CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20658

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

ORIGINAL REVIEW

Submission: **NDA 20-658**

Sponsor: SmithKline Beecham
Four Falls Corporate Center
Rte 23 & Woodmont Ave.
PO Box 1510
King of Prussia, PA 19406

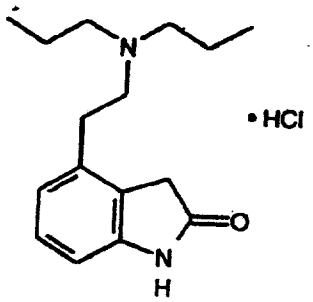
Received: 1/14/96

Reviewer: Brian Ault

Drug: Requip (ropinirole hydrochloride)
SK&F 101468-A
4-[2-(dipropylamino)ethyl]-2-indolinone HCl
ROP refers to ropinirole with doses expressed as mg base unless otherwise noted

Indication: Parkinson's disease

Structure:



mol wt 260 (base), 297 (salt)

Chemical name: 4-[2-(Dipropylamino)ethyl]-2-indolinone monohydrochloride (USAN)
SKF 101468 free base; 101468-A hydrochloride
CAS # 91374-20-8

Table of Contents

Pivotal GLP studies and laboratories where performed:

One-year Oral Toxicology Study of SK&F 101468-A in the Sprague-Dawley (CD) Rat (report TP1003/SKF-101468/2). Batch 11 Lot P9-JSD-811. SmithKline Beecham Labs, King of Prussia, PA.

An Oral Carcinogenicity Study of SK&F 101468-A in CD-1 Mice (report TP1004/SKF 101468/2). Lot P-9-JSD-811.

Two Year Carcinogenicity Study of SK&F 101468-A in the Sprague-Dawley (CD) Rat. Lot P12-JSD-942. SmithKline Beecham Labs, King of Prussia, PA.

1-Year Oral Toxicity Study of SK&F 101468-A in Cynomolgus Monkeys (report TP0015/SKF-101468/1). Lot P-9-JSD-811. SmithKline Beecham Labs, King of Prussia

Male fertility study of SK&F 101468-A in rats (report TP007BA) Batch 11, Lot P9-JSD-811. Smith Kline & French laboratories, Philadelphia, PA.

SK&F 101468-A: Fertility study in the female rat (report TW025BA) Batch 10. SK&F Research Ltd. Welwyn Garden City, Herts., U.K.

Oral teratology study in the rat (report TW023BA) Batch 10. SK&F Research Ltd. Welwyn Garden City, Herts., U.K.

Developmental toxicology study of SK&F 101468-A in pregnant rabbits (report TP004BA). Batch 10. Smith Kline & French laboratories, Philadelphia, PA.

Perinatal/postnatal study of SK&F 101468-A in female rats (report TP-0016/SKF-101468/1). Lot P9-JSD-811

SK&F 101468-A and levodopa: Oral study for toxicological and Embryo-Fetal Developmental Effects in Rabbits (report TP1010/SKF 101468/1). Toxicology Dept., SmithKline Beecham, King of Prussia, PA. Lot #LRS1 and MH-91-127-M1.

BACKGROUND

The primary symptomatology of Parkinson's disease is due to the degeneration of nigrostriatal dopaminergic neurons, and thus a loss of appropriate dopamine neurotransmission in the striatum. Symptomatic relief can clearly be attained using indirectly- or directly-acting dopamine agonists alone or in combination.

Dopamine acts at two families of G-protein coupled receptors designated D1 and D2, normally resulting in activation and inhibition of adenylate cyclase respectively. The D1 class includes D1 and D5 receptors, and the D2 class incorporates D2, D3 and D4 receptors (and splice variants). D2 receptors are present at high levels in the striatum and are thought to mediate anti-Parkinsonian activity. Pharmacological characterization has primarily focused on aggregate D1 and D2 activities, but varying DA receptor subtype agonism may play a role in therapeutic efficacy and side effect profiles of different compounds.

Two direct agonists employed clinically are bromocriptine (Parlodel) and pergolide (Permax). Bromocriptine is reported to be a full agonist at D2 receptors and a partial agonist at D1, while pergolide is a full agonist at both receptors.

Bromocriptine, pergolide and L-DOPA (with peripheral decarboxylase inhibitor) have similar profiles for therapeutic and adverse effects. All these compounds relieve the clinical symptoms characteristic of Parkinson's disease, but as an extension of their pharmacology can cause orthostatic hypotension (particularly on initial treatment), agitation, hallucinations and nausea. These effects are considered to be dopamine receptor mediated.

The metabolism of dopamine generates free radicals and could contribute to the degeneration of nigrostriatal neurons. One therapy directed at this putative mechanism is selegiline (Eldepryl), an MAO B inhibitor. On the basis that L-DOPA increases dopamine metabolism, the early use of direct DA agonists has been advocated as a mechanism to reduce doses of L-DOPA and thus free radical generation.

Direct agonists can also reduce on-off phenomena associated with L-DOPA monotherapy.

Ropinirole (ROP) is being developed for both monotherapy and combination therapy with L-DOPA. It has selectivity for D2-type receptors as compared to D1 receptors, DA-agonist activity in several species, and is efficacious in animal models of

Parkinson's disease.

PHARMACODYNAMICS

Pharmacological Actions Related to the Proposed Therapeutic Indication

Where indicated, studies employed the salt (hydrochloride). The base equivalent is 87.5% of stated dose.

Species	Test	Results/Conclusions
	IN VITRO STUDIES	
1) Receptor Binding and Specificity Studies		
Rat and human	**Binding to D2 and D3 receptors expressed in CHO cells. D2 receptors were the long forms (rat and human). Tables 1-3 PH1004/SKF101468/2**	**Binding showed 2 sites to cloned receptor. The data refer to high affinity sites. Note that there are discrepancies with binding from tissue (below). ROP is D3 selective, with moderate affinity for D2 sites.** Human D2 / Human D3 / Rat D2 / Rat D3 ROP Ki (nM) 1400 / 70 / 950 / 99 **ROP is less potent than bromocriptine and pergolide at D2 or D3 receptors in rat and human. D3 Selectivity (D2Ki/D3Ki) was: Human ROP (20) >Pergolide (5) > Bromo (1.6) Rat Pergolide (15) >ROP (9.6) > Bromo (1.1) The metabolites SKF 89124, 104557, 96990 were of equal or greater potency compared to ROP at D2 or D3 in rat and human. SKF 97930 was inactive. See Tables 1-3**
Human	**D4.4 receptor in HEK 293 cells Tables 1-3 PH-1005/SKF 101468/2**	**ROP Ki 1130 nM, so > 10-fold D3 selective. No other data.**
Rat	**Binding and ACh release (D2 mediated) from striatal slices following electrical stimulation. Table 4 PH0001/SKF101468**	**ROP binding relative to other compounds was fairly consistent with other tissue studies but the IC50 of release showed high affinity (IC50 90 nM) compared to several binding studies. ACh release indicated that bromocriptine and SKF 104557 had low efficacy, while SKF 89124 had higher potency and efficacy compared to ROP. Table 4.**

Human, Rat, Bovine	High affinity binding to wild type tissue homogenate Table 5 PW023BA, PP011BA	<u>Human ROP data</u> caudate D2 29nM Ki platelet α2 9μM IC50 temporal cx βadrenergic >100μM IC50 platelet peripheral BZ >100μM IC50 <u>Rat</u> Cx 5HT2, α1, α2 >10μM Ki <u>Bovine</u> Cortex benzodiazepine >>10μM IC50 Selectivity for D2 was high, but there is a question of relationship with cloned receptor data. DA receptor selectivity analysis should have been performed in normal human tissue. Bromocriptine and pergolide had activity at α1, α2 receptors (50 nM, 560 nM and 90 nM, 52 nM respectively)
Rat Bovine	Receptor binding PW019BA	High ROP selectivity for D2 (Ki 400 nM) over D1 (Ki >100μM), 5HT1 (> 10μM), 5HT2, benzodiazepine or GABA-A. Lack of D1 activity was also seen in a study of adenylate cyclase activation at 10 μM but no inhibition, which should be D2, was presented as a control. Opiate IC50 <10 μM. A study in guinea pigs indicated weak κ (447 nM Ki) and μ (700 nM Ki) activity. Bromocriptine had a Ki of ~100 μM at D1 and <100 nM at 5HT1. Pergolide had a Ki of ~5 μM at D1 and ~150 nM at 5HT1. Metabolites: SKF 89124 20-fold more potent at D2 compared to ROP (180-fold in CHO), and 104557 about half as potent (3-fold more potent in CHO).
Guinea-pig	Renal DOPA decarboxylase inhibition	No effect to 2 mM so ROP should not affect L-DOPA in combination treatment.
Rabbit Dog	Isolated ear artery constriction following nerve stimulation. [3H]-NE release from dog vascular tissue. See Table 7 pp004BA, PP010BA, PP005BA PP006BA	Absolute EC50 values for ROP varied from 27-100 nM. Within-experiment comparison showed SKF 89124 to be 50-fold more potent than ROP and SKF104557 to be 16-fold less potent (432 nM). ROP was antagonized by sulpiride (D2 antag) but the metabolites were not examined. Results are consistent with inhibition of NE release from dog saphenous vein and coronary artery.
IN VIVO STUDIES		
PRIMARY PHARMACOLOGICAL ACTIONS		
1) Mechanistic studies		
Mouse	caudate monoamine levels and metabolites measured following PO or IP dosing 0.03-10 mg/kg. PH1001/SKF101468/1	Both ROP (1-10 mg/kg IP) and bromocriptine (10-30 mg/kg IP) reduced HVA and DOPAC levels with DA unchanged. Consistent with presynaptic D2 receptor agonism.

Mouse Rat	1-100 mg/kg (salt) IP. Spontaneous locomotion. Figs. 1 and 2 PW005BA	Mouse Lower doses (10 mg/kg) inhibited while higher (100 mg/kg) stimulated activity. Sponsor believes data consistent with autoreceptor (low dose) and postsynaptic (high dose) DA receptor agonism (with higher receptor reserve for autoreceptors, presumably with same affinity. Note- there is no reason to assume different receptor reserve since direct agonism may overwhelm dec release). Similar to amphetamine (amphet) or apomorphine (apo). Rat Inc locomotion at 10-100 mg/kg with little stereotypy. Lack of stereotypy suggests lower likelihood of dyskinesia (ie mainly affects extrapyramidal system). Differs from amphet or apomorphine.
Rat	Injection into n. accumbens, or striatum (unilateral) PW005BA	Stimulation of locomotion observed, with circling after striatal injection. Weak effect of other compounds suggests postsynaptic agonist action of ROP greater than that seen with other DA agonists.
Rat	0.3-30 mg/kg (salt) SC Spontaneous locomotion and stereotypy PW034BA	Biphasic change in locomotion, with dec @ 0.3 and increases at 1-30 mg/kg. No effect of naloxone (ROP has some opiate receptor binding activity). Spontaneous activity (sniffing, licking) significant inc 3-30 mg/kg, with max effect about 50% of apomorphine.
Rat HL Marmoset	SKF 104577A studies 30 mg/kg SC normal rat 7.5, 15 mg/kg (salt) SC in unilateral 6-OHDA nigrostriatal lesion rats 0.32-10 mg/kg (salt) SC Locomotion. PH0002/SKF101468/2	Primary metabolite of ROP. 30 mg/kg produced sig decrease in locomotion. No effect on circling (ROP active @ 0.05 mg/kg) Only 10 mg/kg produced small inc in activity (about 50% of effect of 0.1 mg/kg ROP, so <1/100 of the activity, assuming bioequivalence)
Mouse	Unilateral 6-OH DA lesion. 0.001-100 mg/kg (salt) IP Table 6 PW015BA	Doses of ROP >0.01 mg/kg induced contralateral movement or circling. Similar max effect to apo. Suggests direct agonism on supersensitive receptors. Onset <10 min, duration 60-90 min.
Mouse	Unilateral 6-OH DA lesion. 1 mg/kg (salt) IP b.i.d. PW032BA	No tolerance observed to ropinirole-induced circling over 2 weeks.
Rat HL	Unilateral 6-OH DA lesion. ROP and metabolites up to 15 mg/kg IP PH-1002/SKF-101468/2	ROP was equipotent with SKF 89124, the main rat and dog metabolite, for induction of circling (0.05-0.8 mg/kg). SKF 104557, the main human metabolite, was 100-fold less potent (or inactive) vs ROP. SKF 96990 and SKF 97930 (metabolites in cynomolgus monkey with SKF 105557) were inactive to 15 mg/kg. Primary metabolites in man, monkey may not be agonists, or may not be CNS bioavailable.

Rat SD	Recording of single cell firing of nigrostriatal neurons. 1-64 µg/kg IV 0.25 mg/kg IP daily for 21 days PH-1006/SKF-101468/1	IV doses inhibited A9 (s. nigra pars compacta) DA neurons, probably by cell body receptors. Repeat doses inc the # spontaneously active cells and reduced the activity of IV ropinirole. Suggests desensitization of presynaptic receptors, and suggests these receptors are not critical for therapeutic efficacy. Similar reports for apomorphine.
Rat HL	0.032-0.32 mg/kg SC Lever responding and drug discrimination. PH-0005/SKF-101468/2	ROP or other D2 agonists did not show cross-discrimination to putative D1 agonist SKF 81297. SKF 81297 did not substitute for amphetamine in rats trained on amphet vs saline, but ROP was not tested. Data are consistent with non-D1 agonism, but D2 selectivity assessment relies on classification of other compounds that may not be well characterized.
2) Animal Models of Disease		
Rat SD	0.01-10 mg/kg (salt) IP Inhibition of oral dyskinesia induced by a DA agonist dipropylamino-6-dihydroxytetralin. PW027BA	ROP inhibited at 0.01-10 mg/kg IP, tiapride (which is especially effective against human dyskinesia) active at 1-40 mg/kg. This test presumably just indicates that ROP and tiapride block the DA receptors that the agonist activates (not defined)
Marmoset	0.1-1 mg/kg (salt) PO Marmosets treated with unilateral MPTP ic (s.nigra, 20 µg/24hr) over 14 days. Compounds tested over days 7-14 (because monkeys recover from motor deficits after end of infusion) Figs. 3 an 4 and see fig. 6-8 PW022BA	ROP 0.1 mg/kg reversed locomotor and behavioral (responses to stimulation, expression) deficits. 0.5 and 1 mg/kg reversed deficits but caused emesis in at least some animals. 50 mg/kg L-DOPA PO/benserazide 12.5 mg/kg SC reversed deficits and caused emesis. Bromocriptine 1 mg/kg PO had little effect.[Is this dose appropriate or does it indicate nonD2 effects of ROP? Sponsor suggests it has a long onset of action]
Marmoset	0.5 mg/kg (salt) PO BID from day 7-11. Animals treated with MPTP as above, with stable motor deficits at 7 days (94% dec in locomotion, 45% dec in head movement). PW028BA	Reversal of MPTP effects (e.g. return to 64% of normal locomotion) did not show tolerance over 4 days (cardiovascular effects do show tolerance). Mild ptosis observed on day 1 did decrease by day 4. No emesis was observed. The design of this study was poor since 0.5 mg/kg had a similar effect to 0.1 mg/kg in the prior study: limited tolerance would not be observable. Also, data for stability of MPTP effects on days 7-11 are not shown , only days 10-14.
Marmoset	0.01-1 mg/kg (salt) SC Animals as above Fig 5 PW017BA	Threshold 0.05 mg/kg SC; 0.1 and 1 mg/kg almost fully reversed locomotor deficits. Emesis occurred at 0.1 or above.
Marmoset	0.1-1 mg/kg (salt) SC PW016BA	Locomotor and behavioral activity essentially restored @ 0.1 or 1 mg/kg SC. Stereotyped behavior (repetitive wall climbing) was noted @ 1 mg/kg and emesis at both doses. Sponsor states that beneficial effects maintained for 7 days with BID dosing (presumably 0.1 mg/kg), but dose appears supra maximal and data not shown. Bromocriptine @ 0.1 mg/kg produced weak reversal of MPTP symptoms.

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Table 1

Low + High affinity sites in CHO HEK 293 cells

Table 3 Binding of Ropinirole and its Major Metabolites and Comparators to Human D_2, D_3 and D_4 Receptors

Compound	D_2 Ki1(nM)#	D_2 Ki2(nM)#	D_3 Ki1(nM)#	D_3 Ki2(nM)#	$D_{4.4}$ Ki(nM)##
Ropinirole	1380	20300	69.1	455	1130
SK&F 81924	26	1050	2.0	18.1	NT
SK&F 96990	57.8	1440	14.1	104	NT
SK&F 104557	2090	19600	107	792*	NT
SK&F 97930	29100	31000	28100	28000	NT
Dopamine	751	84400	44.4	284	322
Bromocriptine	3.11	3.76	1.94	1.93	NT
Pergolide	8.04	56.2	1.64	2.98	NT
Quinpirole	1040	48500	96.7	101	78.7

D2/D3
20
13
20
17
1.6
4.9

NT - not tested.
Ki1 and Ki2 represent the high and low affinity binding sites respectively (Data from [12]# and [19]##).

Table 4 Binding of Ropinirole and its Major Metabolites and Comparators to Rat Cloned D_2 and D_3 Receptors

Compound	D_2 Ki1(nM)	D_2 Ki2(nM)	D_3 Ki1(nM)	D_3 Ki2(nM)
Ropinirole	948	6990	98.6	3570
SK&F 81924	5.33	318	1.52	8.78
SK&F 96990	31.6	1440	5.64	26.4
SK&F 104557	333	7390	30.6	242
SK&F 97930	28600	29600	33000	32600
Dopamine	669	6280	11.7	290
Bromocriptine	2.64	2.45	2.44	2.44
Pergolide	21.5	54.6	1.5	1.56
Quinpirole	309	6540	29.8	584

Ki1 and Ki2 represent the high and low affinity binding sites respectively (Data from [12])

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Table 2 Cloned receptors

PH1004

Table 7. Effect of species on the pharmacology of D_2 dopamine receptors expressed in CHO cells. K_i represents data for the high affinity site.

Drug	K_i (nM) Rat	Human	Ratio Rat/Human
Quinpirole	310	1000	0.31
Ropinirole	950	1400	0.68
Bromocriptine	2.6	3.1	0.84
Dopamine	670	750	0.89
Quinelorane	49	26	1.9
Lisuride	0.39	0.16	2.4
Pergolide	22	8.0	2.8
Quinagolide	3.0	0.97	3.1
Apomorphine	430	46	9.3
Selegiline	>50,000	>100,000	---
SKF 104557	330	2100	0.16
SKF 89124	5.3	26	0.20
SKF 96990	32	58	0.55
SKF 97930	>25,000	>25,000	---
Domperidone	0.67	1.4	0.48
SCH 23390	820	1000	0.82
Spiperone	0.051	0.053	0.96
Raclopride	6.6	5.5	1.2
Iodosulpride	2.1	1.3	1.6

Note: CHO cells have low G-protein linkage so there may be equivalent of low affinity site in brain.

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Table 3

PH 10-4

Table 8. Effect of species on the pharmacology of D_3 dopamine receptors expressed in CHO cells. K_i represents data for the highest affinity.

Drug	K_i (nM) Rat	Human	Ratio Rat/Human
Dopamine	[redacted]	[redacted]	[redacted]
Quinpirole	30	97	0.31
Quinelorane	0.75	1.1	0.68
Pergolide	[redacted]	[redacted]	[redacted]
Bromocriptine	[redacted]	[redacted]	[redacted]
Ropinirole	[redacted]	[redacted]	[redacted]
Lisuride	0.3	0.19	1.6
Quinagolide	1.2	0.57	2.1
Apomorphine	190	63	3
Selegiline	>40,000	>50,000	----
SKF 104557	31	110	0.28
SKF 96990	5.6	14	0.4
SKF 89124	1.5	2	0.75
SKF 97930	>30,000	>25,000	----
Iodosulpride	3.9	2.6	1.5
SCH 23390	1700	1000	1.7
Raclopride	11	3.9	2.8
Spiperone	1.8	0.36	5.0
Domperidone	76	13	5.8

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Table 4 **Binding of Ropinirole and its Metabolites to D_2 Receptors and Inhibition of Acetylcholine Release From Rat Striatal Tissue**

Compound	Ki (nM)* [^{3}H]-sulpiride	Ki (nM)** [^{3}H]-spiperone	IC_{50} (nM) inhibition of striatal ACh release	Max inhibition
Ropinirole	424.6	29	90	51%
SK&F 81924	21.1	3.7	6	60%
SK&F 104557	966.7	180	>10000	71%
Quinpirole	NT	25	80	60%
Bromocriptine	8.6	28	>10000	20%

* data from [20]; ** data from [18]; NT - Not tested

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Table 5 **Affinity of Ropinirole in Radioligand Receptor Binding Assays**

Receptor site	Tissue	Ligand	K_i (M)	IC_{50}(M)
α_2-adrenoceptors [2]	Human platelets	[^{3}H]-Yohimbine	NC	$9x10^{-6}$
β-adrenoceptors[2]	Human temporal cortex	[^{3}H]-CGP 12177	NC	$>10^{-4}$
5-HT_1 receptors [1]	Rat forebrain	[^{3}H]-5 HT	$>10^{-5}$	NC
5-HT_2 receptors [2]	Rat forebrain	[^{3}H]-Ketanserin	NC	$5x10^{-5}$
Central BZ affinity [2]	Bovine Cortex	[^{3}H]-Flunitrazepam	NC	$>10^{-5}$
Peripheral BZ affinity [2]	Human platelets	[^{3}H]-PK 11195	NC	$>10^{-4}$
$GABA_A$ receptors [1]	Bovine cerebellum	[^{3}H]-GABA	$>10^{-5}$	NC
Muscarinic [1]	Rat forebrain	[^{3}H]-QNB	47% inhibition at 10^{-5}	NC

[1] Ref [20]; [2] Ref [23]; NC - not calculated

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FIG. 1 MOUSE SPONTANEOUS LOCOMOTOR ACTIVITY

Fig 1

PHA-1006/SKF-101468/2 PHA-1006/SKF-101468/2

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FIG. 2 STEREOTYPED BEHAVIOUR IN MOUSE

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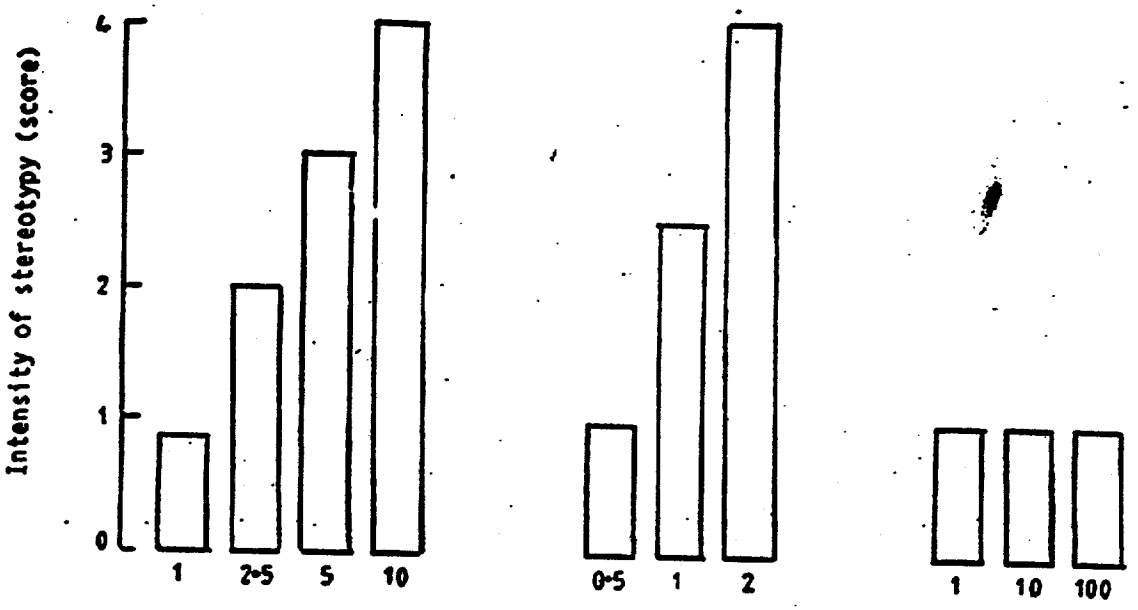

Onset

Within 10 min at 1 mg/kg
Within 5 min at higher doses

Duration

1.0 mg/kg : 60–75 min
10 mg/kg : 90–110 min
100 mg/kg : >2.5 hours

r measurement of stereotyped behaviour mice were placed in individual rspex cages and stereotypy assessed every 5–30 min according to the oring system:

- no stereotypy, behaviour indistinguisable from that of vehicle-treated animals
- periodic sniffing, head or limb movements
- continuous sniffing, head or limb movements

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Table 6

Asymmetric responding of mice having 6-OHDA lesions of the substantia nigra and challenged with SK&F 101468-A, apomorphine or amphetamine.

Drug	Dose (n=6)	Direction of[a] Asymmetry	Intensity of Asymmetry
SK&F 101468A	0.001 mg/kg i.p.	None	None
SK&F 101468A	0.01 mg/kg i.p.	C	1-2 (1.3±0.4)
SK&F 101468A	0.1 mg/kg i.p.	C	2 (2.0±0.0)
SK&F 101468A	1.0 mg/kg i.p.	C	2 (1.8±0.2)
SK&F 101468-A	100.0 mg/kg i.p.[b]	C	2 (2.0±0.0)
Apomorphine	0.5 mg/kg s.c.	C	2 (2.0±0.0)
Amphetamine	1.25 mg/kg i.p.[c]	I	1-2 (1.6±0.3)

[a] C - contralateral, I - ipsilateral, the direction being designated with respect to the side of the lesion.

[b] - prostrate appearance.

[c] - intensity of asymmetry could be increased with increasing dose, but marked stereotypies developed. In contrast, SK&F 101468-A, even at the highest doses, caused only a weak stereotyped sniffing behaviour.

