 (99%)	146 (100%)	142 (97%)	144 (99%)	143 (98%)
13	257 (102%)	251 (100%)	253 (101%)	245 (98%)	**240 (96%)**
27	289 (102%)	283 (100%)	287 (101%)	275 (97%)	**266 (94%)**
39	306 (103%)	298 (100%)	305 (102%)	288 (97%)	**274 (92%)**
53	326 (103%)	317 (100%)	325 (103%)	304 (96%)	**280 (88%)**
65	345 (102%)	338 (100%)	342 (101%)	319 (94%)	**289 (86%)**
79	362 (98%)	368 (100%)	370 (101%)	343 (93%)	**306 (83%)**
91	350 (100%)	349 (100%)	355 (102%)	337 (97%)	**318 (91%)**
103	350 (103%)	340 (100%)	405 (119%)	345 (101%)	318 (94%)

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

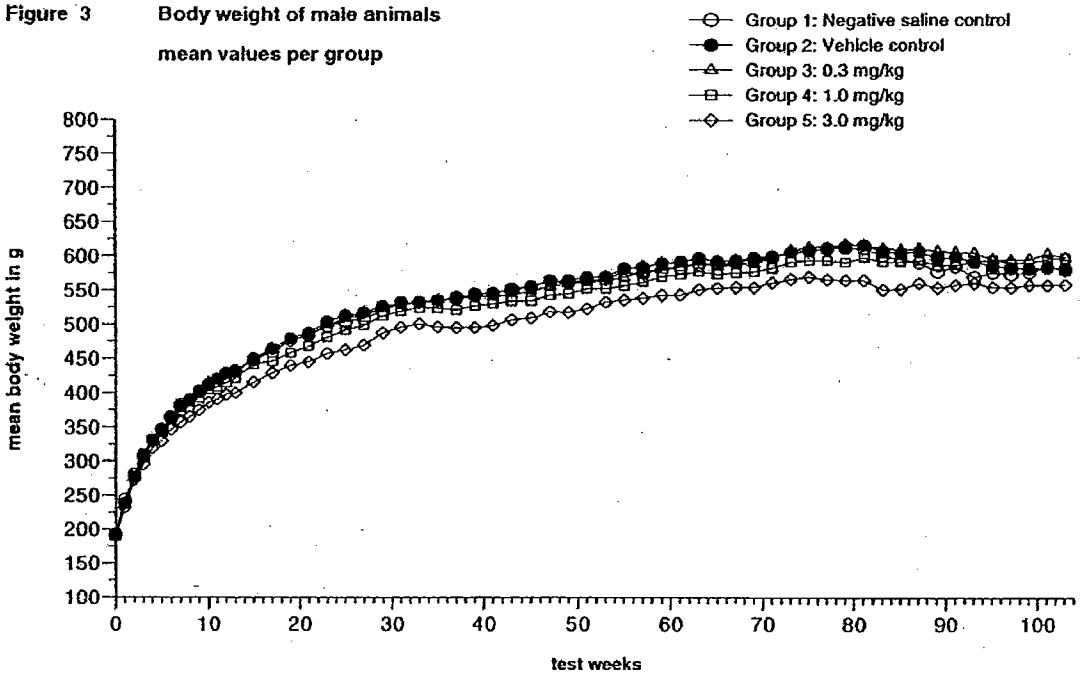


Figure 78, from page 60 of Report 12485/99

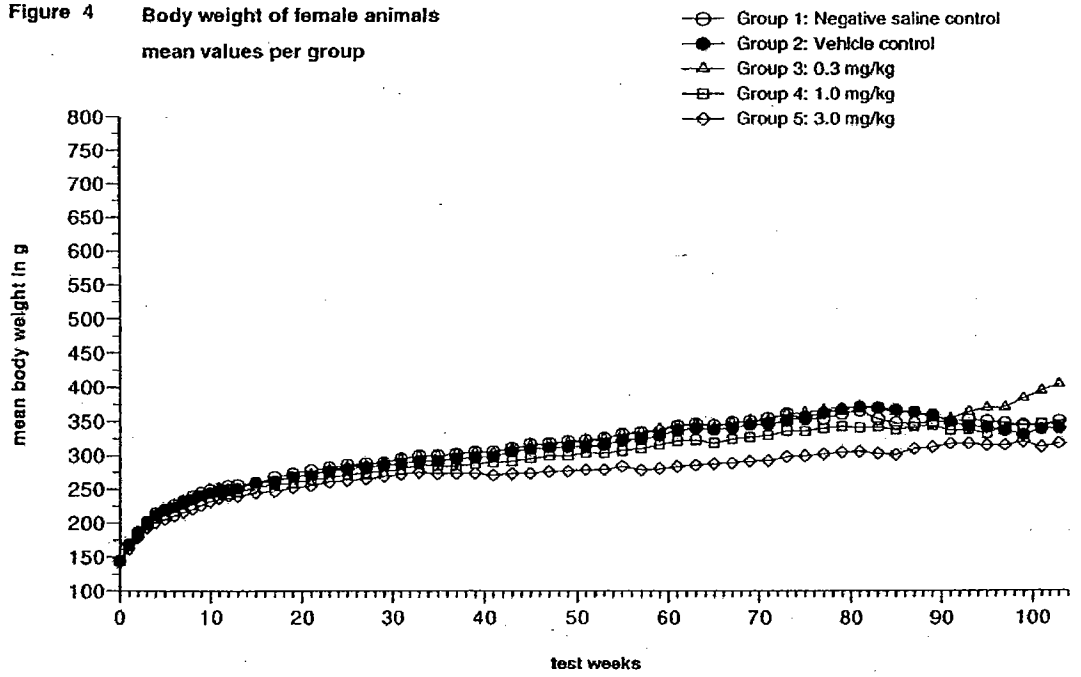


Figure 79, from page 61 of Report – 12485/99

Food consumption:
No significant effects on food or water consumption were observed.

Ophthalmology: Weeks 0, 52, 104; 10 rats/sex/group
No effects were noted on eye or hearing exams

Hematology: Weeks 52, 104
Hemoglobin (Hg), Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), platelets, White Blood Cell Count (WBC) and differential, Reticulocytes

No effects were observed

Clinical chemistry: Weeks 52, 104
Blood Urea Nitrogen (BUN), Creatinine, Glucose (Glu), Alkaline Phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Total Protein (TP), Albumin, Globulin, Albumin/Globulin ratio (A/G), Bilirubin (Bili), Cholesterol, Calcium, Sodium, Potassium, Chloride

No significant effects were observed.

Urinalysis: Week 52

No significant effects were observed.

Gross pathology:
Vesicles with Oily Liquid at Injection Site

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
Males	0/50	25/50	16/50	23/50	29/50
Females	0/50	19/50	6/50	17/50	20/50

Testicular Changes

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