A score of 1 denotes weak asymmetry to one side, 2 denotes marked asymmetry with body bent in one direction with retraction of limbs in one direction. A score of 3 denotes the same as 2 but with pronounced trunk bending such that animal is "nose to tail".

Doses of ropinirole are expressed as the salt.

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The Effect of a Unilateral Infusion of MPTP into the Substantia Nigra of the Common Marmoset.

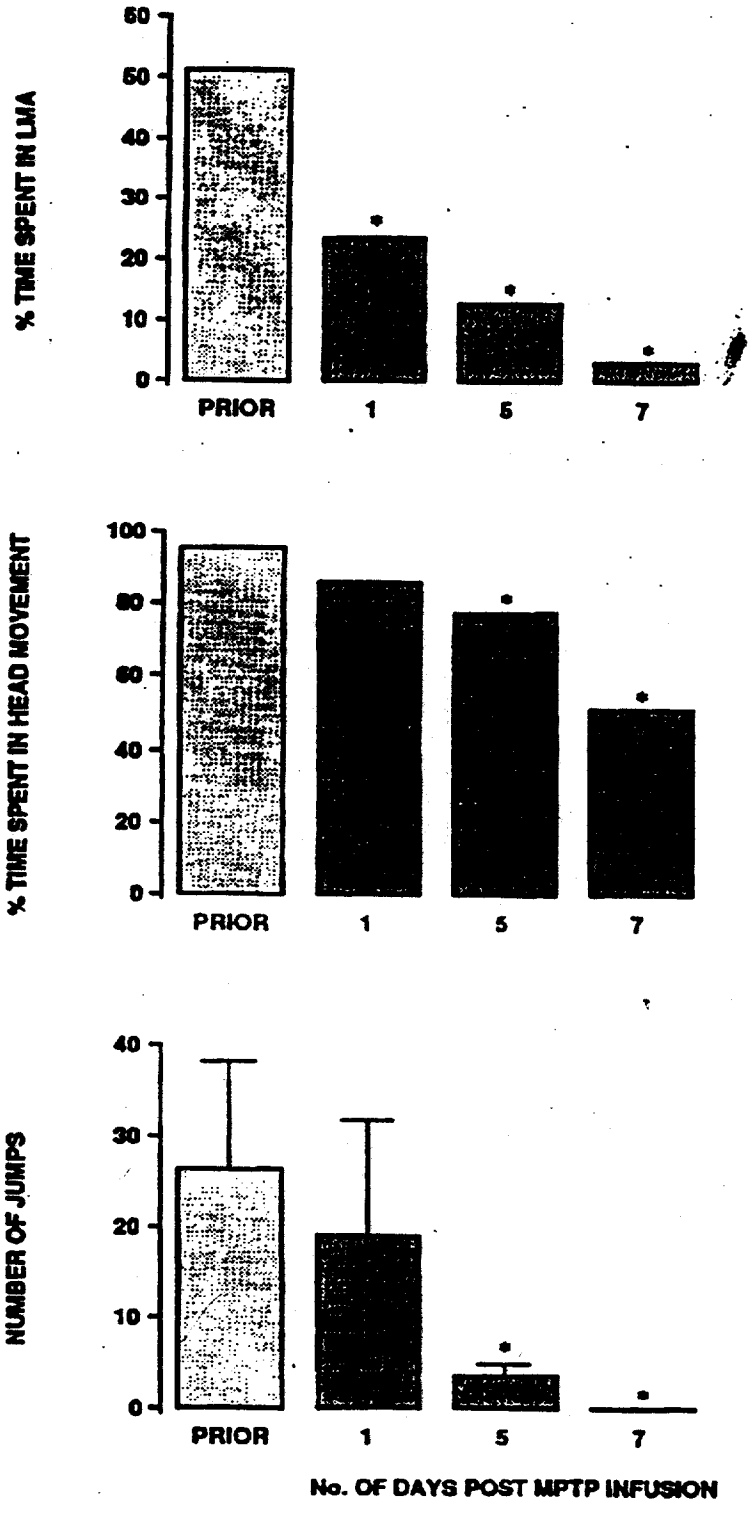

Fig 3

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THE EFFECT OF AN INTRA-NIGRAL MPTP INFUSION IN THE COMMON MARMOSET.

(Days 10-14 of infusion, n=3)

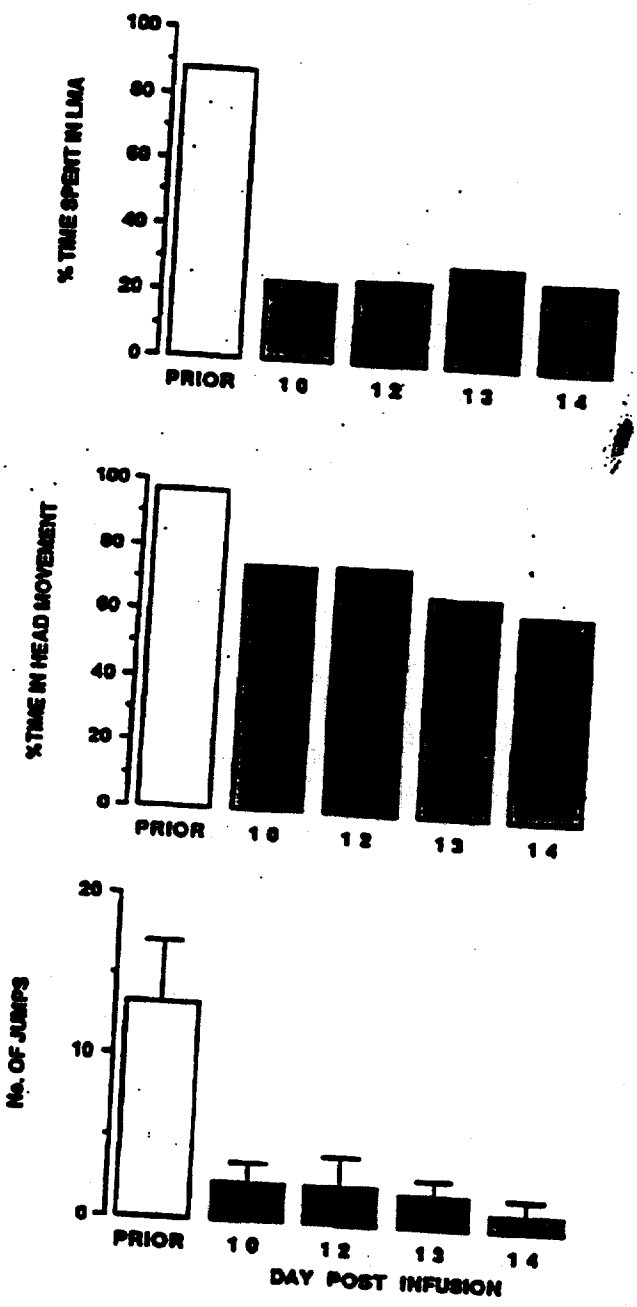

fig 4

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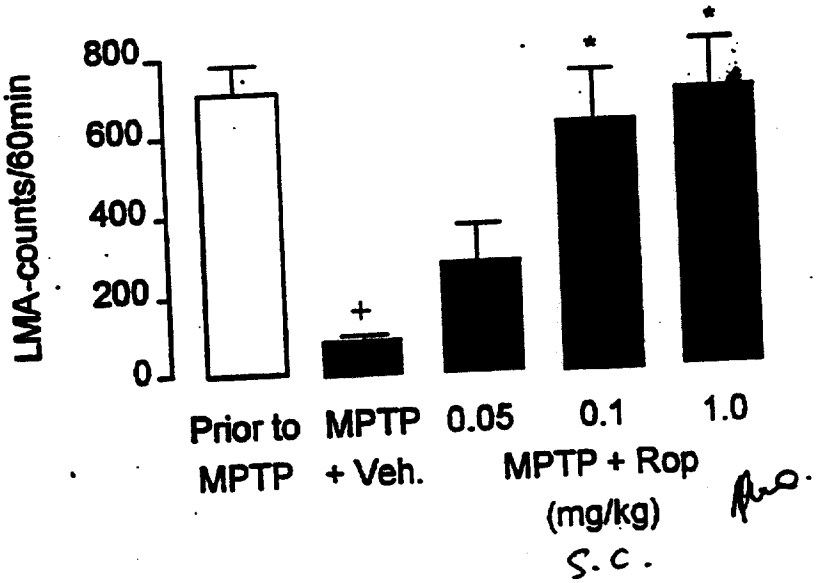
Figure 5 **Dose-response relationship of ropinirole reversal of MPTP-induced locomotor (LMA) deficits in the common marmoset**



Values represent mean ± s.e.m. (n = 3 or 4).
+ P<0.05 MPTP-induced reduction of activity
* P<0.05 reversal of MPTP-induced deficit [69].

Ropinirole given orally at doses of 0.5 and 1.0 mg/kg (doses expressed as salt) also reversed MPTP-induced locomotor deficits in marmosets. However, at the higher dose it caused hyperactivity, emesis and signs of nausea, the lower dose produced evidence of nausea and mild emesis in some animals. After a single acute administration of ropinirole (0.1 mg/kg p.o.; dose expressed as salt) MPTP-induced deficits were reduced but neither emesis nor the associated signs of nausea were seen in any animal. In addition to restoring the general activity of the Parkinsonian marmoset, ropinirole (0.1 mg/kg p.o.) reversed the more subtle behavioral deficits such as slowness of head movement, lack of interest in novel stimuli and the inability of the animal to change its facial expression [10].

L-dopa and bromocriptine were also studied in the MPTP-treated marmoset for comparative purposes. Treatment with a single acute oral dose of 50 mg/kg l-dopa (in combination with benserazide) was able to reverse both locomotor and behavioral deficits induced by MPTP. However, the effect was accompanied by

REVERSAL OF MPTP-INDUCED MOTOR DEFICITS BY SK&F 101468A
0.5 mg/kg p.o. b.d.) IN THE COMMON MARMOSET. (nmf)

0.5 mg/kg b.i.d.

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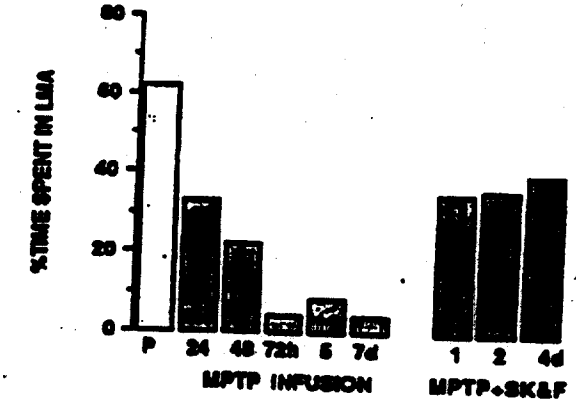

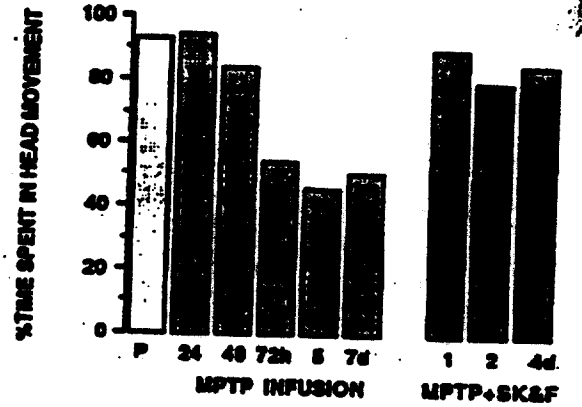

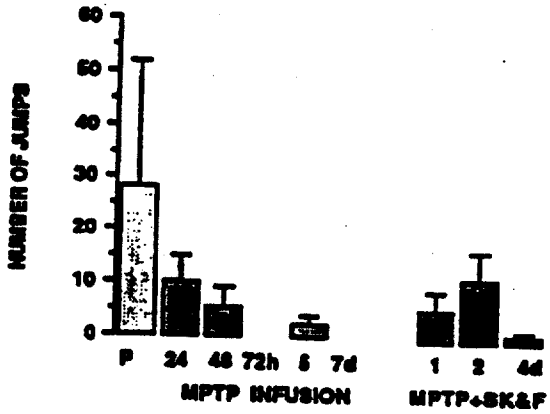

Fig 6

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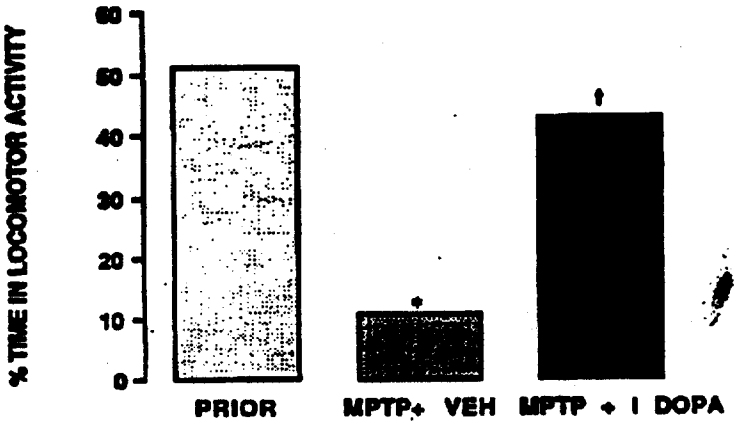
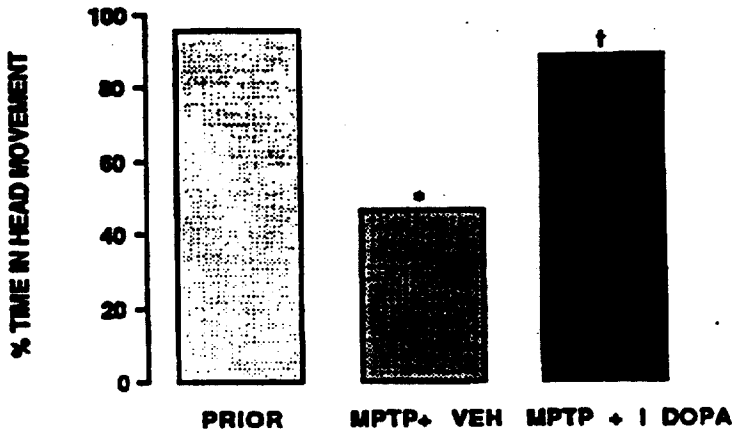
The Effect of l-DOPA on MPTP induced motor deficits in the Common marmoset.



50 mg/kg +60 min



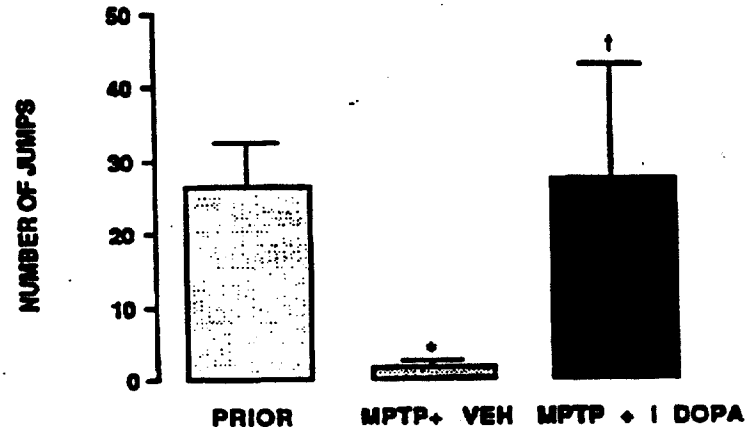


Fig 7

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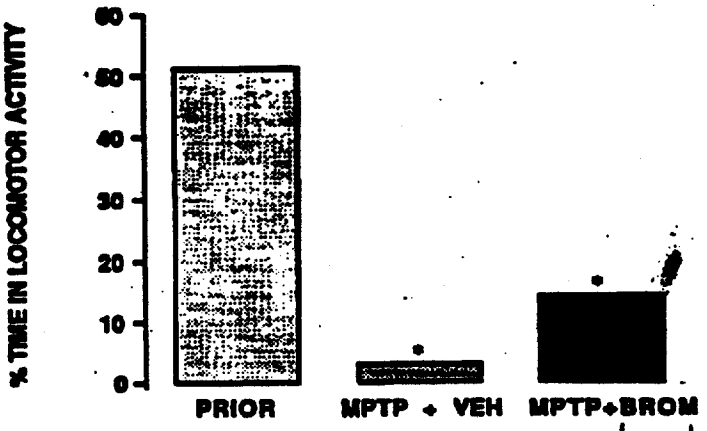
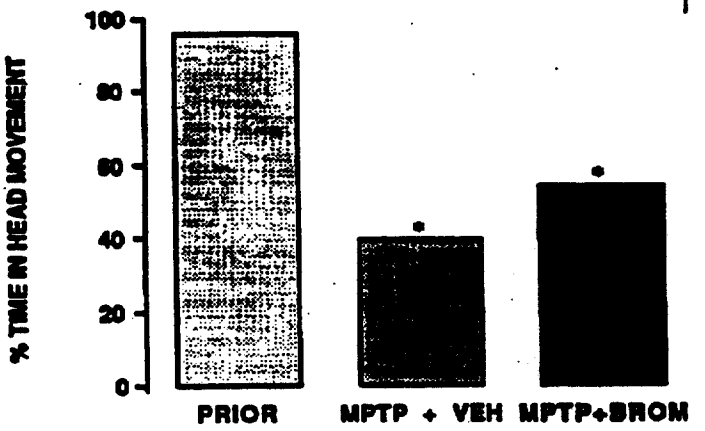
The Effect of Bromocriptine on MPTP Induced motor deficits in the Common marmoset.



1 mg/kg +60 min



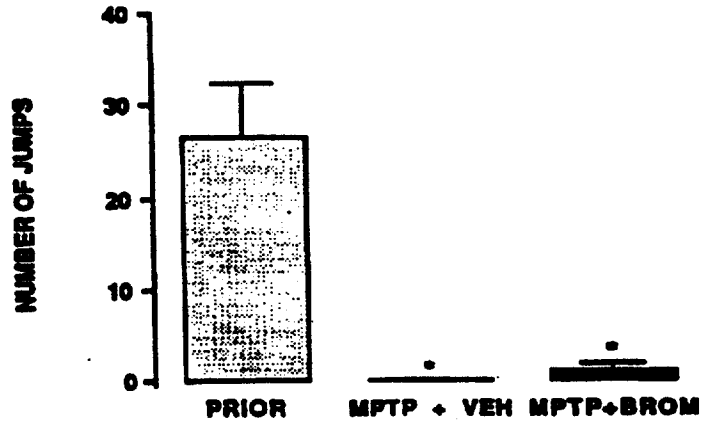


Fig 8

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TABLE 7: Comparison of the potency of various dopamine agonists as inhibitors of the tachycardia induced by electrical stimulation of the cardioaccelerator nerve in the anesthetized dog.

COMPOUND	CARDIOACCELERATOR NERVE* EC_{50} (ug/kg, IV)	RABBIT EAR ARTERY** EC_{50} (nM)
SK&F 85738-H	0.56 ± 0.23 (n=4)	4.0 ± 1.2[1]
SK&F 89124-A	0.98 ± 0.21 (n=4)	1.8 ± 0.3[2]
SK&F 101468-A	7.5 ± 1.8 (n=4)	100 ± 26[3]
SK&F 85174-J	26.4 ± 4.1 (n=7)	122 ± 33[4]
PBDA (SK&F 83797-A)	31.7 ± 4.3 (n=5)	110 ± 20[5]

*Mean ± SEM of ED_{50} values. Values for SK&F 89124-A and SK&F 85174-J differ slightly from previously reported values (Blumberg and Smith, 1982; Blumberg et al., 1985) due to inclusion of additional data points and/or re-analysis of data using more appropriate statistical methods.

**EC_{50} for inhibition of the constrictor response to brief, intermittent field stimulation of adrenergic nerve terminals. Expressed as mean ± SEM of EC_{50} values for individual experiments (n=4-12).

[1]Zeid and Hieble, 1984

[2]Huffman et al., 1983

[3]Gallagher et al., 1985

[4]Blumberg et al., 1985

[5]Steinsland and Hieble, 1979

SECONDARY PHARMACOLOGICAL ACTIONS		
1) Peripheral sympatholytic actions		
Dog Cat	Inhibition of increased heart rate following stimulation of cardioaccelerans nerve in anesthetized dog and cat. Table 7 PP004BA PW003BA	SKF 89124 was 8-fold more active than ROP (EC50 of 0.98 and 7.5 µg/kg IV [dog]. Reduction in stimulated tachycardia was also seen with 10 or 50 µg/kg IV in the cat. Sulpiride sensitive, so consistent with D2 agonism.
Monkey	Inhibition of increased heart rate following stimulation of cardioaccelerans nerve in anesthetized cynomolgus monkey. PW001BA	50 and 150 µg/kg IV decreased the tachycardia response to 0.5 or 1Hz stimulation, but not higher frequencies. 10 µg/kg IV was at the threshold of activity. BP increase was not reduced. These doses also decreased basal BP and HR. Consistent with reduced NE release which can be overcome by high stimulation.
2) Anxiolytic action (PW018BA, PW026BA, PW030BA, PW029BA)		
Mouse	swim test and bright field activity to assess anxiolytic/antidepressant actions of Ropinirole	1-10 mg/kg IP of ROP showed activity in a swim test (despair model) and reduced aversion to a bright open field. Bromocriptine did not share these activities. No tolerance to the anxiolytic action was noted over 14 days, and no rebound anxiogenic effect similar to diazepam was observed following chronic treatment.
Marmoset	0.01-10 µg/kg SC behavioral testing	0.01-0.1 µg/kg significantly decreased responses associated with interaction anxiety.
3) Abuse liability		
Monkey	withdrawal/dependence studies TF1004,TF1005,TF1002, TF1006/SKF-101468	ROP did not modify morphine or phenobarbitone withdrawal @ 15 mg/kg po. Daily administration of up to 7.5 mg/kg po for periods of 28 days did not induce physical dependence. In a study of psychological dependence, the desire to self administer (following lever presses) was studied. ROP was a weak stimulant in 1/4 animals of self administration and probably possess little dependance liability.
4) Miscellaneous studies PP1001, TF1001,/SKF101468/1, PW009BA,		
Mouse	Motor coordination	No effect to 100 mg/kg po
Mouse	Body temperature	Dose-related decrease in core temperature above 10 mg/kg (>5°C @ 100 mg/kg)
Rat	Body temperature	Dose-related decrease in core temperature above 10 mg/kg (significant change of -1.9°C @ 100 mg/kg)
Rabbit	EEG	10 mg/kg po increased awake time. No abnormal waveforms although behavioral changes (hyperactivity) were observed.
Mouse	GI transit	No effect to 100 mg/kg po. Guinea pig ileum contractility was unaffected.

Mouse	Irwin screen	10-100 mg/kg po decreased locomotion. 10-100 mg/kg prolonged hexobarbital sleep time but not anesthesia threshold. Sponsor implicates changes in barbiturate metabolism. No anticonvulsant, proconvulsant or analgesic effects were noted to 100 mg/kg. SKF 89124 po had generally similar effects to ROP (PW013BA) with similar or lower potency.

Safety Pharmacology

Species	Test	Results/Conclusions
1) Cardiovascular studies		
Rat	Coagulation time PP1001/SKF101468	No effect on prothrombin time or activated partial thromboplastin time up to 100 mg/kg
Rat SHR	HR and BP in anesthetized animals 0.025-0.5 mg/kg IV ROP over 6 min Fig. 9 PW007BA, TF1001/SKF104557 PW013BA	Dose dependent reductions in BP and HR were observed (although controls also had dec BP). Significant differences were at 0.025 mg/kg (BP) and 0.1 mg/kg (HR). Maximal changes @ 0.5 mg/kg were -79 mmHg and -64 beats/min, but were probably not the maximum achievable. In a second (bolus) study the max effect of ROP was about -60 mmHg @0.03 or 0.1 mg/kg, the metabolite 104557 was <30-fold less active but did act by D2 (sulpiride antagonized effect). SKF 89124 was active above 5 µg/kg and had about 4-fold greater potency than ROP.
Rat SHR	HR and BP in conscious animals 0.5-5 mg/kg IV over 6 min and 10-40 mg/kg PO Figs. 10+11 PW006BA, PW013BA	Significant reductions in BP were noted for 2.5 and 5 mg/kg IV (24.5-28 mmHg). 20 and 40 mg/kg PO reduced BP significantly (19-25 mmHg). Partial recovery was seen at 2.5-5 hrs. HR was very variable (possibly direct reduction of sympathetic flow with reflex tachycardia after dec BP). SKF89124 was about half as potent as ROP. DA2 antagonists antagonized these effects.
Rat wistar	HR and BP in conscious animals ROP 1-100 mg/kg PO PP1001/SKF101468/1	1, 10 mg/kg no effects 30 mg/kg non-significant inc in HR 100 mg/kg inc spontaneous activity and obscured changes in BP. Apparent inc in HR.
Rat SHR	Tolerance to cardiovascular effects of ROP. 10, 20 and 40 mg/kg bid for up to 14 days followed by 0.5 mg/kg IV under anesthesia. Fig. 12 PW020BA	Resting BP and HR were normal after 2 days bid treatment. Daily treatment had a dose-related effect to reduce the decrease in BP and HR following IV challenge (0.5 mg/kg). After 40 mg/kg the response to IV ROP was similar to saline. Increasing the dose of ROP did not restore the response and cross-desensitization occurred to bromocriptine. Suggests down-regulation of DA receptor/ coupling process.

Rat Normal and SHR	Interaction of ROP with tilt (supine to vertical, head up)-induced hypotension in anesthetized rats (model of orthostatic hypotension). 5-50 µg/kg IV. Figs. 13 and 14 PP007BA	In preliminary expts, doses of 450 µg/kg (normal) or 300 µg/kg (SHR) produced a large potentiation of the tilt-induced fall in BP and blocked normal recovery of BP and inc in HR. In this study 15 µg/kg inhibited BP recovery and 50 blocked recovery. 5 was inactive. 15 µg/kg reduced reflex tachcardia and 50 µg/kg produced bradycardia-hypotension+bradycardia in a tilt test is a syncope-type response. Clonidine also inhibited recovery. Data are consistent with sympatholytic effect of ROP. Should have used bromocriptine or pergolide.
Cat	50 and 500 µg/kg IV over 10 min in anesthetized cat. Hemodynamic effects of vena cava occlusion. PW003BA, PW021BA, PW025BA	50 and 500 µg/kg IV ROP Decreased BP by 18% and 37% respectively. 500 µg/kg IV inhibited the normal recovery of BP following vena cava occlusion, as observed with tilt-induced hypotension. L-DOPA/benserazide caused a decrease in BP/HR but did not affect responses to ROP.
Dog	Hemodynamic effects in anesthetized dog ROP 100 µg/kg IV SKF 89124 10 µg/kg IV Figs. 15 and 16 PP003BA PW012BA	Primary effects were reduction in BP (- 15-25%) and HR (89124 -15%; ROP -6%) resulting in decreased cardiac metabolic rate and coronary flow.
Monkey	Hemodynamic effects in anesthetized cynomolgus monkey and tolerance to BID dosing. 0.1 mg/kg IV, 5 mg/kg po Fig. 17 PW024BA PW001BA	NOEL 10 µg/kg IV. 50 µg/kg and 0.1 mg/kg IV caused decreases in BP (about -30 mmHg) and HR (-16 to 20 bpm). 5 mg/kg po BID for 7-14 days greatly reduced the response, which could not be restored by increasing the dose of ROP. Cross desensitization occurred to bromocriptine (0.3 mg/kg IV produced 33 mmHg fall in BP), supporting receptor /coupling desensitization. Responses to cardiac accelerans stim may have been reduced, but the effect was small initially (and only occurs at low Hz).
Sheep	Purkinje fiber studies in vitro. PW002BA	only 100 µM reduced resting potential, rate or amplitude of depolarization. Prolongation of action potential duration (10-15% max) was observed at low stimulation frequency. Not likely to be arrhythmogenic.
Guinea-pig	Isolated perfused heart and right atria PP009BA, PW008BA	At 10 µM ROP had no effect on contractility of isolated atria but left ventricular contractility and HR was decreased at micromolar levels. Propranolol was about equipotent. SKF 89124 decreased contractility, rate and output (threshold 0.1 µM for dV/dt, 1 µM HR). Coronary flow and isoprenaline effects were not affected
2) Respiration		
Cat	Anesthetized male animals 50 and 500 µg/kg (salt) IV. PW011BA	No changes in respiratory function (rate, vol, airway resistance) at doses that decrease BP (max -15 mmHg) and HR (max -29 bpm).
3) Autonomic nervous system		

Cat	Anesthetized M+F animals 50 and 500 µg/kg (salt) IV. PW004BA	Both doses decreased BP and HR. Reflex tachycardia and inc BP from carotid occlusion was reduced, but responses to NE IV were potentiated. Consistent with decreased NE release and effect of baseline BP, HR. It was unclear whether vagal and ACh IV bradycardia and dec in BP were reduced by changes in baseline or by other mechanisms. The interaction with another agent that reduced BP could be used to analyze this, but there was not a large interaction in any case.
4) Renal studies		
Rat	Conscious normotensive animals 30 µg/kg/min for 20 min and 3 µg/kg/min for 75 min (salt). Figs. 18 and 19 PP001BA	Urine flow and K+ output were significantly decreased in both studies. Urea excretion also was reduced in the second study. BP was not changed in conscious animals (BP was dec in satellite anesthetized rats). GFR did not change but effective renal plasma flow was sig. reduced at 30 min. Sponsor indicates that K+ and urea changes are related to dec urine flow, with the basis for dec urine flow unclear.

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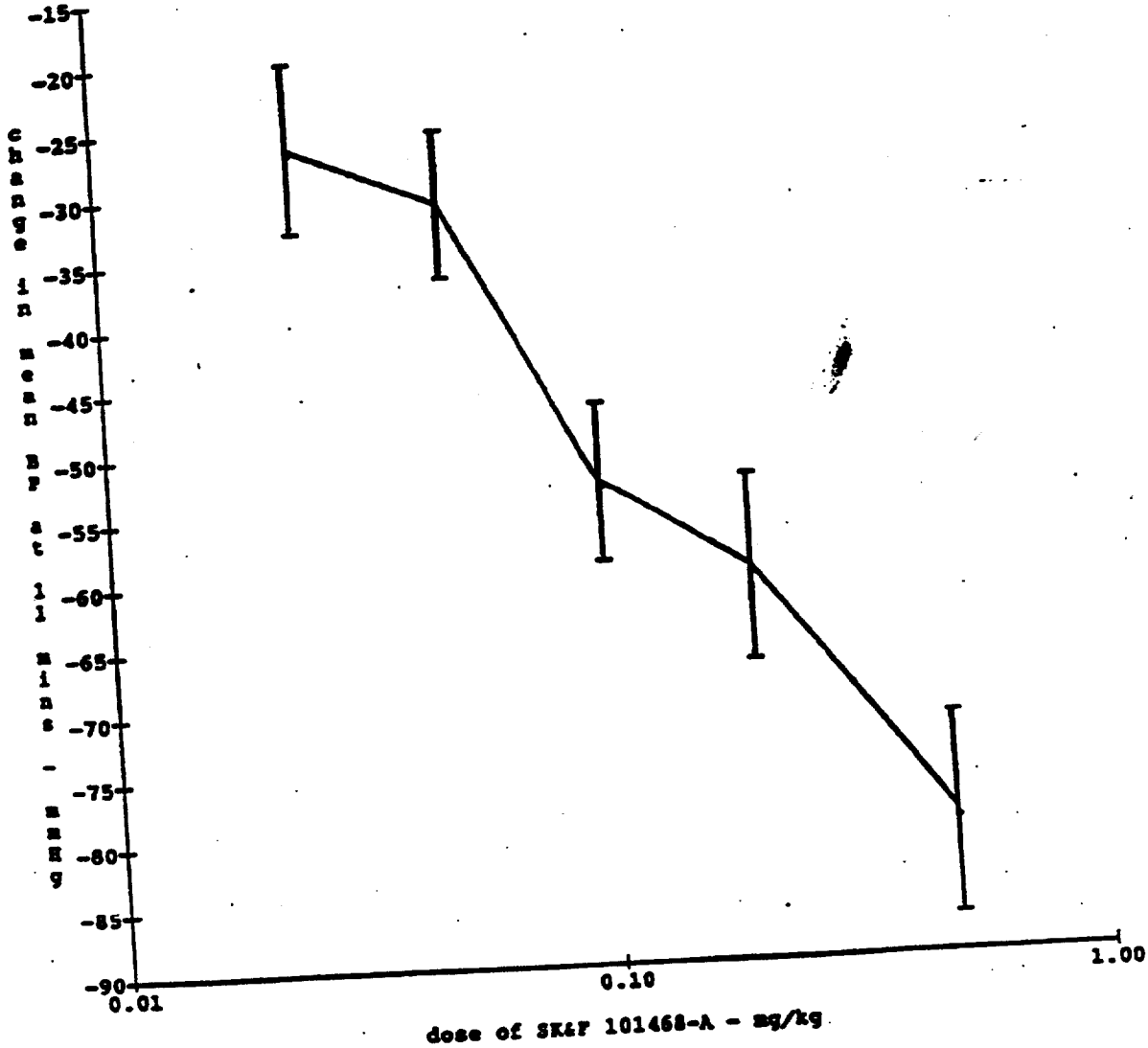

Figure 9 Dose response curve showing reduction in mean blood pressure (BP) at 5 minutes after the end of the 6 minute infusion of SK&F 101468-A.

Values are means ± S.E.M. (n=5)

Anesthetized SHR rat

Fig 9

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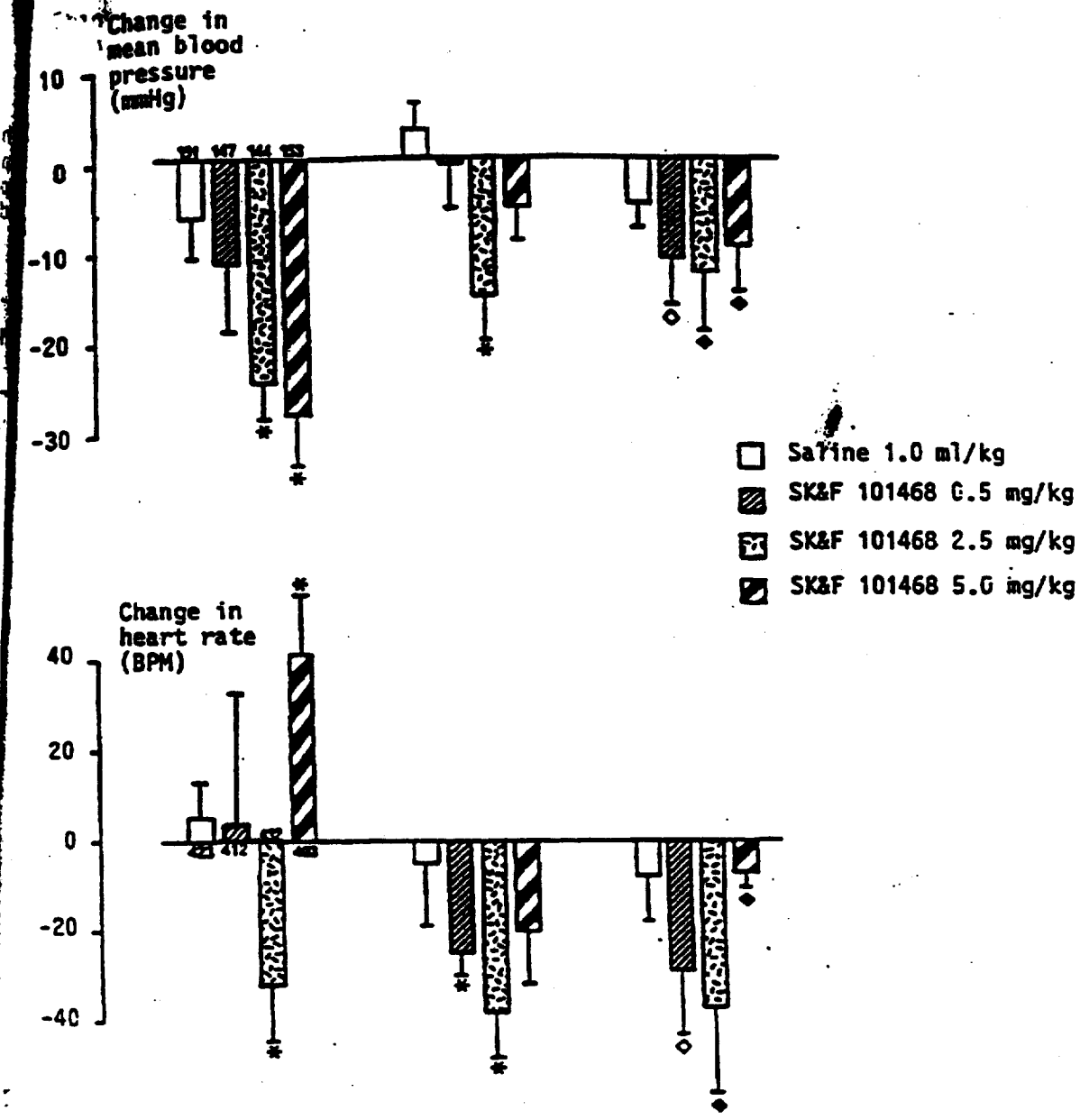

[a] maximum blood pressure change with concomitant change in heart rate

Figure 1 The effect of intravenous administration of SK&F 101468-A on blood pressure and heart rate in the conscious spontaneously hypertensive rat.

Values are mean ± S.E.M., n=6 or ◆ n=5 or ◇ n=4
(numbers denote pre-drug values, mmHg or beats/min [BPM])
* significant change from pre-dose value, $P<0.05$

Concious SH rat IV

fig 10

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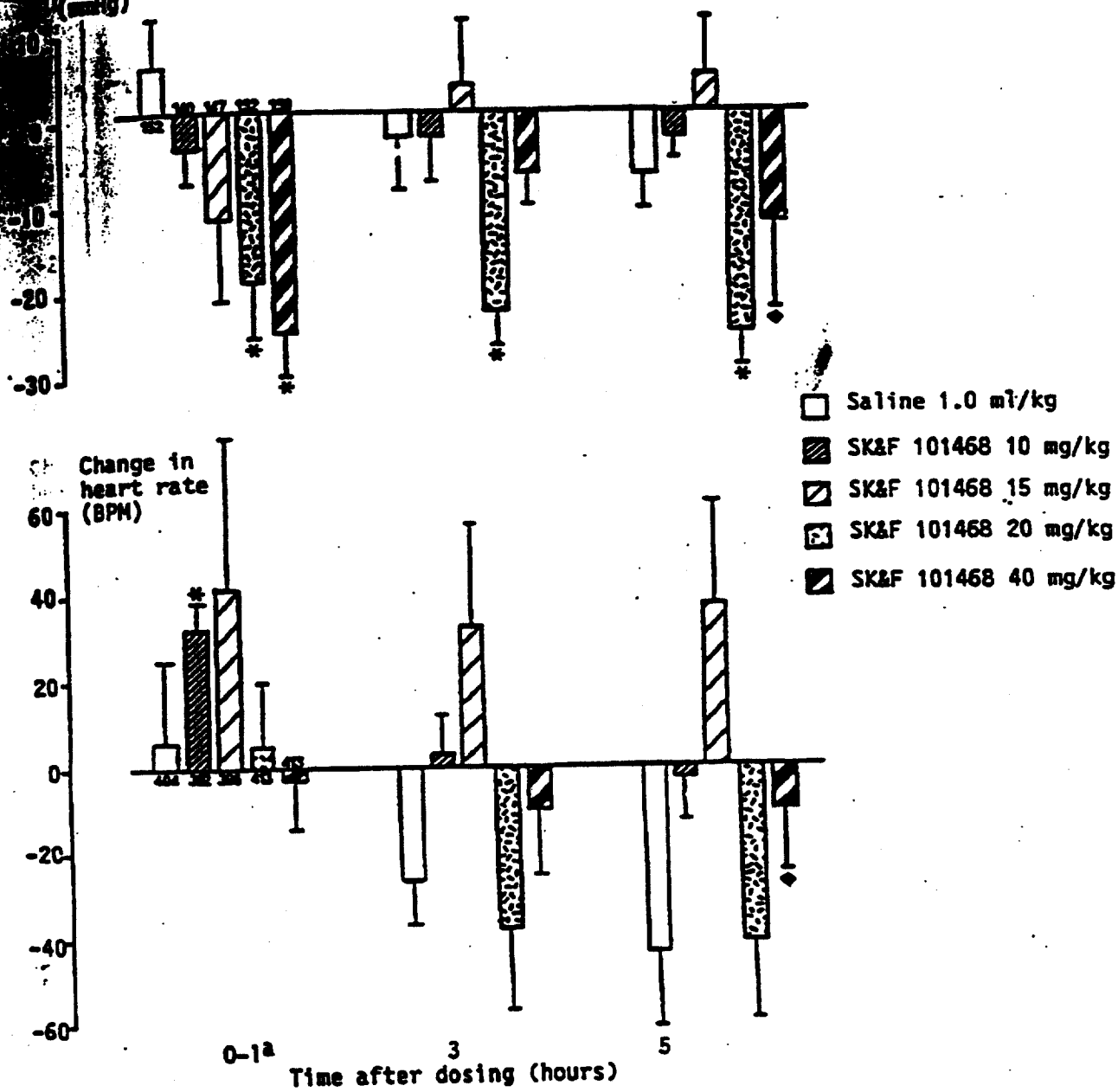

[a] maximum blood pressure change with concomitant change in heart rate

Figure 2 The effect of oral administration of SK&F 101468-A on blood pressure and heart rate in the conscious spontaneously hypertensive rat.

Values are mean ± S.E.M., n=6 or ◆ n=5
(numbers denote pre-drug values, mmHg or beats/min [BPM])
* significant change from pre-dose value, $P<0.05$

Conscious SH rat oral

Fig 11

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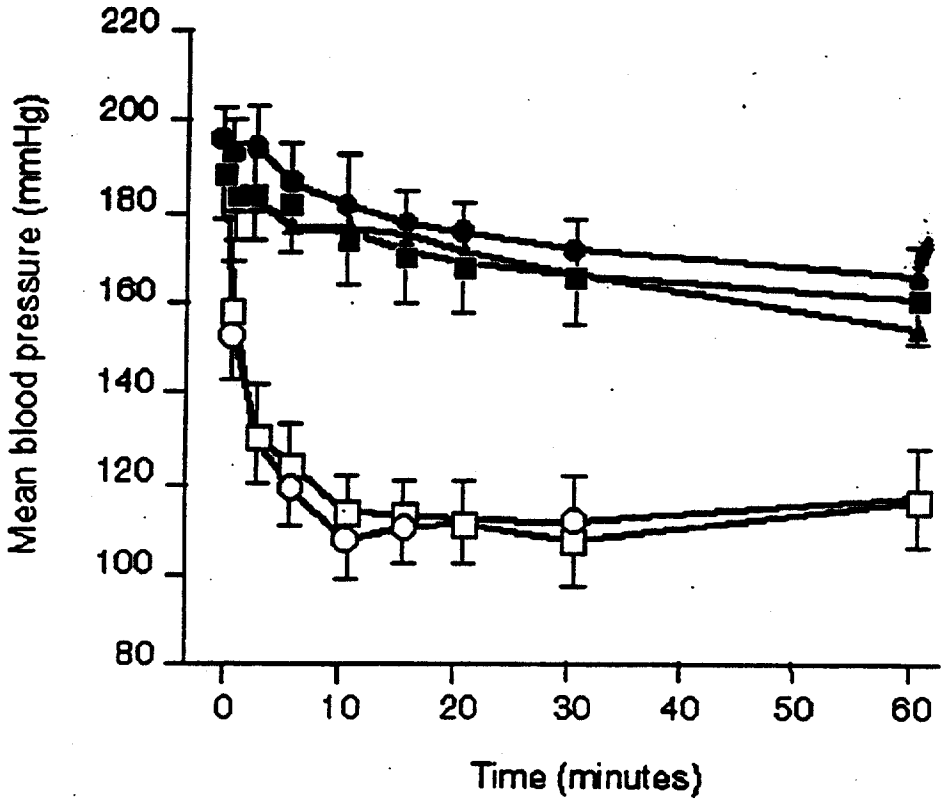
Figure 8 | 2 Effects on blood pressure following chronic dosing with ropinirole to anesthetized SHR



Hypotensive effect of saline i.v. infusion over 5 min (▲) (n=9) compared to ropinirole, 0.5 mg/kg i.v. infusion to anesthetized SHR pretreated twice daily with saline, 1 ml/kg p.o., for 7 (○) (n= 2) or 14 (□) (n=11) days or ropinirole, 40 mg/kg p.o., for 7 (●) (n=10) or 14 (■) (n=11) days. Saline pretreated statistical significances: $p<0.05$, all other points after challenge $p<0.01$. Values represent mean ± s.e.m.

The development of tolerance to the peripheral cardiovascular effects of ropinirole was also investigated in cynomolgus monkeys, a species in which ropinirole has a similar metabolic profile to that in man [59].

Cynomolgus monkeys were treated twice daily with saline or ropinirole 5.0 mg/kg p.o.; (doses expressed as salt) for four days. Tolerance to the hypotensive effect of an intravenous challenge of ropinirole 100 ug/kg was demonstrated after 7 and 14 days pre-treatment which could not be overcome by increasing the i.v. challenge dose ten-fold (doses expressed as salt). Cross tolerance to the cardiovascular effects of an i.v. injection of bromocriptine 0.3 mg/kg, another D_2 agonist, was demonstrated following 27 days pre-treatment.

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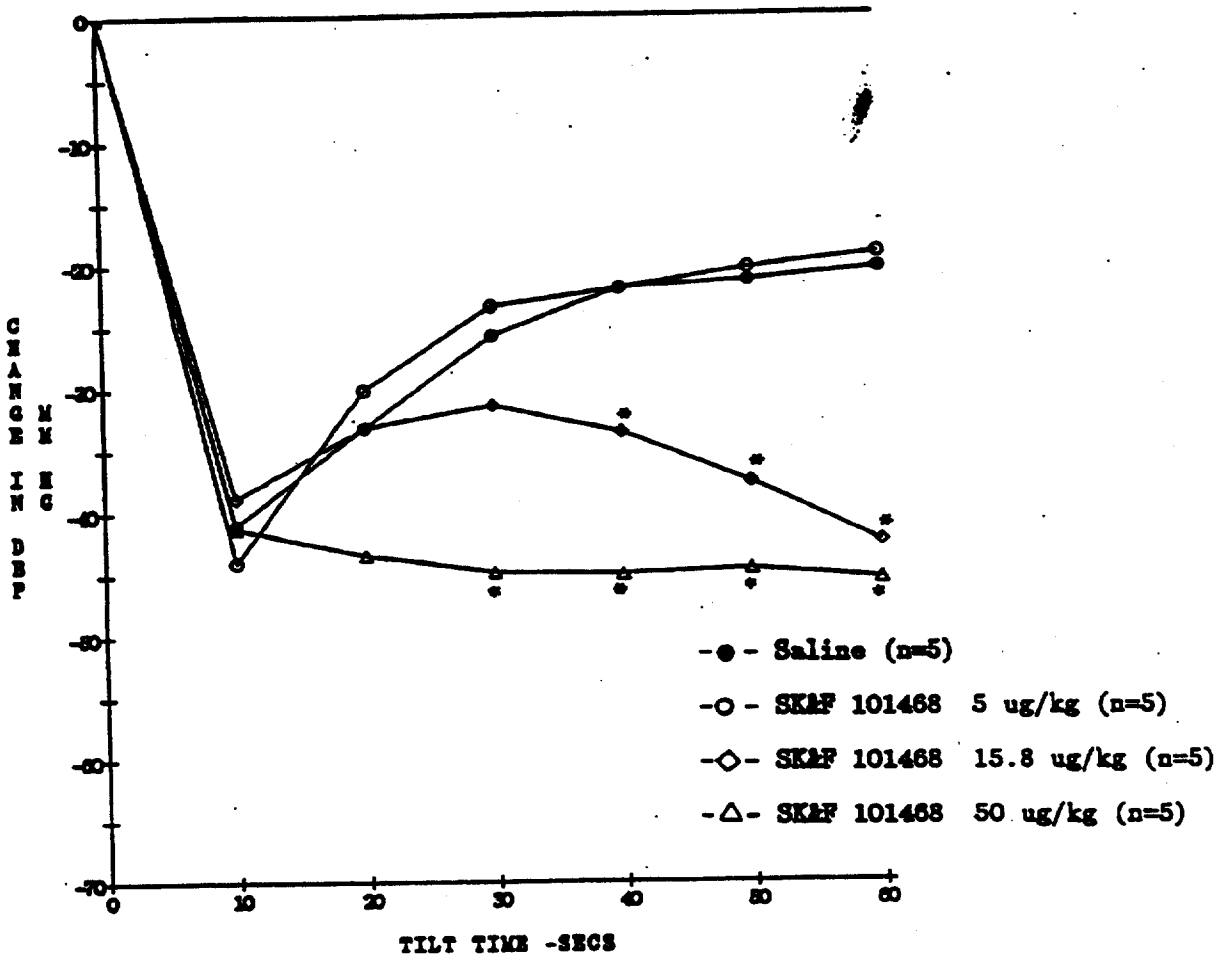

Figure 5: Effect of infusion of 5, 15.8 or 50 ug/kg (total doses) of SKF 101468-A on tilt-induced changes in diastolic blood pressure in the anesthetised SHR rat.

***statistically significant difference from saline treated group $p<0.05$**

Fig 13

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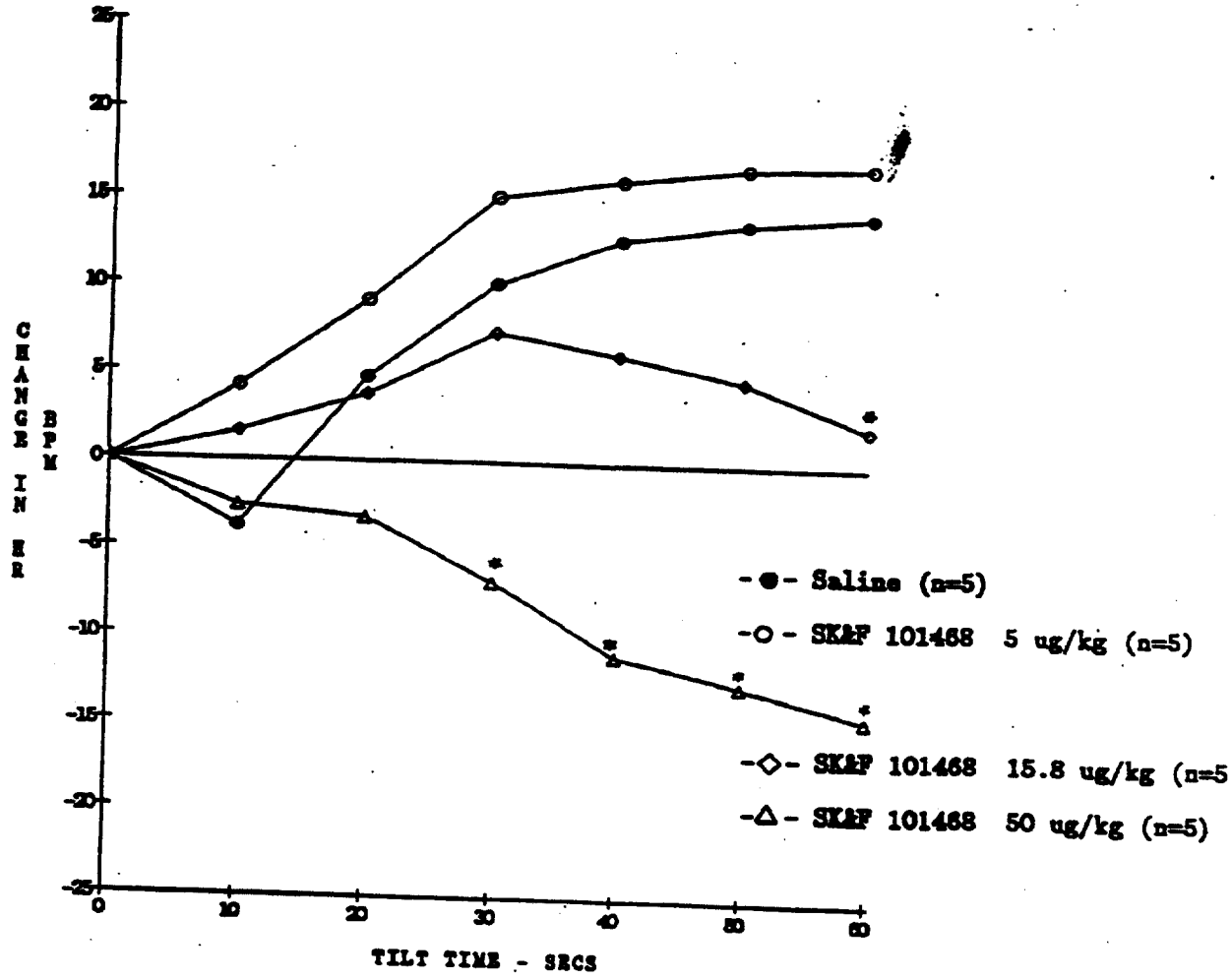

Figure 6: Effect of infusion of 5, 15.8 or 50 ug/kg (total doses) of SK&F 101468-A on tilt-induced increases in heart rate in the SHR rat.