Enlarged	1/50	2/50	4/50	7/50	9/50
Marbled	2/50	1/50	6/50	9/50	8/50
Discolored	2/50	0/50	6/50	10/50	5/50

Uterus Changes

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
Discolored liquid	1/50	0/50	0/50	1/50	6/50
Enlarged	0/50	0/50	0/50	2/50	4/50

Histopathology: Peer Reviewed; Complete in early decedents, high dose and control groups only. The following organs were examined from terminal sacrifice low and mid dose rats: adrenal, heart, injection sites, kidney, liver, lung, mammary gland, ovary, pituitary, stomach, testicle, masses, uterus, and thyroid.

Non-neoplastic:

Incidence of Retinal Atrophy

Sex		Saline	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
Male	Unilateral	3/50	6/50	0/13	0/14	0/50
	Bilateral	1/50	1/50	0/13	0/14	1/50
	Combined	4/50	7/50	0/13	0/14	1/50
Female	Unilateral	4/50	1/50	0/22	0/19	4/50
	Bilateral	1/50	1/50	1/22	0/19	5/50
	Combined	5/50	2/50	1/22	0/19	9/50

Incidence of Cardiac Fibrosis

Sex		Saline	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
Male	Ventricle	33/50	35/50	32/50	31/50	38/50
	Septum	17/50	13/50	1/40	3/50	17/50
Female	Ventricle	21/50	16/50	12/50	20/50	15/50
	Septum	6/50	1/50	2/44	0/50	1/50

The vehicle control group and all animals dosed with rotigotine had chronic inflammatory reactions in the area of the injection sites. Findings included fluid filled blisters, granulation tissue, edema and fibrosis. Findings were more severe in animals that received the highest volume (vehicle control and 30 mg/kg) than in animals receiving lower volumes (3 and 10 mg/kg).

Number of Large Follicles in the Ovary

	Saline Control	Vehicle Control	0.3 mg/kg	1 mg/kg	3 mg/kg
0	20/49	31/50	24/50	23/50	10/50
1-2	17/49	16/50	20/50	21/50	15/50
3-5	12/49	3/50	4/50	4/50	20/50
6-8	0/49	0/50	0/50	0/50	5/50