*statistically significant difference from saline treated group $p<0.05$

Fig 14

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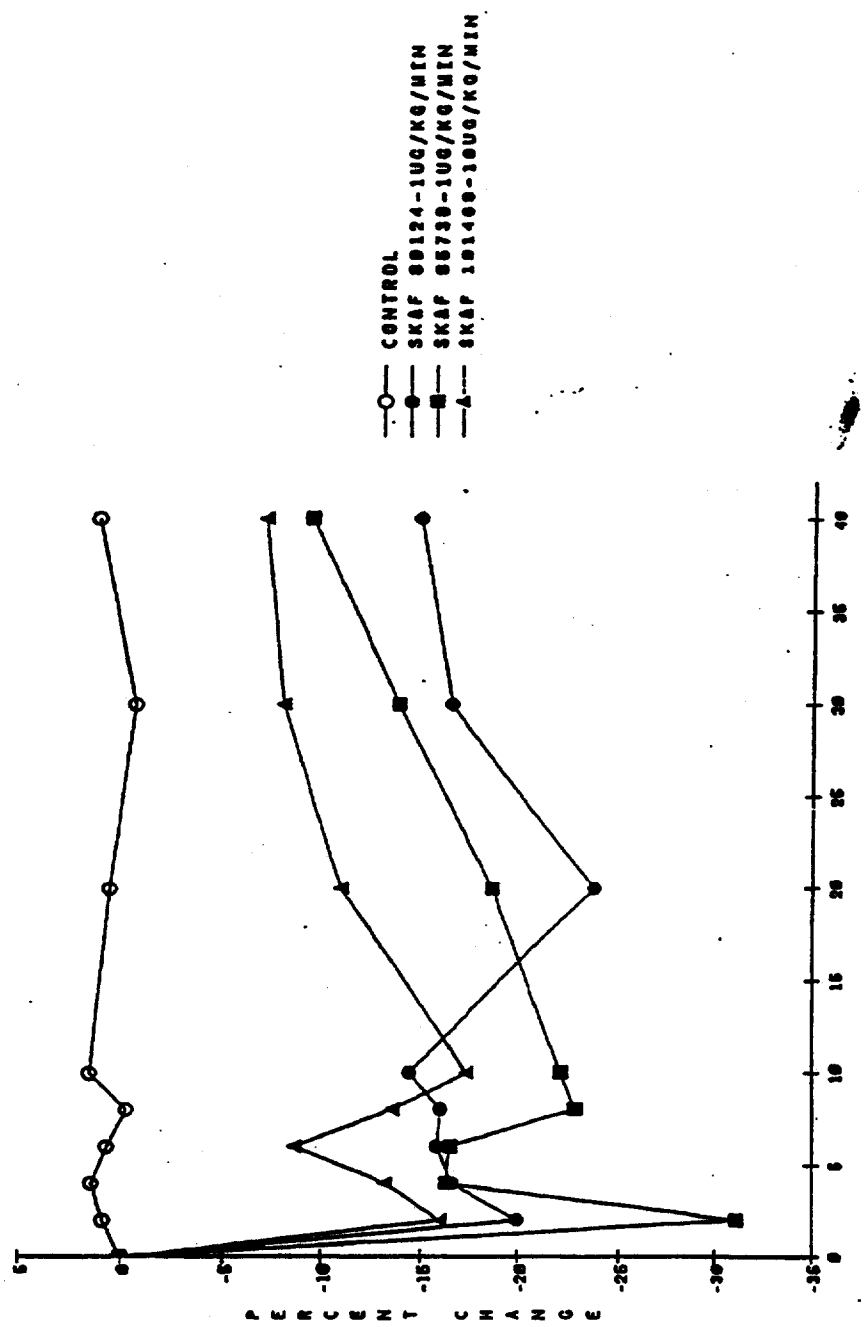

Figure 1: Effect of intravenous infusion of saline (control), SKAF 89124-A, SKAF 85738-H, SKAF 101408-A on mean arterial blood pressure in the anesthetized dog. Drugs infused for 10 min., beginning at time=0.

Fig 15

Fig 15

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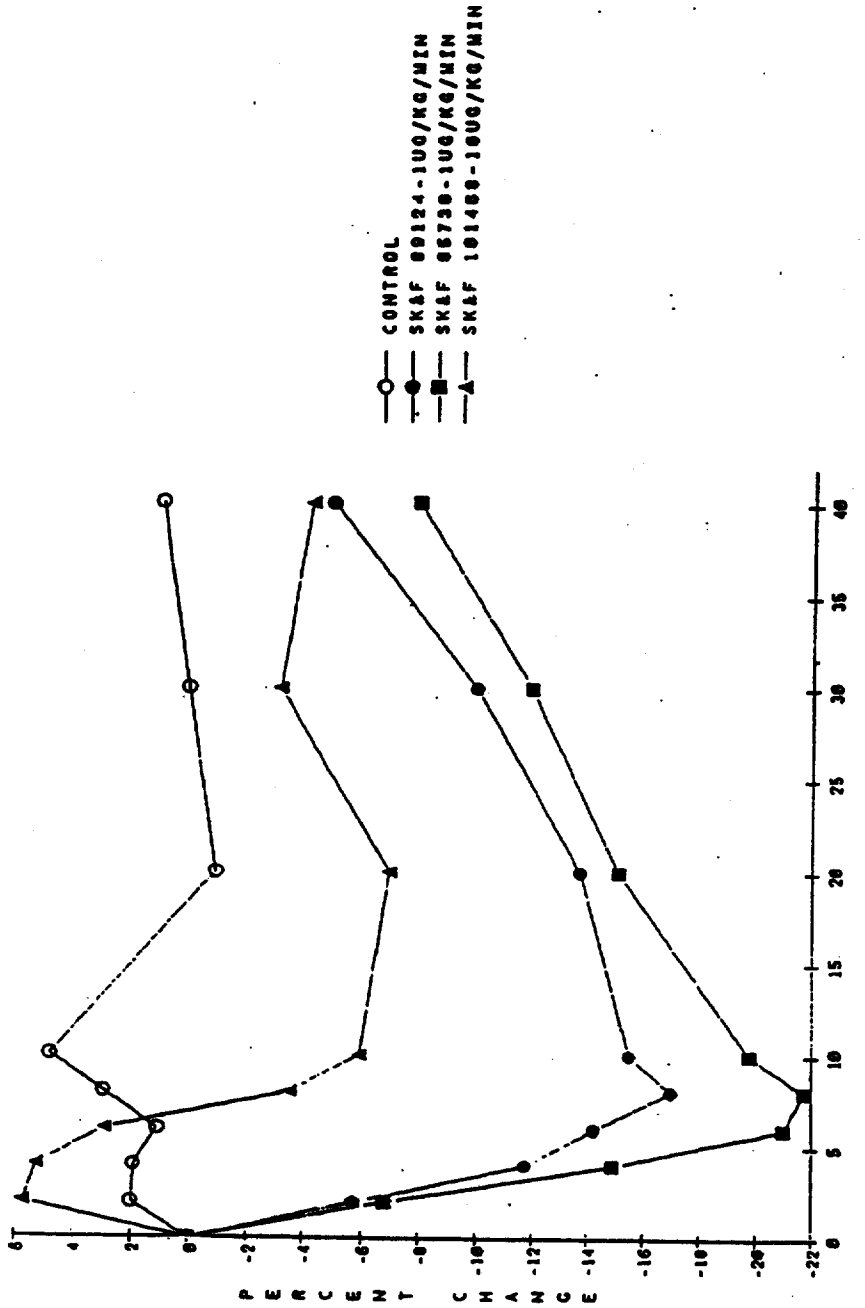

Figure 2: Effect of intravenous infusion of saline (control), SK&F 89124-A, SK&F 85738-H, SK&F 101468-A on heart rate in the anesthetized dog. Drugs infused for 10 min., beginning at time 0.

Fig 16

Fig 16

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Figure 17

Changes in mean blood pressure from values at time -1 min and following administration of SK&F 101468-A, 0.1 mg/kg, i.v., in cynomolgus monkeys pretreated with saline or SK&F 101468-A, 5 mg/kg, p.o., twice daily for 0, 7 or 14 days.

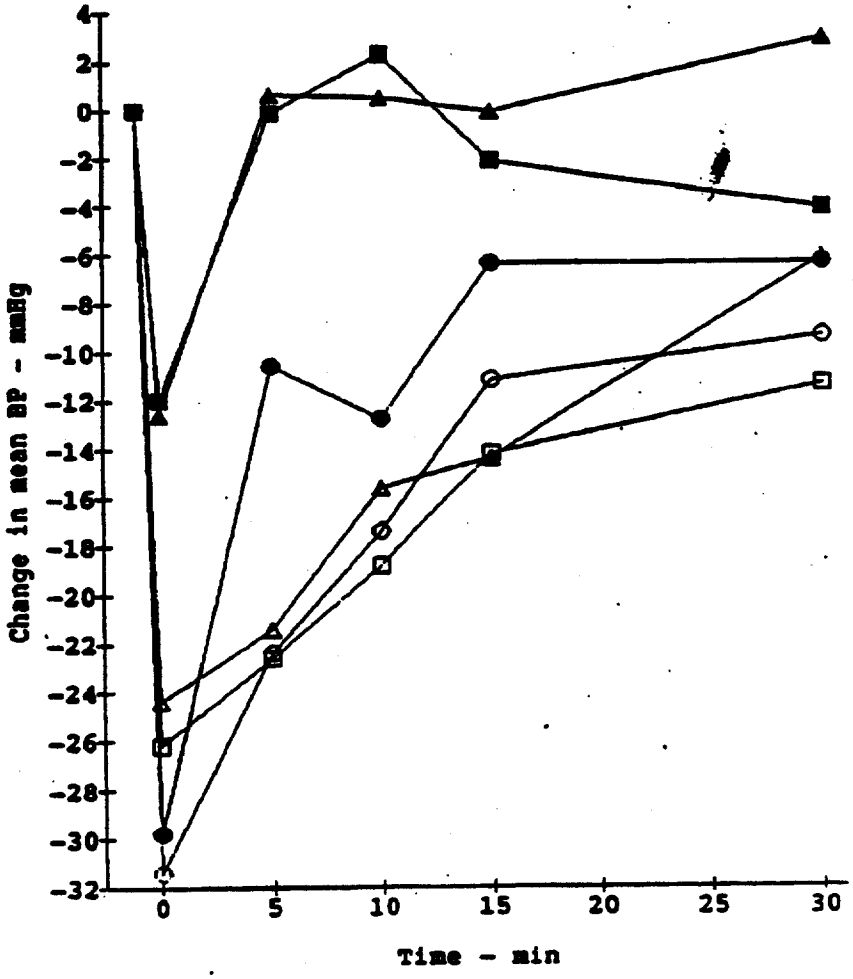

Fig 17

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TABLE 8

EFFECT OF SUSTAINED INFUSION OF SK&F 101468-A
IN CONSCIOUS SPRAGUE-DAWLEY RATS

n=6	CONTROL	SK&F 101468-A 3 µg/kg/min (75 min) SK&F 101468-A	
Body weight g	282 ± 5.7	291 ± 5.5	
Plasma osmolality mOsm/kg/H_2O	293 ± 1.5	293 ± 1.4	
Hematocrit %	41.5 ± 1.4	41.9 ± 1.3	
Mean blood pressure mm Hg	111.7 ± 1.6	111.2 ± 1.2	
Heart rate beats/min	370 ± 5.9	395 ± 8.4	
Filtration fraction GFR/ERPF	0.29 ± 0.01	0.33 ± 0.01	
Urine flow µl/min/100g	15.2 ± 3.2	7.7 ± 2.0	p<0.05
Osmolal clearance µl/min/100g	28.3 ± 4.5	22.1 ± 3.5	p<0.05
Free water clearance µl/min/100g	-13.1 ± 4.0	-14.4 ± 1.9	
Sodium excretion µEq/min/100g	0.73 ± 0.28	0.85 ± 0.30	
Potassium excretion µEq/min/100g	1.39 ± 0.29	0.99 ± 0.17	p<0.05
Urea excretion µmoles/min/100g	3.94 ± 0.42	2.59 ± 0.31	p<0.05

The values listed under CONTROL are the means ± SEM of two clearance periods before infusing SK&F 101468-A; those under SK&F 101468-A are the means ± SEM of two clearance periods obtained during a 1-h infusion of SK&F 101468-A. When P values are not listed, the differences were not statistically significant.

Table 8

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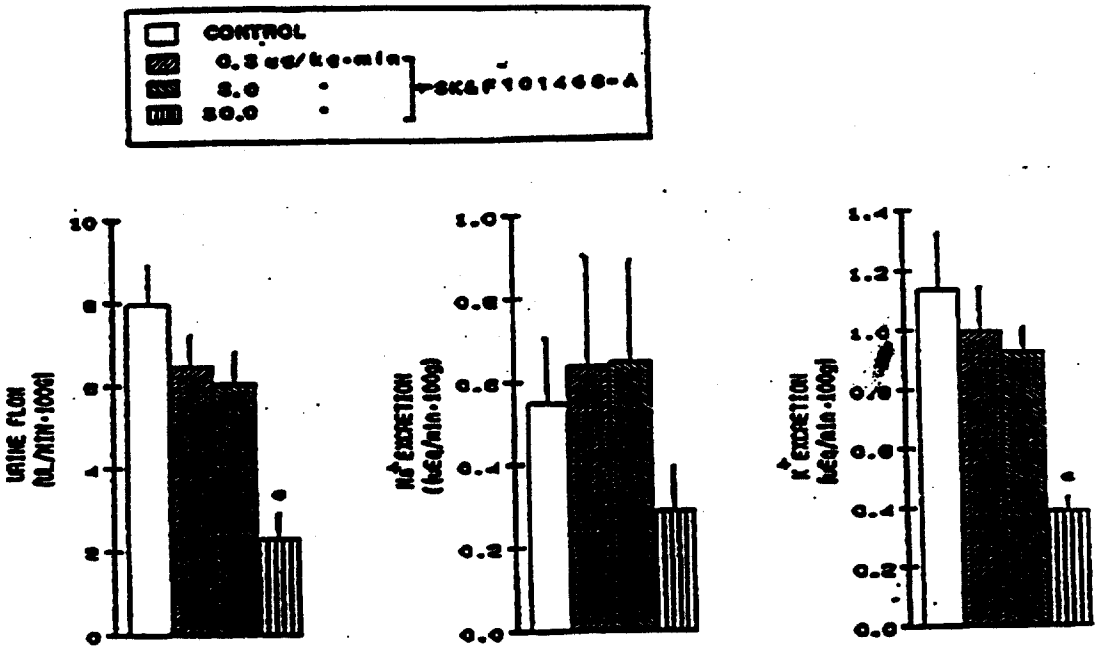

Figure 18

Effect of intravenous administration of SK&F 101468-A on the renal excretion of water and electrolytes in conscious rats. Values are means ± SEM (n=7). Infusions were for 20 min. Significant decrease in urine flow and potassium excretion was recorded with the infusion of 30 µg/kg/min dose.

Fig 18

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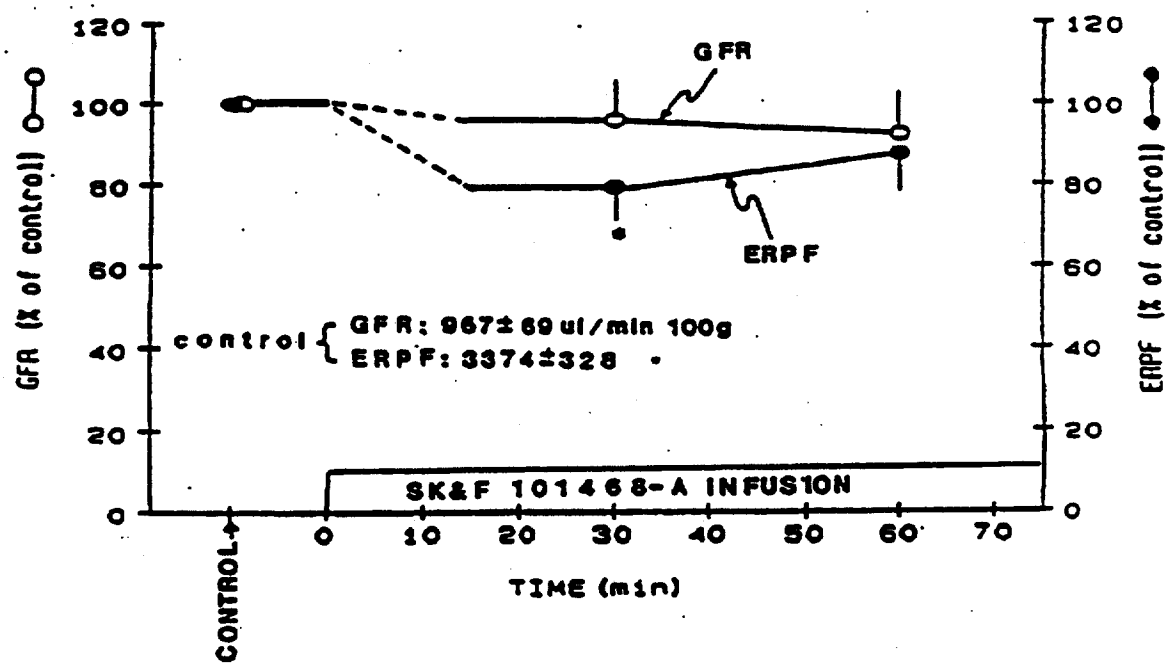


Figure 19

Effect of sustained infusion of SK&F 101468-A (3 µg/kg/min for 75 min) on glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) in conscious, normotensive rats (n=6). Symbols and bars represent averages ± SEM of values during each clearance period. Compared with mean control values, * denotes $p<0.05$.

Fig 19

ABSORPTION AND PHARMACOKINETICS

Analytical Methods

Primary analytical methods were methods could provide a maximum sensitivity (MQL) of about 5 ng/ml for ROP, 10ng/ml for SKF 104557, and 0.1 ng/ml for SKF 89124. Accuracy was better than ±10% and the coefficient of variance between assays was normally 7-12%. in human and monkey plasma provided a ROP MQL of 0.1 ng/ml with accuracy within 5% and between assay coefficient of variation of <10%. Cross reactivity to SKF 104557 and SKF 97930 was <.05%, about 8% for SKF 89124 and 0.5% for the glucuronide of SKF 89124. A specific for SKF 104557 had a MQL of 0.16 ng/ml with accuracy within <7% and inter-assay coefficient of variation of <11%. Cross reactivity to ROP was not observed below 125 ng/ml. Compound stability was at least 1 month in vehicle. ^{14}C-ROP studies were conducted with the label at the C-2 position of the indolone ring, which would not be affected by metabolic pathways.

MOUSE

Single dose studies.

Doses of 5, 15 and 50 mg/kg p.o. gavage, as used in the carcinogenicity study, were examined in 30M and 30F animals (3/s/time point) [study BP/0011]. Exposure increased approximately linearly (Table 9), with Cmax occurring around 1 hr and >90% cleared in 8 hrs. A radiolabelled study using ROP (BW020BA) administered at 10 mg/kg (salt) indicated that 97 % of the oral dose was absorbed.

Repeat dose studies

2 mice/s/g were examined at the end of a 60-day toxicology study that utilized single daily oral doses of ROP (salt) [data also noted in toxicology section, report TP005BA]. ROP was analyzed by RIA at day 61, following the last dose. The peak was at the first time point (30 min) measured, so it may not reflect the true Cmax.

Dose (mg/kg salt) [base]	Plasma concentration with time (ng/ml) 30 min	90	360	AUC (ng.hr/ml)
10 [8.8]	69	15	5	72.8
25 [21.9]	267	62	11	207

50 [43.8]	430	59	18	325
Ratio 1:2.5:5	1:3.9:6.2			1:2.8:4.5

RAT

Single dose studies

One study (BW001BA) utilized 2 and 250 mg/kg (salt) p.o. in 4M rats, approximating the 30 day oral toxicology study dose range, and 2 mg/Kg IV. Absorption was 94% and recovery was >90%. Excretion was >75% in 24 hrs @ 2 mg/kg and 65% @250 mg/kg. About 60% was renally excreted for either dose (Fig. 20). Thus, across doses used in the 30-day toxicology study the absorption and route of elimination were similar. A second study using 0.5, 2, 10, 50 and 220 mg/kg ^{14}C ROP base p.o. indicated Tmax to be 3.7 hr @ 0.5 mg/kg, to 7.3 hr @ 50 mg/kg, with a 30 min transient peak suggesting rapid absorption. There was about a proportional increase in plasma levels to 50 mg/kg, but at the HD of 220 mg/kg an increase was only observable at time points beyond 12 hr. Absorption experiments indicated that transfer was most rapid from the upper small intestine and slowest from the stomach.

In a study (BP008BA) of PK following IV administration the following parameters were determined using ROP 10.4-14.7 mg base, and SKF 89124 4.1-9.5 mg base. 1 animal was used per time point 0 to 5 hrs. Clearance for ROP and SKF 89124 was high, with the latter having a much larger volume of distribution:

	ROP	SKF 89124
CL (ml/min/kg)	52	190
$T_{1/2}$ (min)	27	38
Vd ss (L/kg)	2.0	10.3

Repeat dose studies

A 14 day repeat dose study with PK analysis at days 1 and 14 was undertaken using the doses employed in the 2-year carcinogenicity study (0, 1.5, 15, 50 mg/kg/d ROP base p.o. gavage) with 9/s/group (3 animals/time point 0.5-8 h at 30 min or 1 h intervals). Following a single administration, plasma levels of ROP were generally only measurable at the HD due to metabolism . After 14 days the ROP HD Cmax and AUC greatly increased in both M and F (Table 10), as did the metabolite SKF 104557 to a lesser

degree. Variability was high between animals. In contrast to ROP and SKF 104557, levels of SKF 89124 were stable up to 14 days at the LD but declined at the HD (Table 10). This decline indicates an impaired ability to hydroxylate the parent molecule rather than just enzyme saturation.

A study using 40 mg/kg p.o. ROP b.i.d. for 2 days followed by 0.5 mg/kg IV indicated that plasma levels were higher than in SH rats that received saline b.i.d, ruling out a change in PK parameters as a mechanism for cardiovascular tolerance to ROP.

CYNOMOLGUS MONKEY

Single dose studies

A study utilizing 14C-ROP (reports BW005BA and BW010BA) at doses of 15 mg/kg p.o. and 1 mg/kg ROP salt IV in 4 M monkeys indicated that oral absorption was high (99%). In 3 of 4 monkeys excretion in the urine after both oral and IV administration was about 70%, mainly over the first 24 hr, but was lower in the other animal. Fecal excretion was approximately 10-15% in both cases and occurred mainly over 24-48 hr. This would be consistent with almost complete absorption and subsequent intestinal or biliary excretion after oral dosing. The radioactivity half life was about 60 hr. An abnormal finding (see metabolism below) was that Cmax values for SKF 89124 levels after oral dosing were higher than for ROP (282 and 195 ng/ml respectively). The reason for this result is difficult to determine: the Cmax for ROP was consistent with other studies, and there was no suggestion of chromatographic peaks interfering. After IV dosing the levels of SKF 89124 were very low, consistent with other studies. Total blood radioactivity was much higher than the combined radioactivity for ROP and SKF 89124, which, in the absence of evidence for compound-blood cell binding (blood cell to plasma ratio was about 1.25 for ROP and SKF 89124), indicated the presence of other metabolites. Presumably this was mainly SKF 104557.

The pharmacokinetic profile of ROP was studied in four male monkeys using a crossover design employing oral gavage doses of 1.5, 5 and 15 mg/kg ROP base as well as a single 15 min IV infusion dose of 1.5 mg/kg (Table 11). Analysis of the LD and MD oral doses was limited to 5-6 hrs or less due to low plasma levels, but the standard collection was 13 samples over 24 hrs. ROP increased much more than dose-proportionately, suggestive of saturation of metabolic enzymes or non-linear elimination. Bioavailability correspondingly increased with dose, but was still low (0.4% @ 1.5 mg/kg to 10% @ 15 mg/kg). Clearance was approximately 34 ml/min/kg (2.02 L/hr/kg), which was primarily metabolic clearance since absorption was high and parent compound excretion was low in metabolic studies (4%). Two phases of

disposition could be identified for the HD, an early phase with T1/2 of 64-90 min (oral) and 56 min (IV), and a terminal phase with a half-life of about 11 hr.

The metabolite SKF 104557 was present at higher levels than ROP (Table 12) and had a more dose-proportional increase in plasma concentration. Relative levels and exposure thus decreased compared to ROP with increasing dose:

		Oral		IV
Dose mg/kg	1.5	5	15	1.5
ROP Cmax ng/ml	1.9	20	313	724
ROP AUC0-t ng.h/ml	2.9	41	831	873
Ratio AUC SKF 104557:ROP	337	55	15	0.9

A study utilizing IV (15 min infusion) doses of 0.25 to 1.5 mg/kg in 4 male monkeys generated data broadly in agreement with the above study, with Cmax of 595 ng/ml and AUC of 813 ng.h/ml although the Cmax or AUC in this case increased less than dose-proportionally.

Dose mg/kg	0.25	0.5	1.5
ROP Cmax ng/ml	129	261	595
AUC0-t ng.h/ml	168	394	813

Clearance was about 1.4 L/hr/kg (about half of hepatic blood flow of 2.8 L/hr/kg). Vd was 2.8 L/kg.

Monkey multiple dose studies

From a dose-ranging toxicology study that used 1M and 1F animal/dose for 10 daily doses (BP005BA), PK data were determined on days 8 or 9 (Table 13). Cmax and AUC were comparable with single dose studies at 15 mg/kg, and these values increased more than dose-proportionately as previously observed. SKF 89124 levels were 8-17 fold lower than ROP.

Plasma samples were taken on day 1 and 30 in a toxicity study employing daily doses of 1.5, 5 and 15 mg/kg p.o. using 3/s/gp (BP006BA) Only 3 samples were taken on the first day (2,4 and 8 hrs), and only a small number of HD, usually 2 hr, samples were measurable. More samples were taken on day 30 (0.5-6 hrs), but variability was high (as on day 1). Mean ROP maximum plasma levels on day 30 were 72 ng/ml at 5 mg/kg/day and 249 at 15 mg/kg/day. A comparison of 120 min time point samples where available from individual animals between day 1 and day 30 at 5

and 15 mg/kg showed an increase of 3.9±2.3 (S.D.)-fold, n=10 in ROP plasma levels. However, the accuracy of this observation must be in doubt considering that many day 1 samples were close to MQL and the use of a single time point for determination of plasma drug ratios.

A more detailed analysis was performed on the definitive 1 yr toxicology study using 4/s/gp @ 1.5, 5 and 15 mg/kg/d by gavage (data were collected from all animals). PK data were collected at 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs post dose on days 1, 36 and 366. However, only ROP and SKF 104557 were measured (Table 14). Levels were above MQL to 12-24 hrs except for the initial 1.5 mg/kg dose which was <MQL after 4 hrs.
Data were variable but demonstrated a greater than dose-proportional increase for ROP and SKF 104557. Accumulation of ROP was minimal as assessed by Cmin values at 0 and 24 hr time points (Table 15), but Cmin values were increased on days 36 and 366 for SKF 104557 (Table 16), suggesting some accumulation, consistent with the half life being > 10 hr (Table 12). M + F were comparable. SKF 104557 was present at much higher levels than ROP at all doses, but the ratio decreased as dose increased, suggesting saturation of metabolism (Table 17). The relative concentrations of ROP and SKF 104557 were similar on day 1 to single dose PK data except at the 1.5 mg/kg dose where the ratio was 95, compared to 337 (see above). The sponsor suggests poor characterization of levels of ROP at this dose may underlie this result, which appears possible. The ratios for SKF 104557:ROP were not greatly different on days 1, 36 and 366, indicating a consistency of metabolism and PK parameters. Cmax and AUC values for SKF 104557 were 2930 ng/ml and 11500 ng.h/ml respectively. The half life for ROP was 6.6 hr, 8.8 and 6.8 hrs on days 1, 36 and 366 respectively. Repeat IV dose (0.5-2 mg/kg/day) studies also found that Cmax was similar over 14 days.

HUMAN

In both healthy subjects (14 males with n=1-7 for data points, single oral solution dose 0.32-2.5 mg) and patients (n=4-6 M/F oral tablet of 2-12 mg) Cmax and AUC values increased generally in proportion to dose (Table 17b). Absorption was essentially complete, with a Vd of 7.7 L/kg, somewhat higher than in preclinical species (Table 17c).

Table 17b Human PK following single doses

Dose mg	0.32	1	2	4	6	8
ROP Cmax ng/ml	0.45	2	6	12	10	18
ROP AUC INF ng.h/ml			33	70	65	152
SKF 104557 Cmax ng/ml			3.6	6.4	9.6	16
SKF 104557 AUC 0-t ng.h/ml			40	74	103	167

The Tmax was between 1 and 1.5 hrs. Bioavailability was 46-54%, much greater than in preclinical species, suggesting reduced first pass metabolism. Following an 0.8 mg IV dose, clearance was 1.8 (L/h)/kg.
SKF 104557 had a similar or greater concentration in plasma compared to ROP, and also increased relatively proportionally with dose. Half lives were 5-7 h for ropinirole and 7-11 h for SKF 104557. Upon repeat clinical dosing (t.i.d) with ROP, Cmax and AUC were still dose proportional but were elevated about 2-fold compared to single doses as expected from the dosing interval being similar to half life.

Table 17c Comparative PK data for ROP

Species	T1/2 h	Tmax h	CL (L/h)kg	Vdss L/kg	Absorption
Mouse		0.3-1			97%
Rat	0.5	4-7	3.1	2	94%
Monkey	1	1-3	2	2.2-2.7	99%
Human	5-7	1-1.5	1.3-1.8	7.7	100%

Table 17c cont. Comparative PK data for ROP

Species	Dose (mg base/kg/ day)	Protocol	Cmax ng/ml	AUC 0-t (or 0-24) ng.h/ml	AUC/mg dose
Mouse	43.8	single	296	482	9.6
Mouse	43.8	60 days	430 [11.7]	325 [0.58]	6.5
Rat	50	day 1 of 14 day study	111	226	4.5
Rat	50	day 14 of 14	478 [13]	1580 [2.8]	31.6
Monkey	15	single	313	830	55
Monkey	15	8-9 days	226	475	31.6
Monkey	15	day 1 of 366 day study	195	573	38.2
Monkey	15	day 36 0f 366	107	303	20.2
Monkey	15	day 366 of 366	184 [5]	511 [0.92]	34
Human	0.12 (6 mg)	single	10.3	63	10.5
Human	0.12 (6 mg)	t.i.d.	27	330 (0-24 h)	18.3
Human	0.16 (8 mg)	t.i.d.	36.7	557 (0-24 h)	23.2

Table 9: Model-independent pharmacokinetic parameters for ropinirole in mice following single oral doses at 5, 15 and 50 mg/kg [15].

Parameter (Units)	Parameter value					
Sex	Males			Females		
Dose (mg/kg)	5	15	50	5	15	50
C_{max} (ng/mL)	49.0	204	307	55.6	129	286
T_{max} (h)	0.78	0.30	0.32	0.30	0.53	0.30
AUC_{0-t} (ng.h/mL)	81.6	205	467	83.3	180	498
AUC_{0-inf} (ng.h/mL)	88.3	212	497	102	190	527

Pharmacokinetic parameters were determined in pooled plasma from three animals at each sampling time.

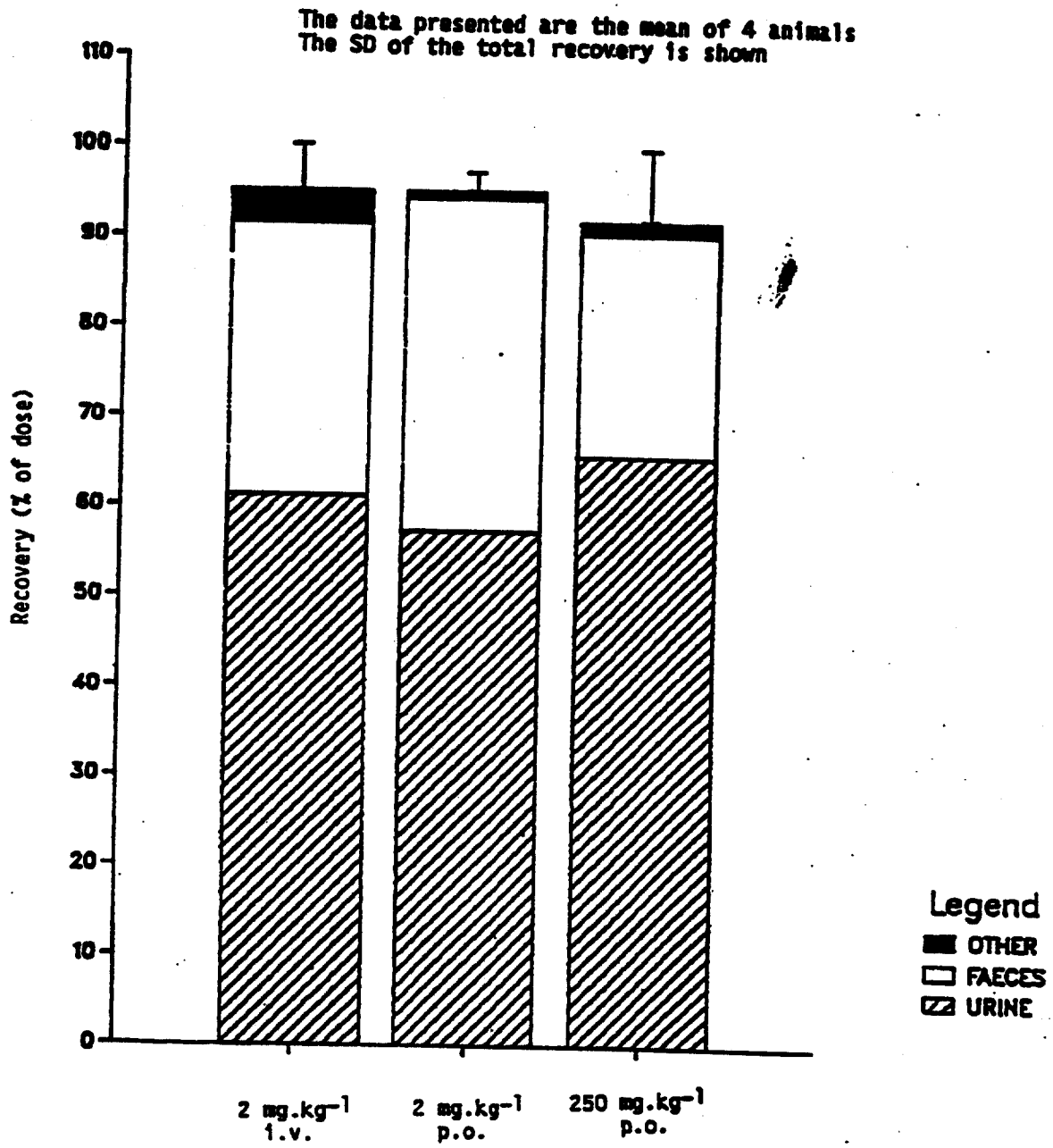
5.C.2.3.3 Pharmacokinetics of ropinirole in mice after repeated oral administration (60 day toxicology study, [16])

A limited number of plasma samples were obtained from mice killed at the end of a 60 day oral dose-ranging study in the mouse following daily gavage administration of ropinirole at doses of 8.8, 21.9 and 43.8 mg/kg. Plasma concentrations of ropinirole up to 6 h after dosing were determined by RIA. As judged by C_{max} and AUC_{0-t} values (Table 2), the systemic exposure to ropinirole increased approximately proportionally with increasing dose.

Figure 2o

BW001BA

Comparison of Routes of Excretion of Radioactivity Following Administration of ^{14}C-SK&F 101468-A to Male Rats. (Target doses 2 & 250 mg.kg^{-1}).



NOTE The urinary recovery at 250 mg.kg^{-1} includes the cage washings for reasons explained in the text.

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TABLE 10 - SK&F 104557 and SK&F 89124 in rats following 14 day repeated oral administration at 1.5, 15 and 50 mg/kg [21]

19c

Parameter (units)		Parameter value				
		C_{max} (ng/mL)		AUC_{0-t} (ng.h/mL)		
		Male	Female	Male	Female	
Ropinirole				% AUC	% AUC	Mean % AUC
50 mg/kg/day	Day 1	22.5	200	106 (14%)	346 (29%)	28.5
	Day 14	394	563	1660 (50%)	1500 (51%)	50.5
SK&F 104557						
50 mg/kg/day	Day 1	39.2	109	222 (29%)	406 (34%)	31.5
	Day 14	284	278	1390 (43%)	1240 (42%)	42.5
SK&F 89124						
1.5 mg/kg/day	Day 1	2.90	1.26	8.20	6.14	
	Day 14	2.56	1.66	9.34	10.2	
15 mg/kg/day	Day 1	83.6	8.93	135	48.0	
	Day 14	9.87	10.7	46.8	60.2	
50 mg/kg/day	Day 1	367	333	448 (58%)	450 (37%)	47.5
	Day 14	50.4	59.8	215 (7%)	181 (6%)	6.5

Pharmacokinetic parameters were derived from composite profiles (3 animals per time point). Ropinirole and SK&F 104557 were generally not detected at 1.5 and 15 mg/kg/day doses

~~shown below~~:

TABLE 11 Mean (SD) MONKEY SINGLE DOSE BLOOD

	1.5mg/kg	5mg/kg	15mg/kg	1.5mg/kg (iv)
Cmax (ng/ml)	1.94 (0.36)	20.5 (5.21)	313 (83)	724 (303)
Tmax* (min.)	60	90	93	15.0
Half-life(λ_1) (min)	64 (30)	76 (31)	90 (15)	56 (12)
Vdss (L)	-	-	-	9.9 (0.9) (~2.2 L/Kg)
AUC (0-T) (ng/ml.min)	176 (39)	2484 (476)	49831 (17667)	52376 (24038)
EAUC (ng/ml.min)	228 (72)	2627 (548)	51389 (18523)	52966 (24422)
Cl (ml/min/kg)	-	-	-	33.7 (15.5) (2.0 L/hr/Kg)
F(%)	0.46 (0.08)	1.7 (0.7))	10 (2.1)	100

Ratios	1.5	5	15
Dose	1	3.3	10
C max		10.5	161

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Pharmaceuticals

monkey single dose
Table 12
JKF 104557 Bloa10

	1.5mg/kg	5mg/kg	15mg/kg	1.5mg/kg (iv)
Cmax (ng/ml)	221 (82)	546 (187)	2737 (802)	90 (33)
Tmax (min.)	60	60	75	66
Half-life (min)	934 (271)	586 (182)	626 (194)	814 (419)
AUC(0-T) (ng/ml.min)	59200 (17000)	131000 (35000)	602000 (35700)	43700 (19200)

Table 13: **Model-independent pharmacokinetic parameters for ropinirole in cynomolgus monkeys following repeated oral administration at 15, 30 and 60 mg/kg/day for up to 10 days [32].**

Parameter (Units)	Parameter value					
Sex	Males			Females		
Dose (mg/kg)	15	30	60	15	30	60
C_{max} (ng/mL)	226	820	1810	315	502	1910
T_{max} (h)	2	2	2	1	1	3
$AUC_{0\text{-inf}}$ (ng.h/mL)	475	3940	9640	657	2720	6390

Pharmacokinetic parameters were determined in 1 male (on Day 8) and 1 female (on Day 9) animals at each dose level.

14

Table 7: Model-independent pharmacokinetic parameters for ropinirole and SK&F 104557 in cynomolgus monkeys following repeated oral administration of ropinirole (1 year toxicology study, [34])

Parameter (units)	Day of study	Parameter value (mean ± S.D., n = 4)					
Sex		Male			Female		
Dose (mg/kg/day)		1.5	5	15	1.5	5	15
Ropinirole							
C_{max} (ng/mL)	1	1.70 ± 0.59	18.4 ± 6.72	147 ± 32.6	2.32 ± 1.05	20.8 ± 12.9	244 ± 177
	36	12.8 ± 12.3	21.1 ± 8.03	79.9 ± 30.6	12.7 ± 6.87	34.0 ± 15.0	135 ± 133
	366	3.11 ± 0.85	19.8 ± 6.60	265 ± 269	8.04 ± 6.19	29.9 ± 10.0	103 ± 84.4
AUC_{0-t} (ng.h/mL)	1	3.64 ± 3.27	58.9 ± 11.2	565 ± 107	3.94 ± 1.28	49.5 ± 25.0	582 ± 229
	36	26.5 ± 9.19	78.1 ± 30.0	266 ± 44.4	24.8 ± 16.9	79.1 ± 34.0	340 ± 123
	366	11.9 ± 4.39	73.7 ± 15.1	688 ± 538	19.9 ± 17.1	64.9 ± 11.5	334 ± 131
SK&F 104557							
C_{max} (ng/mL)	1	35.6 ± 16.7	181 ± 15.7	2610 ± 676	55.3 ± 32.3	625 ± 173	1800 ± 885
	36	288 ± 185	453 ± 162	3200 ± 719	205 ± 160	874 ± 140	1210 ± 706
	366	94.0 ± 39.3	461 ± 300	2960 ± 416	169 ± 26.4	467 ± 30.6	2310 ± 320
AUC_{0-t} (ng.h/mL)	1	256 ± 101	1240 ± 238	12100 ± 1290	249 ± 31.5	2720 ± 474	9970 ± 4960
	36	853 ± 216	2650 ± 1010	21000 ± 4910	780 ± 179	3340 ± 430	6410 ± 1080
	366	776 ± 165	2170 ± 693	11700 ± 724	1010 ± 164	2660 ± 547	11300 ± 1680

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Table 1

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Appendix 50

1-Year Oral Toxicity Study of SK&F 101468-A in Cynomolgus Monkeys

Plasma Concentrations (ng/ml) of SK&F 101468 on Day 1, Day 36 and Day 366 after Oral Administration of 15mg/kg of SK&F 101468

Time (h)	Day 1 M0599	M0600	M0601	M0602	F0603	F0604	F0605	F0606
0.0	NS	NS	NS	NS	NS	NS	NS	NS
0.5	90.9	74.1	9.46	14.2	14.3	154	37.2	508
1.0	53.4	96.9	68.3	42.4	131	160	43.1	413
2.0	107	180	134	165	21.7	112	176	114
4.0	28.3	70.9	43.3	46.8	11.9	28.4	95.9	23.8
6.0	14.3	35.1	24.7	32.4	5.86	13.0	28.9	15.7
8.0	16.5	23.5	16.7	24.4	7.64	9.58	19.7	11.9
10.0	15.7	16.9	9.58	8.73	5.71	6.05	13.8	4.13
12.0	19.3	11.2	7.60	6.81	12.2	4.04	12.0	2.26
24.0	3.36	1.76	3.20	2.13	5.24	5.29	4.42	0.95

Cmin

Time (h)	Day 36 M0599	M0600	M0601	M0602	F0603	F0604	F0605	F0606
0.0	3.29	1.70	1.68	2.69	0.64	0.25	1.68	0.69
0.5	7.86	37.9	15.7	6.50	25.7	20.9	3.44	40.8
1.0	29.6	64.1	43.1	77.3	15.7	94.2	7.53	329
2.0	116	53.3	46.7	92.6	14.0	84.5	89.2	106
4.0	20.7	19.6	28.8	25.0	28.5	24.6	55.9	16.2
6.0	5.85	9.15	13.2	7.94	22.3	11.3	19.8	6.99
8.0	3.52	3.11	11.2	7.97	6.96	6.09	10.0	5.98
10.0	2.71	2.88	4.74	6.60	4.78	3.75	9.59	3.47
12.0	2.09	1.50	3.28	6.04	2.64	2.17	9.39	2.71
24.0	1.18	0.50	2.09	1.88	0.89	0.34	3.07	1.81

Time (h)	Day 366 M0599	M0600	M0601	M0602	F0603	F0604	F0605	F0606
0.0	1.79	0.48	2.39	0.75	1.14	0.73	0.77	0.53
0.5	35.8	28.7	349	18.6	17.1	40.3	2.51	59.1
1.0	131	34.2	663	133	53.5	37.0	52.8	228
2.0	121	78.3	401	190	68.1	44.8	69.1	186
4.0	31.9	37.1	71.3	43.8	53.5	18.9	25.1	17.3
6.0	14.6	17.5	31.9	20.0	20.0	8.36	8.70	5.28
8.0	12.0	11.0	17.8	6.18	12.2	8.32	8.08	5.98
10.0	4.75	3.61	8.66	4.78	6.86	4.56	3.64	3.06
12.0	3.00	3.14	6.51	3.38	6.83	3.39	2.00	2.04
24.0	1.42	0.53	2.84	1.41	1.97	1.00	0.72	0.89

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Table 16

Appendix 53

1-Year Oral Toxicity Study of SK&F 101468-A in Cynomolgus Monkeys

Plasma Concentrations (ng/ml) of SK&F 104557 on Day 1, Day 36 and Day 366 after Oral Administration of 15mg/kg of SK&F 101468

Time (h)	Day 1 M0599	M0600	M0601	M0602	F0603	F0604	F0605	F0606
0.0	NS	NS	NS	NS	NS	NS	NS	NS
0.5	677	546	400	357	370	1210	521	573
1.0	632	1090	1100	865	336	1480	874	2640
2.0	2280	3340	1840	2990	622	1670	2260	2660
4.0	966	1320	736	871	332	535	1920	719
6.0	348	831	513	553	247	280	954	433
8.0	426	520	393	672	103	213	1070	269
10.0	389	395	245	440	255	197	488	197
12.0	335	55.6	253	300	247	58.8	314	158
24.0	236	63.0	225	261	212	NS	276	78.0

Time (h)	Day 36 M0599	M0600	M0601	M0602	F0603	F0604	F0605	F0606
0.0	40.0	93.2	NS	37.0	14.0	23.1	49.0	NQ
0.5	264	1045	1320	431	386	288	654	360
1.0	3420	1740	1230	1000	382	1240	128	2190
2.0	1910	2170	3330	372	290	1230	744	1480
4.0	3550	1610	2160	3800	648	528	726	509
6.0	1620	1290	975	1910	341	503	422	191
8.0	1100	637	NS	1400	371	193	308	176
10.0	609	380	1230	1240	305	247	197	178
12.0	412	260	355	1190	123	133	150	97.2
24.0	130	78.8	194	286	88.0	NQ	90.8	231

Time (h)	Day 366 M0599	M0600	M0601	M0602	F0603	F0604	F0605	F0606
0.0	171	65.4	263	73.2	140	79.0	111	55.5
0.5	555	303	2560	440	426	1100	89.0	1580
1.0	1860	572	3190	3364	1450	845	1230	1850
2.0	2420	2880	2270	1400	1850	2470	2370	2570
4.0	1150	1330	747	672	840	1330	895	739
6.0	446	608	543	774	496	590	658	498
8.0	377	572	294	696	276	534	511	188
10.0	208	527	325	324	188	272	443	122
12.0	136	192	248	397	353	182	425	114
24.0	53.6	46.8	112	171	182	89.4	123	132

Table 17

1-Year Oral Toxicity Study of SK&F 101468-A
in Cynomolgus Monkeys

Mean and Median Ratio of SK&F 104557 AUC (0-T)
to SK&F 101468 AUC (0-T)

Ratio	1.5mg/kg			5mg/kg			15mg/kg		
	Day 1	Day 36	Day 366	Day 1	Day 36	Day 366	Day 1	Day 36	Day 366
Total									
Mean	95.4	44.8	85.6	52.0	50.1	43.7	23.4	58.8	37.1
Median	91.2	41.1	89.0	36.6	46.8	40.4	25.1	57.4	33.6
SD	59.6	18.5	35.7	39.1	19.3	17.4	5.80	37.7	19.4
CV (%)	62	41	42	75	39	40	25	64	52
Min.									
Max.									
Male									
Mean	116	41.0	84.5	25.5	43.8	36.8	26.0	93.2	27.9
Median	114	36.9	86.2	25.7	42.6	32.3	26.6	95.6	27.9
Min.									
Max.									
Female									
Mean	79.9	48.6	86.7	78.5	56.4	50.6	20.9	24.3	46.3
Median	63.7	47.6	90.3	70.5	57.6	45.0	20.3	21.7	49.6
Min.									
Max.									

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Table 17d Comparative PK data for major metabolites

Species	Dose (mg base/kg/ day)	Protocol	Cmax ng/ml	AUC 0-t (or 0-24) ng.h/ml
SKF 104557				
Rat	50	day 14 of 14 day study	281 [8.5]	1315 [2.2]
Monkey	15	day 366 of 366 day study	2635 [79.5]	11500 [19]
Human	0.48 (8 mg)	t.i.d.	33	605
SKF 89124				
Rat	50	day 14 of 14 day study	55.1 [42.4]	198 [10.3]
Monkey	15	day 366 of 366 day study	Not determined	Not determined
Human	0.48 (8 mg)	t.i.d.	1.3	19.2

[] ratio to human exposure @ 8 mg p.o.

DISTRIBUTION

Protein binding

In vitro binding was mainly investigated by ultrafiltration. Binding of ROP was low in all species, from 10-40% (Table 18). When examined together, monkey and man were quite similar, about 10% bound, but other studies indicated up to 40% bound. SKF 89124 also had low binding, but SKF 104577 was not examined. SKF 104577 had very weak pharmacological activity, so low protein binding, and thus high free levels, would not be expected to contribute to observed activity. However, the high plasma levels of this metabolite could potentially interact with other highly bound drugs if protein binding was much greater than for ROP or SKF 89124.

Volumes of distribution

	Rat	Monkey	Human	
ROP	2.0	2.8	7.7	L/kg

Distribution was extensive for both ROP and SKF 89124 following single IV doses.

Tissue distribution

Whole body autoradiography was used to examine distribution in rats of radioactivity following ^{14}C-ROP administered at a target concentration of approximately 1.9 mg/kg (salt), 1.7 mg/kg base, as a single IV dose or 5 daily oral doses. After 6 hrs both routes had similar extensive distribution profiles with highest levels in liver and kidney. At 30 min post dose, IV administration produced highest levels in endocrine and exocrine glands, urinary system and gut. Oral dosing produced highest early levels in oral/esophageal mucosa, gut, urinary system and liver. CNS levels were lower after oral dosing compared to IV.

Quantitation of ^{14}C-labelled ROP in rat tissue samples generally indicated highest tissue levels 1 hr after IV administration, and 6 hr after oral dosing. After oral administration high levels were found in the gut, liver and kidneys while brain was below MQL (Tables 19 and 20). IV administration (Tables 21 and 22) also showed high levels in these tissues, but CNS distribution was relatively higher (about equivalent to heart).

In the monkey, limited tissue analysis after a 6 hr IV infusion of 1 mg/kg ^{14}C-ROP indicated high label levels in kidney and liver (Table 23). The levels relative to brain were about 20:1 and 10:1 respectively; comparable to those in rat (about 11:1 and 8:1).

Single IV (1.6 mg/kg) and oral (1.8 mg/kg) doses of ^{14}C-ROP salt in hooded Lister rats examined the affinity of compound and metabolites for pigmented tissues. Pigmented tissues (eye, skin) had high radiolabel levels at 6 hr relative to non-pigmented tissues in albino rats, whereas other tissues had similar levels:

	Lister		Albino	
	IV	PO	IV	PO
skin nmol/g	3.7	.38	.28	.28
eyes nmol/g	23.2	.23	.1	.1

Binding to pigmented tissue was greater following IV administration. The levels in the eye declined with a half life of 16-20 days, and thus were still detectable at 28 days. Since only single dose studies were performed, the potential for accumulation in pigmented tissues is unknown. The sponsor notes

the demonstrated affinity of lipophilic molecules with basic groups, which is believed reversible and not commonly associated with toxicity. Chloroquine and chlorpromazine can bind to melanin and have been associated with retinopathy (especially chloroquine), but the relationship between these effects is unknown and binding in the eye is much longer than observed with ROP, with compound retention up to 1 year.

Distribution into brain was measured in 4 M rats following IV administration of 2.2 mg/kg (0.6 mg/kg bolus and infusion of 0.6 mg/kg/h for 2.5 h) 14C-ROP or SKF 89124. IV was used since levels were below MQL following oral dosing. The difference between oral and IV dosing may be explained by the low bioavailability of ROP due to first pass metabolism, and thus a low transfer into brain compared to IV administration. Penetration was high in rat since the brain:blood ratios were around 1 (Table 24), and different brain areas only varied by a factor of 2. This would be anticipated in light of the positive logP (2.4). ROP appeared to be the primary entity transferring into brain since the penetration of SKF 89124 was 3-4 fold lower in rat (Table 24), and only ROP was found in monkey brain after IV infusion of 1 mg/kg (although an extraction efficiency for SKF 104557 appears not to have been defined). In a 10-day oral toxicology study in monkeys using 0, 15, 30, 60 and 240 mg/kg/day p.o., csf 2 hrs post-dose on days 9 or 10 contained mainly ROP (247 ng/ml @ 15 mg/kg) and some SKF 89124 (29 ng/ml), with a csf:plasma ratio of around 1, similar to brain penetration in rats (Table 25).

Placental transfer was examined following daily oral administration at 150 mg/kg/d for days 10-15 of pregnancy. The maternal radioactivity showed high glandular levels, which was previously observed after IV dosing at 1 mg/kg, but was otherwise comparable to non-pregnant animals. Fetal levels were comparable to plasma, as in the case of brain, indicating a low barrier to diffusion. Radioactivity was well distributed in the fetus, with no organ concentration.

Drug transfer into milk occurred following single oral dosing of 3 lactating rats at 0.5 mg/kg ^{14}C-ROP on day 12 after parturition, but levels were 2-9-fold lower than plasma and the total transfer was about 0.01% of total dose over 8 hrs.

METABOLISM

Liver enzymes

In rat, induction of enzymes was examined following 3 days @ 220 mg/kg/d or 14 days @ 44 and 100 mg/kg ROP base p.o. and preparing liver S9. Total CYP450 was measured by reduced CO binding spectrum, and activities for ethylmorphine N-demethylation (EDM) and 7-ethoxycoumarin O-deethylation (ECOD) were determined. ROP induced a dose-dependent increase in CYP450 levels, with a 28%

rise in F only @ 44 mg/kg/d, to a maximum of 43-48% in both M and F at the higher doses. EDM and ECOD activities were increased to a maximum of about 2-fold. These increases were regarded as moderate compared to other inducers such as phenobarbitone, but only data for ECOD, which can be elevated to a three-fold greater level than by ROP, were supplied.

ROP and its main metabolites (20-500 μM) were directly examined for inhibition of EDM and ECOD. Only the highest concentration of SKF 89124 had an effect, on EDM, indicating minimal likelihood for activity in vivo.

Human liver microsome metabolism was much higher than for kidney or lung microsomes, with formation of SKF 104557 being about 4-fold higher than for SKF 89124. This reflects in vivo metabolism where 56% of an oral dose of ROP undergoes depropylation compared to 11% being hydroxylated. Furafylline inhibition indicated high affinity CYP1A2 as the major metabolizing enzyme. ROP had little inhibitory effect on P$%) enzymes, even on CYP1A2, as assessed by metabolic probes (Table 26) since the affinity for ROP was relatively low (up to 270 μM).

Biotransformation and excretion

ROP was extensively metabolized. Primarily, phase I metabolism was by hydroxylation to SKF 89124 (Fig 21) or depropylation to SKF 104557 (with some subsequent metabolism to SKF 96990 and 97930; Fig 21). These metabolites were then glucuronidated, especially SKF 89124. SKF 89124 was the primary metabolite in rat, with SKF 104557 being the primary metabolite in mouse, monkey and man.

In the rat, after single oral doses of 0.5-1.75 mg/kg ROP, parent compound was not detectable in plasma, but comprised 17% after 220 mg/kg, a similar level to that observed following 1.75 mg/kg IV, indicating saturable first pass metabolism (Table 27).

A 14 day rat repeat dose study with PK analysis at days 1 and 14 using 0, 1.5, 15, 50 mg/kg ROP base p.o. showed that at the HD ROP Cmax and AUC greatly increased over 14 days in both M and F (Table 10), as did the metabolite SKF 104557, while levels of SKF 89124 were stable or declined by 14 days (Table 10). This decline indicates an impaired ability to hydroxylate the parent molecule rather than just enzyme saturation.

In a satellite to a segment II study, the metabolite profile of ^{14}C-ROP in rats after treating for days 10-15 of pregnancy at a dose rate of 150 mg/kg/d was similar to the 14 day studies noted above (Table 27). Urine constituents generally paralleled plasma levels, with SKF 89124 glucuronide being the primary metabolite (Tables 27 and 28). Unconjugated SKF 89124 comprised 2-10% of

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dose.

In monkey the major metabolic route was depropylation to SKF 104557, and SKF 97930 was a more prominent end product (Table 27). Urinary metabolites were similar to plasma metabolites, with SKF 104557 and SKF 97930 comprising 24% and 11% respectively of oral doses (Table 28).

Radiolabelled metabolites in human plasma were usually below MQL, but urinary content of SKF 104557 and SKF 97930 at 40% and 9% respectively was proportionally most similar to the mouse and monkey (Tables 27 and 28). Glucuronidated SKF 89124 comprised about 9% but the level of SKF 89124 itself was low (<5%). ROP was present at 7% of dose. Urinary metabolites were similar following IV or oral dosing, consistent with first pass metabolism being less important in humans.

Following 2-12 mg single doses of ROP p.o. in man the levels of SKF 104557 were similar or greater than for ROP, but SKF 89124 constituted <5% of total dose (Table 27).

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Table 27 Metabolic profile of ropinirole

			% radioactivity or % identified compounds				
Species	Dose mg/kg (base)	Assay	ROP	SKF 104557	SKF 89124 (+glucuronide)	SKF 96990 (+glucuronide)	SKF 97930
Mouse	0.88 IV, and p.o.	Plasma 0.5-6 h	6	39	18	15	
Rat	1.75 p.o.	Plasma 1 h	ND	ND	65		
Rat	1.75 IV	Plasma 1 h	19	ND	60		
Rat	220 p.o.	Plasma	17	<5	52		
Pregnant Rat	150 p.o. for 6 days	Plasma 2 h d6	17	28	55		
Monkey	13 p.o.	Plasma 1 h	2	17	6	16	20
			% Total dose (see also Table 28)				
Mouse	0.88 IV	Urine	8	42	6	5	
Rat	1.75 p.o.	Urine	ND	ND	57		
Rat	220 p.o.	Urine	3	9	16		

ND= not detected

Excretion of radioactivity (% dose)

Species	Dose/route mg/kg	Urine	Feces
mouse	8.8 IV	89	4.5
mouse	8.8 p.o.	86	6
rat	1.75 IV	61	30
rat	1.75 p.o.	57	37
monkey	0.88 IV	72	14
monkey	13 p.o.	72	12
human	0.0016 IV	90	3
human	0.012 p.o.	88	3.5

Table 10: The *in vitro* binding of radiolabeled ropinirole and SK&F 89124 to plasma proteins in the rat, dog, cynomolgus monkey and man [42,43].

Compound	Concentration range (ng/mL)	Percentage plasma protein binding			
		Rat	Dog	Monkey	Man
[^{14}C]SK&F 89124	6-1300	23.8 ± 2.09	27.4 ± 2.75	-	24.7 ± 4.95
[^{14}C]ropinirole	9-5800	29.6 ± 2.65	38.6 ± 2.64	-	39.1 ± 3.77
[^{3}H]ropinirole	0.03-300	-	-	-	11.0 ± 3.05
[^{3}H]ropinirole	0.03-3000	-	-	9.87 ± 1.74	-

Values are means ± S.D. of 16-20 determinations. - not determined

The *in vitro* blood to plasma partition ratios for ropinirole and SK&F 89124 in rat, dog, monkey and man were essentially independent of the concentration used and are given in Table 11 [42,43].

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TABLE 12: Peak Radioactivity Concentrations in Selected Tissues After Oral Gavage Administration to Male, Albino Rats of SK&F 101468-A, Target Dose 2 mg.kg^{-1}

Results are expressed as a percentage of the administered dose contained in the whole tissue and are the mean of the values for 4 animals.

Tissue	Time (h)	Peak Radioactivity Concentration (% dose.wet tissue^{-1})	Range (% dose.wet tissue^{-1})
Heart	6	0.004	
Lungs	6	0.015	
Liver	1	2.66	
Spleen	6	0.002	
Stomach	1	0.450	
Small Intestine	1	8.50	
Large Intestine	6	0.962	
Kidney	6	0.134	
Adrenal Glands	6	0.001	
Testes	6	0.014	
Eyes (pair)	6	0.001	
Brain	72	0.004	
Skeletal Muscle	6	0.246	
Whole Blood	6	0.417	
Plasma	6	0.309	
Salivary Glands	6	0.004	
Thyroid #	1	0.001	
Bone Marrow	6	0.006	
Skin Dorsal +			
Skin Ventral +			
Fat +			
Gut Contents	1	78.2	75.5 - 80.9
Pituitary Gland	6	0.000	0.000 - 0.000***

Key :- *** :- >= 75% of samples N.D
** :- >= 50% of samples N.D
* :- >= 25% of samples N.D
N.D. :- Not detected
+ :- Total weight of tissue not known
# :- peak concentration at 1 h expressed in % dose.wet tissue^{-1} but at 6 h expressed in nmole equiv.g^{-1} (Table 11)

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TABLE 14: Tissue Distribution of Radioactivity in Male, Albino Rats 6 Hours After Oral Gavage Administration of ^{14}C-SK&F 101468-A. Target Dose 2 mg.kg^{-1}

All results given are the mean of 4 animals.

Tissue			Radioactivity Concentration Mean (nmole equivalents SK&F 101468.g^{-1} wet tissue)	Range (nmole equivalents SK&F 101468.g^{-1} wet tissue)	(nmole equivalents SK&F 101468. total wet tissue^{-1})	(% dose.g^{-1} wet tissue)	(% dose. total wet tissue^{-1})
Heart			0.105		0.078	0.006	0.004
Lungs			0.230		0.272	0.013	0.015
Liver			2.03		20.5	0.107	1.07
Spleen			0.074		0.041	0.004	0.002
Stomach			2.17		3.08	0.130	0.188
Small Intestine			7.60		34.1	0.423	1.93
Large Intestine			7.68		17.7	0.409	0.962
Kidney			1.52		2.52	0.081	0.134
Adrenal Glands			0.150		0.010	0.008	0.001
Testes			0.183		0.231	0.006	0.014
Eyes (pair)			0.103		0.023	0.006	0.001
Brain			N.D.		N.D.	N.D.	N.D.
Skeletal Muscle	W		0.062		4.37	0.004	0.246
Whole Blood	W		0.412		8.24	0.021	0.417
Plasma	W		0.775		6.20	0.039	0.389
Salivary Glands			0.145		0.063	0.008	0.004
Thyroid			0.165		0.005	0.008	0.000
Bone Marrow	W	***	0.416		0.416	0.006	0.006
Skin Dorsal	+		0.279			0.015	
Skin Ventral	+		0.277			0.016	
Fat	+		0.089			0.005	
Gut Contents			69.3		696	3.45	36.1
Pituitary Gland		***	0.159		0.002	0.011	0.000

Key :- W - Default tissue weight used
+ - Total weight of tissue not known
*** - >= 75% of samples N.D.
** - >= 50% of samples N.D.
* - >= 25% of samples N.D.
N.D. - Not detected

25d

21

TABLE 6: Peak Radioactivity Concentrations in Selected Tissues After Intravenous Bolus Administration to Male, Albino Rats of ^{14}C-SK&F 101468-A, Target Dose 2 mg.kg^{-1}

Results are expressed as a percentage of the administered dose contained in the whole tissue and are the mean of the values for 4 animals.

Tissue	Time (h)	Peak Radioactivity Concentration (% dose.wet tissue^{-1})	Range (% dose.wet tissue^{-1})
Heart	1	0.020	
Lungs	1	0.077	
Liver	1	2.53	
Spleen	1	0.037	
Stomach	1	0.125	
Small Intestine	1	10.2	
Large Intestine	6	1.63	
Kidney	1	0.552	
Adrenal Glands	1	0.014	
Testes	1	0.330	
Eyes (pair)	1	0.007	
Brain	1	0.065	
Skeletal Muscle	1	3.40	
Whole Blood	1	0.631	
Plasma	6	0.393	
Salivary Glands	1	0.063	
Thyroid	1	0.002	
Bone Marrow	1	0.108	
Skin Dorsal	+		
Skin Ventral	+		
Fat	+		
Gut Contents	1	49.3	38.6 - 53.4
Pituitary Gland	1	0.001	0.001 - 0.002

Key :- + :- Total weight of tissue not known

TABLE 6: Tissue Distribution of Radioactivity in Male, Albino Rats 1 Hour After Intravenous Bolus Administration of ^{14}C-SK&F 101468-A; Target Dose 2 mg.kg^{-1}

All results given are the mean of 4 animals.

Tissue		Radioactivity Concentration Mean (nmole equivalents SK&F 101468.g^{-1} wet tissue)	Range (nmole equivalents SK&F 101468.g^{-1} wet tissue)	(nmole equivalents SK&F 101468. total wet tissue^{-1})	(% dose.g^{-1} wet tissue)	(% dose. total wet tissue^{-1})
Heart		0.403		0.253	0.032	0.020
Lungs		0.897		0.986	0.070	0.077
Liver		3.36		32.2	0.264	2.53
Spleen		0.962		0.476	0.075	0.037
Stomach		1.32		1.61	0.103	0.125
Small Intestine		31.9		129	2.51	10.2
Large Intestine		2.19		4.88	0.172	0.384
Kidney		4.92		7.02	0.386	0.552
Adrenal Glands		2.52		0.171	0.198	0.014
Testes		2.33		4.20	0.183	0.330
Eyes (pair)		0.477		0.085	0.037	0.007
Brain		0.444		0.830	0.035	0.065
Skeletal Muscle	W	0.621		43.5	0.049	3.46
Whole Blood	W	0.402		8.03	0.032	0.631
Plasma	W	0.547		4.37	0.043	0.344
Salivary Glands		2.18		0.803	0.171	0.063
Thyroid		0.739		0.029	0.058	0.002
Bone Marrow	W	1.38		1.38	0.108	0.108
Skin Dorsal	+	1.44			0.113	
Skin Ventral	+	1.24			0.098	
Fat	+	1.88			0.147	
Gut Contents		49.5		630.	3.87	49.3
Pituitary Gland		3.02		0.014	0.237	0.001

Key :- W - Default tissue weight used
+ - Total weight of tissue not known

Table 23 Concentrations of Radioactivity in Selected Tissues of Monkeys M60 and E48B Following Intravenous Infusion of ^{14}C-SK&F 101468-A (1 mg.kg^{-1}, free base)

Tissue	Concentration of radioactivity* Monkey M60	Monkey E48B
Liver	18.2	13.5
Kidney	41.0	19.8
Brain#	1.9	2.1
Pituitary gland	2.9	13.5

* Expressed in nmole equivalents.g^{-1} wet tissue weight

\# Determined one half of the entire brain only

Table 12: **Concentrations of radioactive material in the brain of male rats following an intravenous dose of either [^{14}C]ropinirole or [^{14}C]SK&F 89124A at 2.2 mg/kg [49].**

Sample	[^{14}C]ropinirole		[^{3}H]SK&F 89124A	
	Concentration	Ratio#	Concentration	Ratio#
Blood	181 ± 71	-	241 ± 30	-
Olfactory bulb	138 ± 51	0.76	88 ± 43	0.37
Cortex	296 ± 97	1.64	90 ± 30	0.37
Striatum	142 ± 30	0.78	86 ± 41	0.36
Thalamus & hypothalamus	141 ± 36	0.78	92 ± 35	0.38
Cerebellum	273 ± 36	1.51	94 ± 29	0.39
Hippocampus	126 ± 37	0.70	75 ± 22	0.31
Mid brain	137 ± 28	0.76	77 ± 32	0.32
Brain stem	216 ± 80	1.19	78 ± 35	0.32
Whole brain	260 ± 36	1.44	88 ± 33	0.37

** Concentrations are in terms of ng of either of ropinirole or SK&F 89124 free base equivalent/g or mL and are mean values ± S.D. from 4 animals. # - Ratio of the concentration in the brain to that in blood.*

Ropinirole-related material was also shown to cross the blood brain barrier in the cynomolgus monkey at the end of an intravenous infusion of [^{14}C]ropinirole at 1 mg/kg [50]. Plasma and brain concentrations of radioactivity were very similar, and although, ropinirole accounted for the majority of the radioactivity in the brain, the compound only represented a very small proportion of the radioactivity in the plasma.

The cerebrospinal fluid (CSF) of cynomolgus monkeys obtained at necropsy (2 h after dosing on Day 9 or Day 10) in a ten day oral toxicity study at doses of 15, 30 and 60 mg/kg/day (Table 13 was found to contain both ropinirole and

SK&F 89124, [32]). The concentrations of both these compounds in the CSF increased with increasing dose. The ratios of the concentrations of ropinirole in CSF to those in plasma ranged from 0.8 to 1.2. The concentrations of SK&F 89124 in the CSF were in all cases lower than those in the plasma, and the CSF to plasma concentration ratios of the compound ranged from 0.4 to 0.8.

Table 15: Concentrations of ropinirole and SK&F 89124 in the cerebrospinal fluid (CSF) of cynomolgus monkeys at necropsy following oral administration of ropinirole at 15, 30 and 60 mg/kg/day for up to 10 days [32].

Dose (Sex)	Ropinirole			SK&F 89124		
	Concentration (ng/mL)		Ratio#	Concentration (ng/mL)		Ratio#
	CSF	Plasma		CSF	Plasma	
15(M)	80.3	80.9	1.0	ND	<10	-
30(M)	1620	1370	1.2	44.8	71.8	0.6
60(M)	3450	4490	0.8	117	302	0.4
15(F)	247	282	0.9	18.1	29.1	0.6
30(F)	428	501	0.9	NS	29.8	-
60(F)	1710	1550	1.1	147	196	0.8

Doses are in terms of mg/kg/day. Data are for a single male (M) and a single female (F) animal at approx. 2 h after dosing on Day 9 or Day 10. # - Ratio of the concentration in CSF to that in plasma. ND - not detected. NS - No sample. - not determined.

5.C.2.5.5 Placental transfer of drug-related material in pregnant rats [51]

Placental transfer of drug-related material into fetal tissues was examined in pregnant Wistar rats following repeated daily oral administration of [^{14}C]ropinirole at 150 mg/kg/day for 6 days (Days 10-15 of pregnancy).

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Table 28 **Radiometabolite patterns in 0-24 h urine of mouse, rat , monkey and man following single oral or intravenous doses of [^{14}C]ropinirole [14,27,63]**

Compound	Percentage of dose							
	Oral				Intravenous			
	Mouse	Rat	Monkey	Man	Mouse	Rat	Monkey	Man
	8.8 mg/kg	1.75 mg/kg	13 mg/kg	0.6 mg	8.8 mg/kg	1.75 mg/kg	0.88 mg/kg	0.08 mg
Ropinirole	<1	ND	1	7	8	1	3	9
SK&F 89124	ND	10	ND	1	ND	2	<1	<1
SK&F 104557	42	ND	24	40	42	ND	12	34
SK&F 96990	ND	ND	NS	<1	ND	ND	2	1
SK&F 97930	4	ND	12	9	3	ND	11	8
SK&F 89124 gluc.	7	47	NS	9	6	55	2	8
SK&F 96990 gluc.	5	ND	5	1	5	ND	7	1
SK&F 104557 carbamyl gluc.	ND	ND	6	5	ND	ND	6	4

ND - Not detected. NS - Not separated; in monkey urine SK&F 96990 and SK&F 89124 glucuronide did not separate under the HPLC conditions used and together accounted for approx. 5% of the dose. Data for the rat are for a pooled sample from 4 animals and those for the mouse, monkey and man are mean values (n=2-6). gluc - glucuronide.

Table 28

000350

Excretion was primarily renal in all species examined (Table 27), and was similar in the rat from 1.75 up to 220 mg/kg p.o. (57-67% of total dose). With bile duct cannulation, at an oral dose of 20 mg/kg, urinary excretion was 23% and 76% was present in bile. Although a corresponding dose level appears not to have been examined in normal rats, the substantial difference in urinary excretion with bile duct cannulation compared to bracketing doses in normal rats indicates significant entero-hepatic circulation.

ACUTE/SUBACUTE TOXICITY

Species	Route	Approx LD50 mg/kg
Mouse	p.o.	667
	IV	46
Rat	p.o.	862
	IV	66

Clinical signs:
Mortality was associated with convulsions and respiratory abnormalities. Other clinical signs were consistent with CNS DA receptor activation, including hyperactivity, stereotyped behaviors (head movements), tremors and convulsions. The dose-response relationship for these signs was not delineated. Highest non-convulsive doses were 400 mg/kg p.o. in mice and 800 mg/kg p.o. in rats (salts).

Acute toxicity of metabolites

The primary metabolite in man, mouse and monkey, SK&F 104557-A, had no observable adverse effect in mice at doses up to 10 mg/kg IV, the highest nonconvulsive IV dose level of SK&F 101468. SK&F 104557-A is further metabolized to SK&F 96690 and 97930. About 10 % of SK&F 101468 is excreted as the latter compound, and <1% as SK&F 96690. At doses up to 10 mg/kg IV, SK&F 97930 had no observable adverse effects in mice.

In the rat the major metabolite of ROP is SK&F 89124. About 10 % of oral ROP in man is excreted as SK&F 89124 in urine, as compared with up to 47% in rat (dose-dependent). Doses of 1-10 mg IV SKF 89124 in rats induced transient clinical signs consistent with DA-receptor stimulation, including ptosis and hyperactivity, but no other observable toxicological effects.

Previous reviews of subacute studies (30-day and shorter range-finding studies) indicated that primary toxicity was related to CNS activity, with clinical signs as observed in acute studies. Target organ toxicities identified included ground-glass appearance of centrilobular hepatocytes of male rats @ 250 mg/kg or higher, dose-related increase in liver, prostate and ovarian weights in rats, and adrenal wt in female rats. Monkeys showed diffuse hepatic and renal lipidosis and/or thymic lymphocyte depletion @ 240 mg/kg. Reticulocytosis increased at 30 mg/kg, although the stimulus was not clear, leading to an elevated erythron. ALT and AST increased in monkeys at 30 mg/kg or higher. Rats showed elevated serum AP and ALT at doses that caused hepatocyte changes, which may have been related to microsomal induction. Blood glucose decreased in concert with decreased weight gain and lower food consumption. BUN increased with no histopathological correlate and the A/G ratio was increased. Ovarian weight increased along with inhibition of corpora lutea lysis. Adrenal weight increased in F and HD M, with increases in the width of zona fasciculata and zona reticulata.

MOUSE CHRONIC TOXICITY

90 Day Oral Toxicity Study (report TW027BA)

DOSE SELECTION

Selected from a 7-day sighting study:

mg/kg/d p.o.	
200	Max non-lethal dose
100	Max non-convulsive dose
100	Ptosis, hyperactivity
25	NOAEL

METHODS

Species:
CD-1, 16/s/g

Treatment:
0, 25, 100, 200, 250 mg/kg/d p.o. in water for 30 (4 M/4 F interim sacrifice) or 90 days 16/s/group
Batch 10.

Observations:
Clinical signs, hematology and serum chemistry (at day 30 sacrifice of 4 animals from each group), food and water consumption (4 measurements over 90 days), body weight (weekly),

macroscopic and microscopic pathology (day 31 or from full necropsy on day 91).

RESULTS

Mortality:

Dose	Males	Females	Time of death excluding gavage-related
0	0/16	0/16	
25	2/16	0/16	
100	3/16	1/16	1,3,7 weeks
200	4/16	5/16	2,6,7,10 wks
250	13/16	12/16	1-6, 9 weeks, mainly 1-3 wks

In 11 animals death was observed preceded by clinical signs (subdued behavior, abnormal gait, tremors, convulsions). Others were found dead without premonitory signs.

No drug-related effects were noted for body weight, food consumption, hematology, clinical chemistry or pathology.

EVALUATION

100 mg/kg produced substantial mortality over 90d, including mortality in <7 days, which was not observed in a 7-day study, indicating that it is too high a dose for the carcinogenicity HD. Clinical signs were consistent with CNS DA receptor activity and toxicity was not associated with histologic lesions. The only dose below this, 25 mg/kg was a NOAEL.

60 day range finding (report TP005BA)

DOSE SELECTION

In a 90-day study mortality was seen at 100 mg/kg/d, but not in a 7-day study. No effects were seen @ 25 mg/kg/d. This study was designed to determine dosages for the mouse carcinogenicity study.

METHODS

Species:
CD-1, 10/s/g

Treatment:
Doses of 0, 10, 25, 50, 100, 200, 250 mg/kg/d salt p.o. in water @ 10 ml/kg

Observations:
Mortality, clin signs (daily), food consumption (weekly), body weight (daily), macroscopic pathology only (since no histologic lesions were observed at 90 days) on termination and pharmacokinetics on all mice @ 10, 25 and 50 mg/kg (and some controls).

RESULTS

Mortality:
Observed at and above 50 mg/kg/d, with 1(F)/20 dead @ 50; 1(F)/20 @100; 2(M)/20 @ 200; 7(2F,5M)/20 dead @ 250.
Usually preceded by convulsions. Only females died @ 50 and 100 mg/kg/d (1/10), but more males died @250 (5/10). 5 deaths, including all 50-200 mg/kg/d groups were <4 weeks, while 5/7 deaths in the 250 mg/kg/d occurred in weeks 5-7.

Clinical signs:
Line listings were not provided. Hyperactivity occurred in all drug-treated groups, usually within 1-2 hours post dose, and was dose-dependent and time dependent, increasing over the first 8 days and then decreasing in incidence. On day 2 only a few mice @ 200 and 250 mg/kg were hyperactive but by day 8 almost all were. At day 8 25 mg/kg only occasionally produced activity and the other doses showed a dose-related effect. After 14 days hyperactivity was not seen @ 25 mg/kg/d. From day 6 convulsions were observed in at least 1 animal in the 250 mg/kg/d group, but less frequently @ 200 and not at lower doses.

Food consumption/body weight:
Transient decreases were noted in all drug groups for up to 3 weeks, with no clear dose-dependence, then values returned towards control. At study termination males at and above 25 mg/kg and females at 200 mg/kg had weight decreases of about 5%.

Other:
No macroscopic pathology noted.

Pharmacokinetics:

Performed by at day 61 following last dose. The peak was at the first time point (30 min) measured, so it may not reflect the true Cmax.

Dose (mg/kg)	Plasma concentration with time (ng/ml) 30 min	90	360	AUC (ng.min/ml)
10	69	15	5	4369
25	267	62	11	12423
50	430	59	18	19511
Ratio 10:25:50	1:3.9:6.2			1:2.8:4.5

An Oral Carcinogenicity Study of SK&F 101468-A in CD-1 Mice (report TP1004/SKF 101468/2)

DOSE SELECTION

50 mg/kg was expected to be the highest dose that would not show limiting clinical signs and mortality since mortality was 1/20 @ 60 days and 4/32 @ 90 days in prior studies.

METHODS

Species:
CD-1 mice from 42±1 day of age housed 1/cage, grouped in racks with rack position moved but not animal position, 60/s/group.

Treatment:
0, 0, 5, 15, 50 mg/kg/d base equivalent administered as HCl salt in water by gavage for 104 weeks. Solution was made weekly with stability >14 days. 10 ml/kg/d.

Observations:
Clinical signs (daily, then weekly after 4 weeks). Food consumption (weekly for 13 weeks then every 90 days), body weight (weekly). Palpation for masses (weekly after wk 26). Terminal studies were hematology (RBC/WBC), gross pathology and histopathology (all controls and HD animals; animals that died or were sacrificed preterminally; all gross lesions)

RESULTS

Mortality
No significant increases in mortality were noted as shown in Table 29 and Figs 22 and 23. A significant decrease in mortality was found @ 5 mg/kg/d compared to combined control groups.

Clinical signs
Hyperactivity with intermittent vigorous movement and stationary licking/grooming/biting was noted on at least 1 occasion from weeks 36-37 for HD groups for 44% (27/60 M; 27/60 F) animals. Only 3/60 F showed hyperactivity @ 15 mg/kg. The HD group hyperactivity was noted much later than in the 60-day study in which hyperactivity was noted from 8 days onward in some mice from the 50 mg/kg/d dose group. There are no raw data to compare hyperactivity rates or severity. Alopecia increased with dose from 32% and 23% in M and F controls respectively to 67% in HD M or F mice. Lower dose F also showed increased incidence (40-47%). This may be partly due to increased licking and/or grooming. Palpable masses were not increased in treated groups, with controls having an incidence of 9/120 and 4/120 compared to 8/120, 6/120 and 5/120 in LD, MD and HD groups respectively.

Body weight/food consumption
There was often a significant decrease compared to combined controls (Fig 24)in mean body weight (usually about 1.5 g/4%) between weeks 28 and 60 in the 15 and 50 mg/kg/d dose M groups. By week 99 the difference was still consistent but usually not significant. HD M body weight gain was 10% and 18% less than control groups. HD F also often showed significant decreases in mean body weight compared to one control group, but not the other, over weeks 28-60. At the study termination there was little difference in mean body weights (Fig 25).

Sporadic differences in food consumption were observed, with the HD and MD groups usually being slightly elevated compared to controls, but for the whole study mean weekly intakes were similar (+2% for HD males, +4% HD females).

Hematology
Only HD males had a significant decrease in WBC count (42% and 56% vs the control groups). No group had abnormal RBC counts.

Pathology
Tissues sampled are shown in Table 30. Preterminal and terminal data are combined.

Neoplastic lesions. A significant increase in benign endometrial stromal polyps was observed compared to one control group or both control groups combined (10/120 controls, 14/60 HD F; Tables 31 and 33). LD and MD groups were not different to controls. The sponsor notes that this tumor is relatively common and the increase may be incidental to treatment. A lack of dose-dependence with the other dose groups was observed. Lung alveolar/bronchiolar adenomas and liver adenomas and carcinomas were relatively common (Tables 31 and 32) and did not show increased incidence.

Non-neoplastic lesions. No clear dose-related pathological findings were observed. Interstitial cell hyperplasia of the testis was elevated (7/60 in HD compared to 6/120 controls), but not significantly (Table 34). Retinal degeneration was not increased in the HD group (3/120 control males, 0/60 HD F; 5/120 control females, 2/60 HD F).

EVALUATION

Clinical signs consistent with DA receptor agonism were observed at the HD, although the HD produced less hyperactivity than was observed in a 60-day dose-ranging study.
HD M had a decrease in body weight gain of 10-18% over the study compared to control groups while females were not different to controls at study termination. No increase in mortality was observed, with both controls and HD having about 40% survival. Increased exposure could probably have been achieved by dietary administration. The HD appears appropriately selected based on observed body weight changes and mortality observed in prior toxicology studies @ 100 mg/kg/d (1/20 in a 60-day study and 4/32 in a 90-day study). However, variability was observed between the dose ranging studies. The 2-year study suggests the 90-day study exhibited exaggerated mortality. An intermediate dose between 50 mg/kg/d and 100 mg/kg/d, or possibly higher with dietary administration, would have been a better selection, but repeating the study would be unlikely to provide a much improved risk assessment.

An increase in benign endometrial polyps was observed in HD F only. The sponsor considered this incidental, indicating that there was no difference to one control group and the incidence was within historical control values. However, historical data were not provided and the incidence was significantly different to combined controls. This effect should therefore be regarded as drug-related, especially as the HD was not necessarily the MTD and incidence may have risen with a higher dose. Hyperplasia of the urinary bladder epithelium was noted only in drug-treated animals (1F, 2M), but males only had small abnormal foci.

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RESULTS AND DISCUSSION

1. Mortality (Table 2, Figures I and II)

A total of 166 males and 169 females died or were sacrificed for humane reasons during the 104 weeks of treatment (not including 13 mice replaced during the first 28 days of treatment). The incidence of mortality and the corresponding survival was as follows:

Group	Males Mortality	Males %Survival	Females Mortality	Females %Survival
1 Vehicle Control	30	50	38	37
2 Vehicle Control	40	33	36	40
3 SK&F 101468-A, 5 mg/kg/day	26	57	32	47
4 SK&F 101468-A, 15 mg/kg/day	31	48	29	52
5 SK&F 101468-A, 50 mg/kg/day	39	35	34	43

TABLE 29 MOUSE 104 wk CA study

Analysis of the overall incidence of deaths revealed a statistically significant decrease in mortality for Group 3 males when compared to control animals (combined Groups 1 and 2). This decrease was not considered to be of any biological significance. Statistical comparison of the survival distribution of drug-treated animals against that of the combined control groups revealed no intergroup differences.

2. Clinical Signs (Alopecia/ Thinning of the Fur - Table 3)

A total of 32 mice had cutaneous or subcutaneous masses still present at time of death (preterminal & terminal animals). Of the 300 male and 300 female mice used in this study, the distribution of animals bearing palpable masses (not drug-related) was as follows:

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32

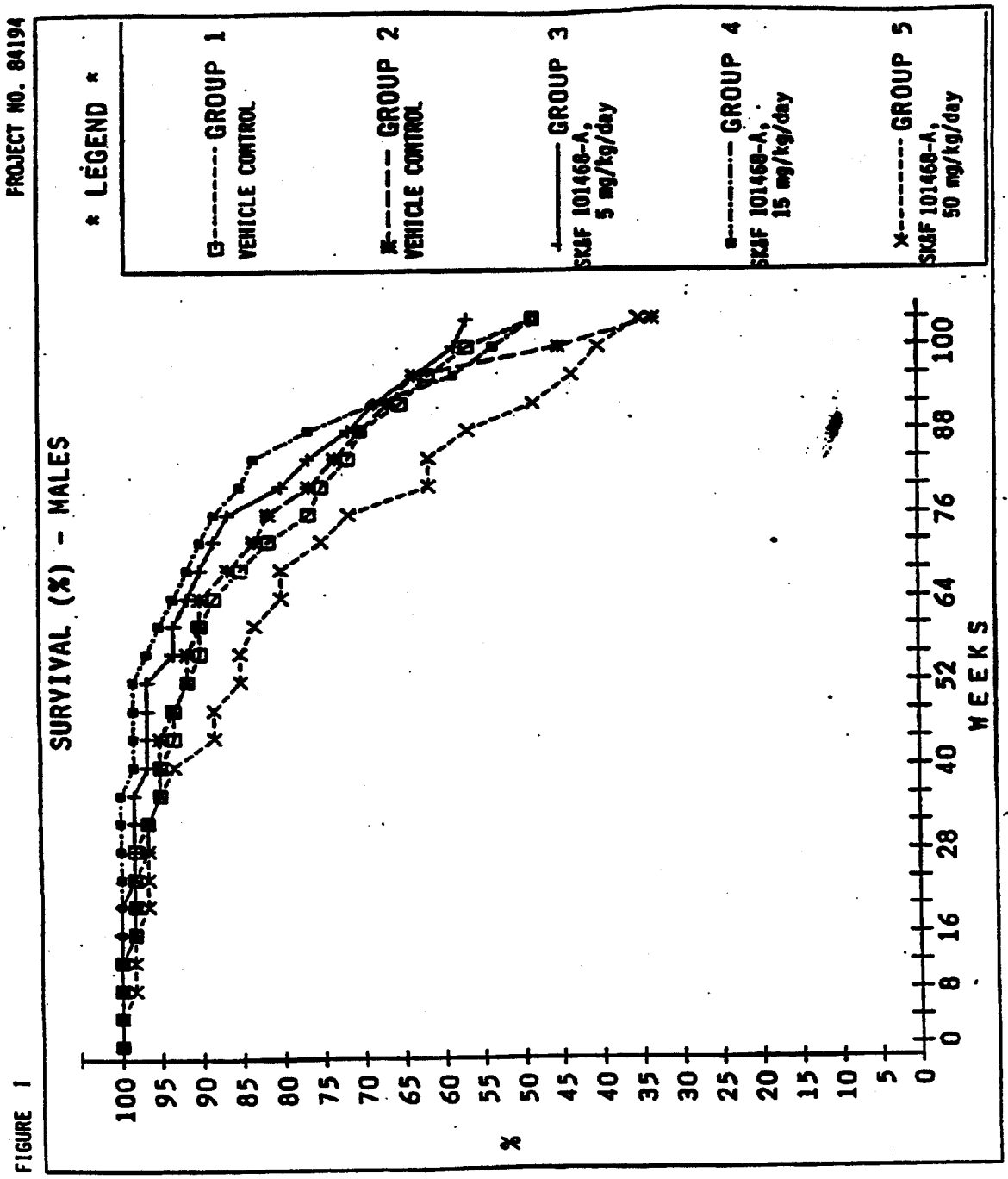

FIGURE 1

Fig 22

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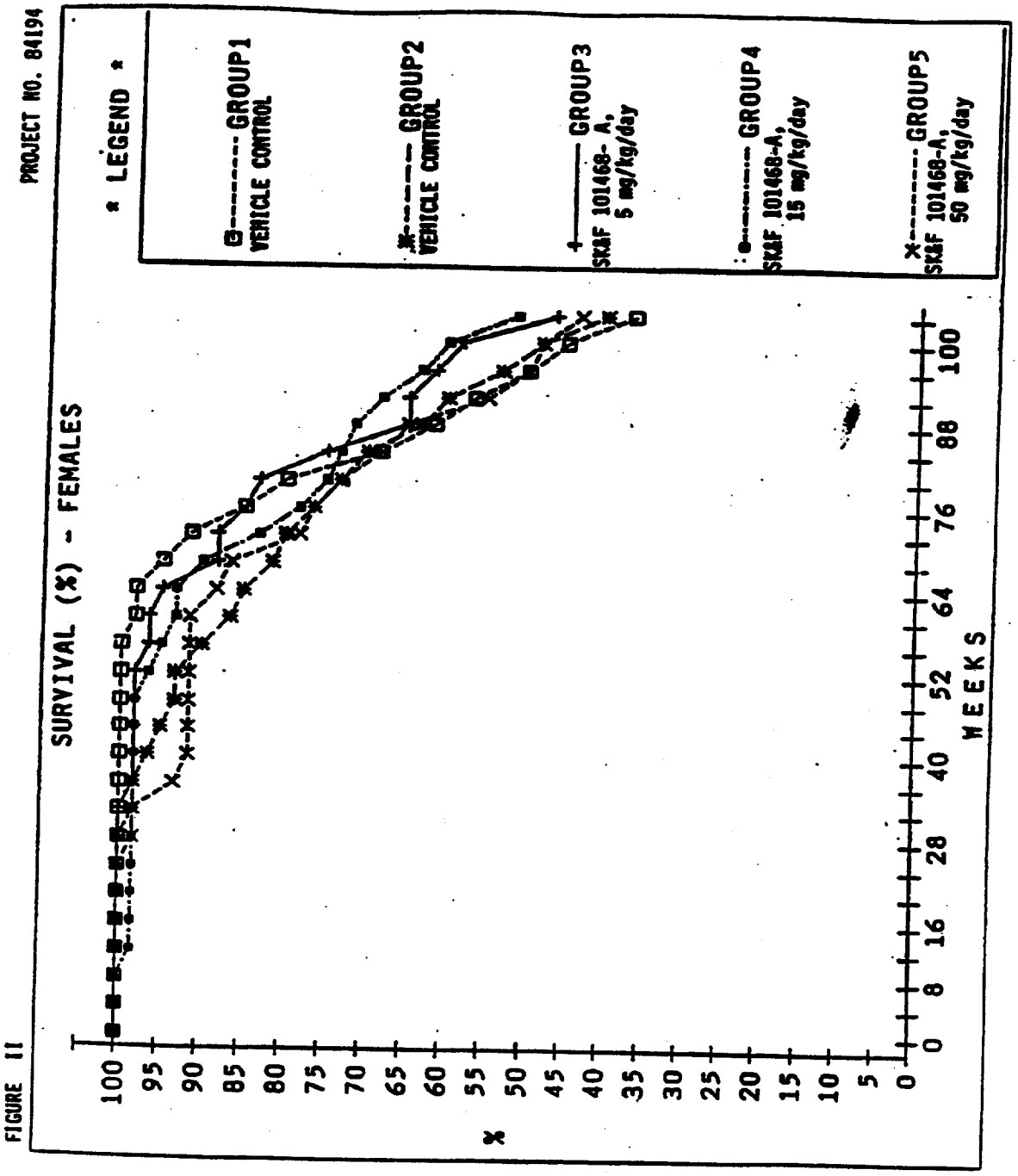

FIGURE 11

Fig 23
1

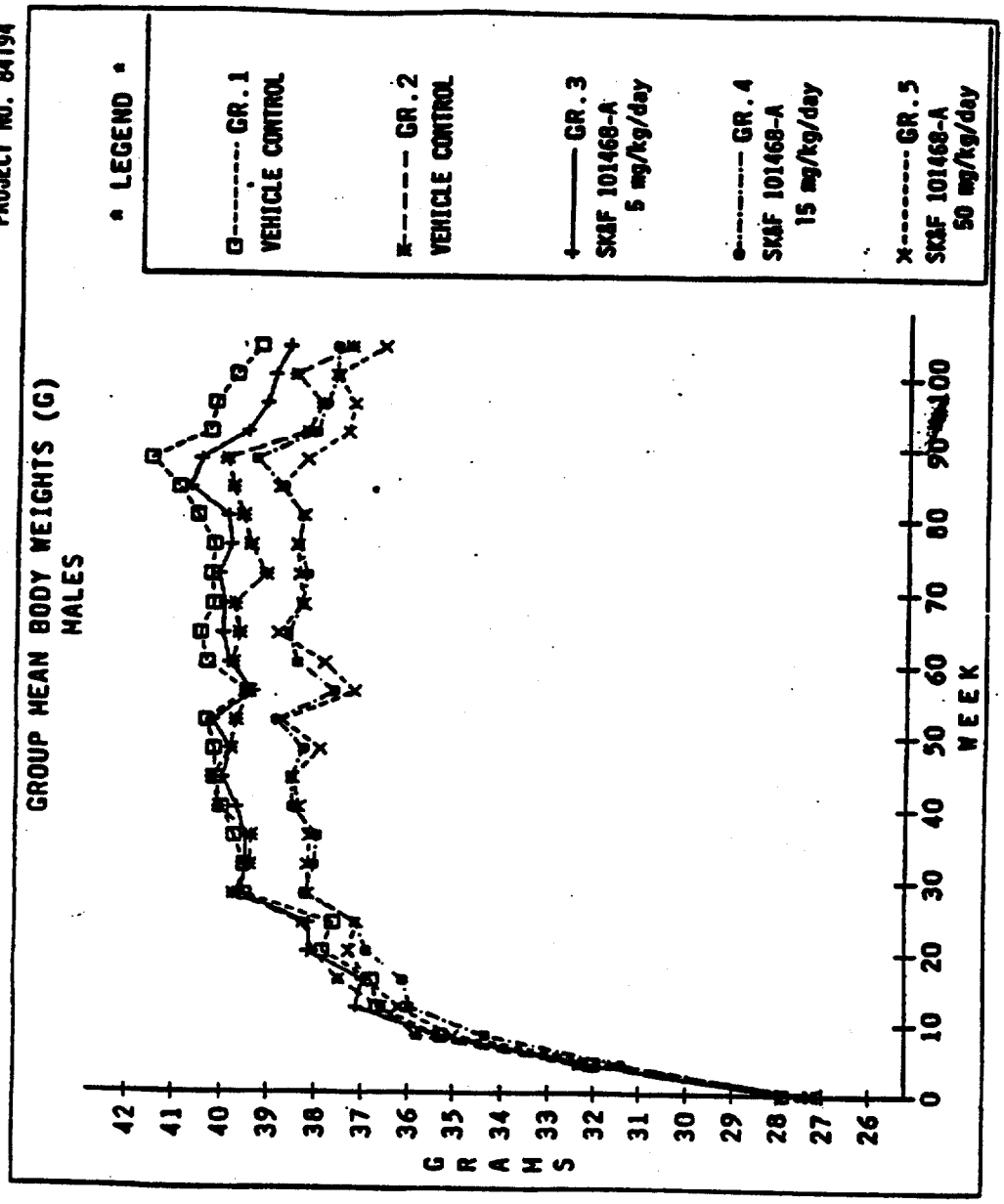

FIGURE III

Fig 24

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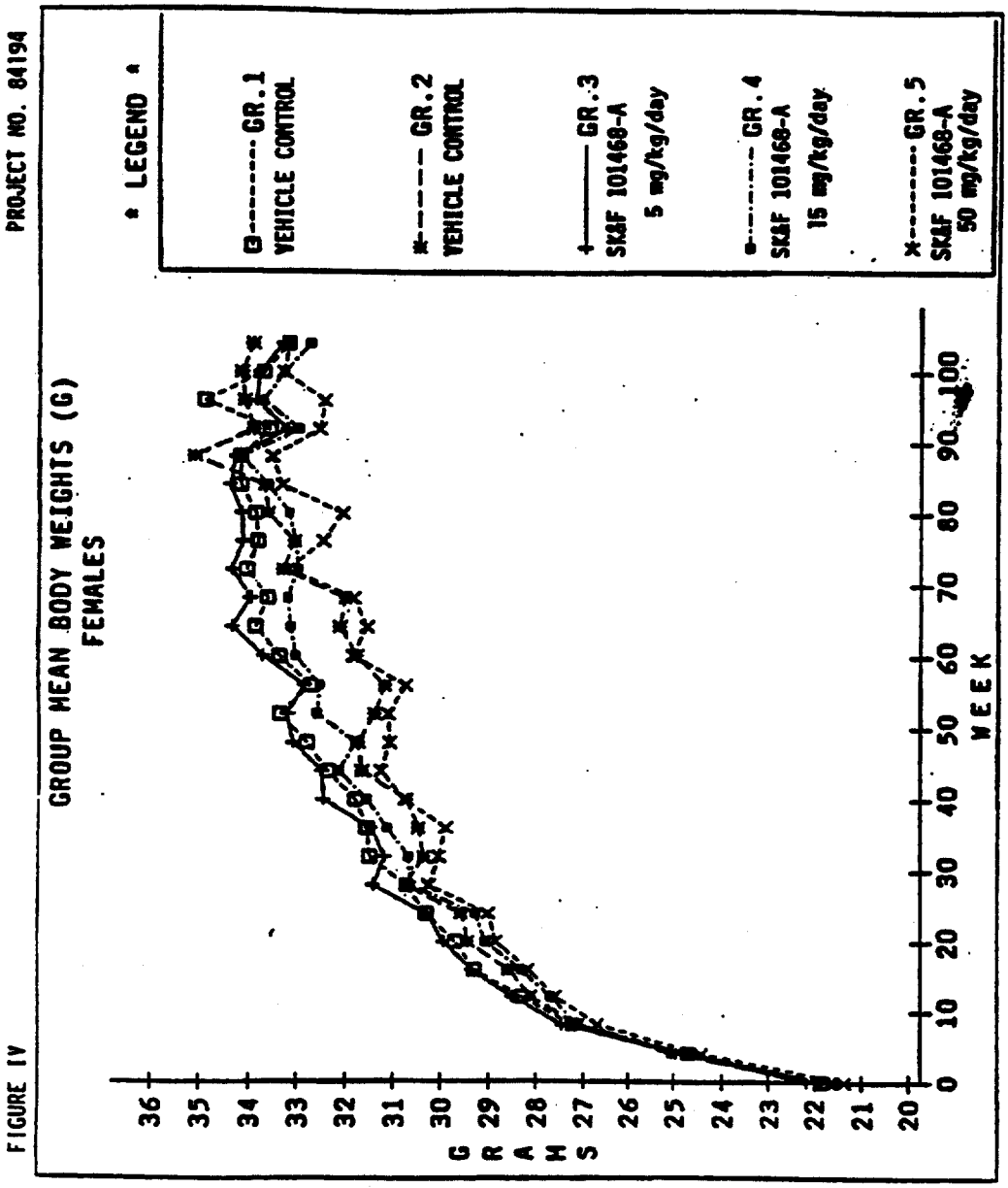

FIGURE IV

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Table 30

Gross pathology mouse CA

formalin was used for fixation and preservation unless otherwise indicated.

adrenals
animal identification++
aorta (thoracic)
bone and marrow (sternum)
brain (3 levels)
cecum++
cervix++
clitoral glands
colon
duodenum
epididymides *
esophagus
eyes *
femurs
gall bladder
harderian glands
heart
ileum
jejunum
kidneys
larynx++
liver (sample of left and right lateral lobes)
lungs (sample of 2 lobes) +
lymph nodes (mandibular and mesenteric)
macroscopic lesions
mammary gland
optic nerves *
ovaries
pancreas
pituitary
preputial glands
prostate
rectum
rib (costochondral junction)++
salivary glands (mandibular, sublingual, parotid)
sciatic nerve++
seminal vesicles
skeletal muscle
skin
skull: base, ear canals, nasal turbinates
spinal cord (cervical)
spleen
stomach
testes *
thymus
thyroid lobes (and parathyroids)
tongue
trachea
urinary bladder
uterus
vagina

Histopathology mouse CA

eosin. Histopathological examination was performed on the following list of tissues:

adrenal glands
aorta
bone and marrow (sternebrae)
brain (3 levels)
clitoral glands**
colon
duodenum
esophagus
eyes
femur
gall bladder
harderian glands **
heart
ileum
jejunum
kidneys
liver, right lateral lobe
liver, left lateral lobe
lung (sample of 2 lobes)
lymph node, mesenteric
lymph node, mandibular
macroscopic lesions
mammary gland +
nasal cavities (2 levels)
optic nerves +
ovaries
pancreas
pituitary
preputial glands **
prostate
rectum
salivary glands: parotid, sublingual, mandibular
seminal vesicle
skeletal muscle
skin
spinal cord, cervical
spleen
stomach
testes/epididymides
thymus
thyroid (and parathyroid) +
tongue
trachea
urinary bladder
uterus
vagina

+ If after routine processing, mammary gland, optic nerves or parathyroids were occasionally missed, the block was resectioned once or the tissue re-embedded for resectioning. If the tissue was still missing, the block was not resectioned since the missing tissue was not determined to be a target organ.

** Tissues were preserved but examined histopathologically only if lesions were observed during gross examination.

All of the aforementioned tissues from all animals in high dose and control groups (Groups 1, 2 and 5) and all animals in the low and intermediate dose groups (Groups 3 and 4) which died or were sacrificed before study termination plus all gross findings in all groups were examined microscopically.

Palpable masses were not increased. Thus, only benign endometrial polyps were identified as drug-related.

RAT CHRONIC TOXICITY STUDIES

14-day, 30-day and 6-month studies were performed with Wistar rats. 14-57-day and 1 year studies were performed with Sprague-Dawley rats

6-month oral toxicity study in rats (report TW012BA)

DOSE SELECTION

In 30-day studies using 10, 50 or 250 mg/kg/d no mortality was observed. Clinical signs such as hyperactivity and transient weight loss were dose-related and observed at 50 and 250 mg/kg/d. Minor pathological changes were observed, including elevated adrenal and liver weight (associated with abnormal hepatocyte cytoplasm in the latter case). An increase in the number of corpora lutea was noted at all drug doses. These dose groups were continued into the 6-month protocol, with an additional lower dose.

METHODS

Species:
SK&F Wistar rats (bred on site).

Treatment:

Two subgroups were used at staggered times. Mortality in the studies induced changes in the HD groups

Group A

#/s/group	Dose (mg/kg/d)
15	0
10	10
10	50
15	250 (days 1-4) → 200 (days 5-56)→ 125 (days 57 on)

Group B

15	0
10	10
10	50
25	200 (days 1-4) → 200 (days 5-56)→ 125 (days 57 on)

An additional 20 females were treated with 2 mg/kg/d.
The HD group is referred to as 125 mg/kg/d.

10 animals/s for control and HD groups were allowed 6 weeks recovery before sacrifice.

Observations:
Clin signs, body weight, activity 5 hr post-dose (weekly), food/water consumption, ophthalmoscopy (pre-dose and week 26), hematology/serum chemistry and urinalysis (weeks 5,13,26 and week 32 for recovery rats), prolactin (2 hr post-dose, weeks 1,6,10,14,22,32).

RESULTS

Mortality:
Deaths in the 250 and 200 mg/kg/d groups (17 deaths) prompted a reduction in dose to 125 mg/kg/d. From week 9 in the HD group(125 mg/kg/d) there were 11 deaths. Deaths mainly occurred <5 hrs post-dose (20/28) with the remainder being found dead in the morning. There was no overt cause of death histologically, but aggregated alveolar macrophages were suggested by the sponsor to indicate possible repeated inhalation of drug. 5 deaths at 50 mg/kg were ascribed by the sponsor to incidental problems with blood sampling for prolactin (which took place after drug administration) or gavage.

Clinical signs:
50 (MD) and 125 mg/kg/d (HD) groups were hyperactive and, to a lesser extent, aggressive. 14/80 HD animals convulsed at least once, an effect that had not been observed at this dose in previous studies. No hyperactivity was seen at 10 mg/kg/d or below. Body weights for MD and HD M were usually less than control, but this was significant for both groups only at week 27 (6-8%). Food and water consumption were higher (max increases of 46% and 65% respectively) than for controls.

Serum chemistry/hematology/urinalysis:
Mainly transient increases at week 5 in ALT and alkaline phosphatase in M+F (about 50%; ALT 93 to 133 IU/L and AP 91-135

IU/L) were noted, with some significant increases at weeks 13 and 27 in females. HD F platelets were significantly reduced at week 5 (10%) and stayed below control levels (2-8%). HD M platelets were also below control (up to 7%), but not significantly. Prolactin control values varied, with group means between 11 and 36 ng/ml for males and 6-11 ng/ml for females. All drug treatment groups showed reduced levels, with almost all samples below MQL (0.8 ng/ml) for the whole study.

Pathology:
Gonadotroph cells (staining for chromogranin A was consistent with this identification) were increased in the pars distalis of the pituitary (similar to effect of castration and may be caused by decreased prolactin reducing testosterone levels-prolactin and LH stimulate testosterone production in rat) in all treated groups, which reversed with washout. Ovaries showed a dose-dependent increase in weight (125% and 170% at 50 and 125 mg/kg/d respectively) and redness (1/20 @ 2, 15/19 @ 50, 21/21 @ 125) probably due to an increase in corpora lutea (mean of 28 in controls and 51, 104 and 110 respectively at 10, 50 and 125 mg/kg/d).

Liver weight increases were marked in HD females (25%) and occurred in HD males (10%), with reversal on washout. This corresponded to centrilobular hypertrophy in 4/15 M and 3/21 F HD rats.

Adrenal cortical hyperplasia of the zona reticularis and fasciculata were noted and were reflected in an increased organ weight at 50 and 125 mg/kg (+20% and 30% in M; +12% and 49% in F respectively).

Uterine distension increased in incidence and severity in a dose-dependent manner, with inflammation (endometritis and pyometra) observed in 1/19 and 4/21 HD F respectively.

Vaginal epithelium indicated more animals were in pro-estrous in all dose groups (8-12 per group) compared to controls (5/20).

Hyperplasia (papillomatous) of the u. bladder epithelium was seen in 1 HD F, with 2 HD M showing small hyperplastic foci. This may be drug-related since it is unusual, but was not seen in recovery animals. Hyperplasia of the urothelium can be caused by other toxic agents e.g. cyclophosphamide and are thought to be reversible.

White foci in the lungs were alveolar macrophages. While not unusual, the incidence was above control (7/15 HD M, 9/21 HD F

compared to 1/60 controls) and lower in recovery animals, possibly due to intermittent drug inhalation.

EVALUATION

Mortality in this study was much higher than anticipated from the 30-day study. The cause of death was unclear for many animals, except that pulmonary inflammation, possibly due to drug inhalation was implicated. Housing at 5/cage was also noted as a potential contributing factor. Clinical signs were mainly an extension of DA receptor stimulation and consistent with acute studies. Only minor enzyme elevations (ALT and alkaline phosphatase) were noted and may reflect increased liver weights and centrilobular hypertrophy. Abnormalities were noted in pituitary gonadotrophs, adrenal cortex hyperplasia, uterine distension and ovary weight (increased number of corpora lutea) that were probably related to prolactin suppression in all dose groups (i.e. > 93% inhibited)). These changes mainly reversed on washout and may not be relevant to humans because prolactin does not play a comparable role to that in rats.
Epithelial hyperplasia in the urinary bladder in HD animals may indicate drug-dependent cellular transformation.

One-year Oral Toxicology Study of SK&F 101468-A in the Sprague-Dawley (CD) Rat (report TP1003/SKF-101468/2)

DOSE SELECTION

Previous studies indicated that 125 mg/kg/d produced convulsions and mortality. 100 mg/kg was therefore selected as the HD.

METHODS

Species:
Sprague-Dawley rats, virus antibody free (VAF) were obtained from . Age was about 6 weeks and weight range was 175-209 g M and 135-171 g F.

Treatment:
0, 5, 50, 100 mg/kg/d base equivalent of HCl salt in water (10 ml/kg) by gavage. 25/s/g

Observations:
Morbidity (daily), clinical signs (weekly), body weight/food consumption (2x weekly/ weekly respectively for 13 weeks then

monthly), urinalysis/hematology (months 3,6,9,12) with plasma hormones at 6,12 months, pathology and ophthalmology (necropsy). All animals were necropsied as noted in Table 39.

RESULTS

Mortality:
Mortality increased in the HD (36%), and possibly MD (12%), compared to the control group (8%) as shown in Table 35. Deaths were distributed from 3-12 months. In the HD group 5/18 deaths were associated with convulsions (Table 36).

Clinical signs:
Convulsions were occasionally observed, in 1, 3 and 8 animals respectively in LD, MD and HD groups. Of 8 HD rats with seizures 5 died on the same day. Other signs such as hyperactivity, ptosis and abnormal posture were dose related (observed at 50 and 100 mg/kg/d) had also been noted in 6-month studies at 50-125 mg/kg/d. Additional observations that were dose related were salivation, Straub tail, ocular discharge and urine wet fur (see Table 37). Aggression and alopecia were also noted in MD and HD groups. Most signs were observed up to about 6 hrs. Palpable masses were not increased (Table 37).

Body weight/food consumption:
HD M had a persistent reduction in weight gain and had a reduced body weight (about 13%) at study termination. HD F had a similar decrease in terminal body weight, but had increased weight from about week 2-28. Food consumption increased in MD and HD groups, with overall increases of 5% and 13% for males and 24% and 27% for females. The LD groups were not affected.

Ophthalmology:
No dose-related effects. No retinal degeneration.

Hematology/Serum Chemistry/Urinalysis:
No clear dose-related effects were noted but there was a small (up to 4 mg/dl) increase in serum urea nitrogen for MD and HD M and HD F. There was no other indication of changes in renal function. This change could be related to a decrease in effective renal plasma flow noted in safety pharmacology studies at 3 μg/kg/min ROP for 75 min (Fig 19)

Endocrinology:
Plasma hormone concentrations were only analyzed at 12 months. HD M prolactin levels were about 6-fold lower than controls, but females were not significantly affected. Progesterone decreased and estradiol increased so the ratio of these hormones was

elevated (see Fig 28). It is notable that terminal prolactin levels were much higher than in the 6-month Wistar rat tox study. This may have been related to the time of blood collection (24 hr post dose compared to 2 hrs in the 6-month study) since the assay methodology was similar, or different rat strains.

Organ weight:
Absolute weights for adrenals were increased (53% and 50% for MD and HD, M+F combined) as well as relative weights (68% and 72% for MD and HD, M+F combined). LD animals were not affected. Liver wt was also elevated in F (+18% and 11% in absolute wt for MD and HD, about 25% in relative wt). HD M may have also showed some change (18% inc in relative wt at HD).

Pathology:

Brain. No abnormality detected at 3 levels, but no details of the sections appear to have been provided.

Erosion or ulceration of the gastric mucosa was noted macroscopically and microscopically (below) in a dose-related fashion

Dose	0	5	50	100 mg/kg/d
Males	1/25	2/25	2/25	6/25
Females	1/25	2/25	6/25	5/25

Urinary bladder. Distension was observed in about one third of MD and HD M.

Liver. Hepatocellular alterations (foci) were noted in F rats in a dose-related manner (8, 11 and 12/25 in LD, MD and HD vs 3/25 and 0/25 in controls respectively). Hypertrophy was not observed in controls but was present in 6/25 F rats at the HD Acute congestion, and possibly vacuolation, were also elevated.

Ovary. Increased numbers of corpora lutea, and enlarged ovary, were seen in 1 F of each dose group. Luteal cells had some apparent drug-related changes in appearance in 4/25, 7/25 and for LD, MD and HD animals compared to none in controls. Cysts were noted in 7/25 and 6/25 at MD and HD, rates which were 2-3 times control.

Pituitary. Pituitary gland enlargement was decreased in females, and not commonly observed in males. This corresponded to a decrease in adenomas in the pars distalis (HD F 1/25 vs control 6/25).

Testis. Leydig cell hyperplasia was observed in the testis (11/25 and 9/25 for MD and HD vs 1/25 controls, Table 38).

Uterus. Endometrial hyperplasia increased from 2/25 to 14/25 and 6/25 for MD and HD groups. The MD group included 5 cases (vs 1 in controls) of cystic hyperplasia (Table 38).

Vagina. Drug-treated females showed morphological changes in the epithelium, with keratinization noted for 20/25 and 17/25 in MD and HD vs 11/25 in control. 8/24 MD and 2/22 HD F had an unusual patchy appearance to the vaginal epithelium.

EVALUATION

The HD selected produced increased mortality and body weight decrease compared to control. The MD showed intermediate effects and the LD was essentially a NOAEL.

Many of the changes noted in clinical signs and pathology in the pituitary, vagina, ovary, uterus and testes can be ascribed to an extension of the direct DA agonist action of ROP or the secondary decrease in prolactin. However, the substantial difference between data in this study for prolactin and those observed in the 6-month study of Wistar rats raises potential questions about consistency of action (which was the reason the species under study was changed for the 1-year test). Other explanations are species differences, altered time of sampling or decreased pituitary sensitivity which occurs in aged female rats.

Increased adrenal weight was ascribed by the sponsor to potential changes in ACTH, via the influence of estrogen or stress. The latter may be involved in gastric erosion/ulceration, although comparable gastric effects were not observed in the 2-year carcinogenicity study.

Liver hypertrophy in females was previously observed in the 6-month study (where it also occurred in males). The incidence of neoplasia was not elevated, and the sponsor attributes the change to enzyme induction and adaptive responses.

Two Year Carcinogenicity Study of SK&F 101468-A in the Sprague-Dawley (CD) Rat.

DOSE SELECTION

Doses above 50 mg/kg/d were associated with mortality in toxicity studies (36% @ 100 mg/kg/d for 1 yr).

METHODS

Species:
Sprague-Dawley rats (virus-antibody-free,
approx 6 weeks of age,

Treatment:
0,0, 1.5, 15, 50 mg/kg/d base equivalent HCl salt in water by gavage 10 ml/kg. 70/s/g

Observations:
Morbidity (daily), toxicity e.g. ptosis, hyperactivity (weekly), clinical condition (monthly), palpable masses (monthly), body weight (weekly except monthly weeks 13-54), food consumption (weekly for wks 1-13 and 104 then 3 monthly), hematology (RBC/WBC) and pathology at necropsy. HD, controls and dead animals from MD and LD had full necropsy (macroscopic and microscopic). MD and LD animals had macroscopic exam and microscopic exam of eye, liver, ovary, pituitary, testes/epididymis.

RESULTS

Mortality:
Due to high rates of mortality in all groups the study was terminated at 100 weeks. Deaths were comparable or reduced in treated groups compared to controls, with the MD F group having significantly higher survival (Figs 29 and 30). Control survival was 21-29% in M and 24-31% in F. HD survival was 31% (22/70) in M and 33% (23/70) in F. MD survival was 29% and 49% respectively for M, F.

Clinical signs:
Convulsions were observed in all groups, with MD and HD group incidences higher than controls (4/140 and 6/140 compared to 5/280). 13/18 animals with seizures were found dead or sacrificed before study termination, although only 2 deaths were on the day of seizure. Consistent dose-related signs (below) were reported as post-dose in the HD (LD was close to control levels and the MD showed intermediate effects):

	Control		50 mg/kg/d	
	%Incidence	%occurrence	%Incidence	%occurrence
post-dose salivation	0	0	100	90
stereotyped activity (including hyperactivity)	0	0	100	99
ptosis	<5	<0.1	95	8
abnormal posture (hunched, low)	<3	<0.1	65	5
urine-wet fur	17	0.4	99	59
ocular discharge (red or colorless)	28	7	70	10
aggression.	<5	<0.1	44	1

%Incidence= %of all animals exhibiting event, %occurrence = % of observations in which event was noted.

Footpad lesions and alopecia were also noted in MD and HD groups.

Body weight/food consumption:
Body weight gain was reduced in the HD M and F groups by 21% (272% vs 343% in control) and 14% (241% vs 279.5 in control) respectively (Figs 31 and 32). BW gain in MD M and F was reduced by 16% and 5.6% respectively. Food consumption was correspondingly increased in most drug groups with the HD M and F being 19% and 21% higher than controls.

Hematology:
Only red and white cell counts were determined, and means were not different from controls. Two rats (1 con, 1 MD F) had myelomonocytic leukemia, which the sponsor notes spontaneously occurs in this strain.

Pathology:
Macroscopic. Tissue samples are noted in Table 30(i). Palpable masses were not increased in treatment groups, with an overall incidence of 43% in controls and 49%, 30% and 27% in LD, MD and HD groups respectively. Bladder distension and footpad lesions were increased. Pituitary enlargement was decreased (control M 71/140, MD M 24/70, HD M 13/70; control F 111/140, HD F 39/70).

Microscopic.
i) Neoplastic findings. Testicular interstitial (Leydig) cell benign tumors and hyperplasia were significantly increased in a dose-related manner (below and Table 37(i)). Tumors were usually accompanied by hyperplasia. The LD group only showed an increase in hyperplasia.

Incidence of proliferative interstitial (Leydig) cell lesions (70/group) and skin fibromas in males

Dose (mg/kg/d) Lesion	0	0	1.5	15	50
Adenoma (total)	2	3	2	12	32
Adenoma (only)	1	2	0	2	3
Hyperplasia (total)	6	8	17	30	44
Hyperplasia (only)	5	7	15	20	15
Adenoma + hyperplasia	1	1	2	10	29
Adenoma or hyperplasia	7/70	10/70	17/70	32/70	47/70
Skin fibromas (M)	2/70	2/69	4/59	7/60	7/70

Skin fibromas were also significantly elevated, but only in males. One case of uterine endometrial carcinoma, in the HD group, was observed.
Pituitary adenomas (pars distalis) were decreased in incidence (controls 77/138 M, 110/140 F with HD M 12/70 and HD F 40/70) as well as in size, as assessed by the incidence of brain compression and ventricular dilation. Conversely, the incidence of hyperplastic foci in MD and HD F was elevated (12/140 controls vs 14/70 HD; M were similar to controls). This increase in F could indicate decreased progression to tumor development.
Mammary tumors were decreased in incidence in HD females (18/69 fibroadenomas vs 74/140 in controls; 0/69 adenomas vs 20/140 in controls).

ii) Non-neoplastic findings.
a) Testis. HD M showed increased degenerative changes in seminiferous tubules but only in the HD group (32/70 vs 37/140 in

controls).

b) Ovaries. Decreased ovarian quiescence (absence of c. lutea/follicles) was noted (2/70 HD F vs 19/138 con) and decreased Sertoliform hyperplasia was noted, although abnormal corpora lutea (amorphous/eosinophilic) were evident in 5/70 and 9/70 animals for MD and HD groups compared to none in controls.

c) Uterus. Endometrial hyperplasia was only observed in HD (3/70) and MD (1/70) animals

d)Liver. Centrilobular hypertrophy was noted, in 12/140 MD animals and 26/140 HD rats compared to 5/280 controls, consistent with 1 yr toxicity studies. Eosinophilic foci were observed in F only (25/70 at the HD compared to 22/140 controls).

e) Pituitary. An increase in vacuolation of gonadotrophs in pars distalis was noted in MD and HD M (15/70 and 23/70 respectively vs 14/138 in controls) as observed in toxicity studies.

f) Eye. Retinal atrophy, usually described as mild or moderate, was noted only in drug-treated rats after 1.6 -2 yrs.

Dose (mg/kg/d)	0	1.5	15	50
M	0/140	1/69	1/69	7/70
F	0/140	3/68	2/68	9/70

The sponsor notes that most of these rats 18/23) were housed at highest light intensity, suggesting photo-sensitization.

EVALUATION

Clinical signs due to DA receptor agonism were observed in nearly all HD animals, with an increased incidence of seizures, the majority of which were associated with preterminal mortality. The decrease in body weight gain for the HD group was -17%.
The study was terminated early (100 weeks) to limit mortality, which was high in all groups (about 20 survivors in each group, Figs 29 and 30). The question of statistical validity with low survival needs to be addressed. Apart from the number of surviving animals the HD was an appropriate MTD for single daily dose administration.

Leydig cell tumors and hyperplasia were increased in a dose-related manner, with the LD M only showing hyperplasia. 32/70 M had adenomas in the HD group, mainly accompanied by hyperplasia.

Decreased prolactin levels (which were observed in rats treated for 1 yr but not monitored in this study) from a variety of agents have been associated with increases in Leydig cell tumors. The mechanism of action has not been clearly delineated (Kovacevic et al., Int J. Andrology 10, 1987, 773-784), but may involve prolactin receptors on Leydig cells causing down regulation of LH receptors. Subsequent reduction in LH-dependent testosterone release elevates serum LH and induces a mitogenic response.Sponsor studies indicated 50 mg/kg/d reduced Leydig cell LH receptor density, but serum LH levels were not affected in an 8-day study. A literature study indicated that serum LH levels do not increase until after about 4 weeks treatment. These data are therefore consistent with hypoprolactinemia leading to Leydig cell proliferation but are not definitive of such an effect. A lack of prolactin receptors on Leydig cells in humans may indicate a lack of cross-species predictability.

Benign skin fibromas were also increased (4/139 controls and 4/59, 7/60 and 7/70 in M LD, MD and HD groups). There was no increase in F. The sponsor considers this an incidental finding, noting the lack of effects in F and absence of related malignant tumors, but both MD and HD groups were affected and this should therefore be considered drug-related.

A single case of malignant uterine carcinoma occurred, in the HD F group. While not statistically significant a treatment-related effect is possible since malignant endometrial or myometrial tumors are associated with administration of other dopamine agonists, bromocriptine and pergolide. A difference in the incidence rate between DA agonists, if pharmacologically-mediated, could have been influenced by gavage administration of ROP compared to dietary dosing for bromocriptine and pergolide. The induction of uterine tumors has been ascribed to the increased estrogen/progesterone ratio induced by these dopamine agonists (resulting from prolactin inhibition), and a similar estrogen dominance was observed in 1 year studies in rats at doses of 50 and 100 mg/kg/d ROP. However, such endocrine effects are not observed in humans and thus the predictive value of these observations for human carcinogenicity risk is not known.

Hyperplastic foci were increased in the pituitary of MD and HD F, but this was associated with a decrease in tumor incidence, suggesting reduced progression due to DA receptor inhibition of cellular activity. Although hypertrophy was observed in the liver, there was no significant increase in neoplasia.

Retinal atrophy, possibly associated with abnormally high light levels, was only observed in drug-treated groups. A possible relationship to melanin binding should be borne in mind.

44a

Table 3 of the Rat CA tissue samples

Macroscopic Observations: The following tissues were collected at necropsy:

Adrenal Glands*	Pancreas*
Animal Identification	Parathyroids*
Aorta, thoracic*	Pituitary*
Blood Smears**	Preputial/Clitoral Gland
Bone Marrow Smears**	Prostate*
Brain*	Rectum
Cecum	Rib (CCJ)
Cervix	Salivary Gland, mandibular*
Colon*	Salivary Gland, sublingual*
Duodenum*	Salivary Gland parotid (both sets)*
Epididymides*†	Sciatic Nerve
Esophagus*	Seminal Vesicles*
Eyes*††	Skeletal Muscle (sternal)*
Femurs	Skin*
Heart*	Skull, base, nasal turbinates, ear canals and accessory tissues
Harderian Gland	Spinal Cord, lumbar*
Ileum*	Spleen*
Jejunum*	Sternebrae* (and bone marrow*)
Kidneys*	Stomach*
Larynx	Testes*†
Liver, left lateral lobe*	Thymus*
Liver, right lateral lobe*	Thyroid*
Lung* (right caudate and left lobes)	Tongue
Lymph Nodes, mandibular*	Trachea*
Lymph Nodes, mesenteric*	Urinary Bladder*
Macroscopic Lesions*	Uterus*
Mammary Gland*	Vagina
Nasal Cavity	
Ovaries*	

* Tissues processed and examined microscopically (see below)
** Prepared but not examined from each animal killed terminally or, when feasible, from animals killed in extremis
† Testes and epididymides were fixed in Bouin's fixative.
†† Eyes were fixed in Davidson's fixative.

All tissues were fixed in 10% Modified Millonig's Buffered formalin unless otherwise noted.

Microscopic Observations: The tissues that are marked with an asterisk in the above list were examined microscopically for all rats in both control groups and the high-dose group (50 mg/kg) and for all decedent rats in the low- and mid-dose groups (i.e., animals that died or were killed during the course of the study). For low-and mid-dose group rats in the terminal kill, the following tissues were examined: eye, liver, ovaries, pituitary, testes with epididymides and macroscopic lesions. The microscopic findings were peer reviewed.§

§ See memo from Dr. H.A. Solleveld to Dr. C.J. Fish, March 16, 1993.

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It is concluded from the results of this study that administration of ropinirole at dosages of 5, 15 or 50 mg/kg/day for 104 weeks produced no evidence of a carcinogenic effect at any of the dosages tested.

Table 31

Neoplastic Lesions Occurring with an Incidence >5% in Control and/or High-Dose Groups*

Lesion	Sex	Control I	Control II	5 mg/kg	15 mg/kg	50 mg/kg
Liver cell	M	18/60	19/60	18/44	12/44	10/60
adenoma	F	2/60	6/60	0/45	1/46	1/60
Liver cell	M	3/60	5/60	7/44	4/44	0/60
carcinoma	F	1/60	0/60	1/45	0/46	1/60
Bronchoalveolar	M	22/60	13/60	18/42	25/50	15/60
adenoma	F	12/60	7/60	20/47	12/41	6/60
Bronchoalveolar	M	2/60	2/60	0/42	0/50	1/60
carcinoma	F	1/60	2/60	1/47	0/41	1/60
Mammary gland	M	0/60	0/59	0/26	0/31	0/60
adenoma	F	0/60	0/60	0/32	1/30	0/60
Mammary gland	M	0/60	0/59	0/26	0/31	0/60
carcinoma	F	3/60	0/60	3/32	3/30	0/60
Ovarian adenoma	F	2/60	1/60	1/49	3/51	3/60
Stromal polyp, uterus	F	3/60	7/60	5/58	6/57	14/60
Stromal sarcoma, uterus	F	4/60	6/60	1/58	6/57	3/60
Hemangioma, uterus	F	0/60	0/60	1/58	1/57	0/60
Hemangio-sarcoma, uterus	F	0/60	3/60	0/58	0/57	1/60

*Only taking into account routinely processed tissues

5.B.3.3.3 Rat

Two-Year Carcinogenicity Study in the Sprague-Dawley (CD®) Rat [TP-1005; May 1993]

Study Design

The objective of this study was to evaluate the carcinogenic potential of ropinirole (Lot: P12-JSD-942) when administered orally to Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) for 2 years. Ropinirole was given daily, by oral gavage, to male and female (70/sex/group) rats at dosages of 1.5, 15 or 50 mg/kg/day (doses expressed as the base) for approximately 23 months (706-709 days).[50(1)] Two additional groups (70/sex) received an equivalent volume (10 ml/kg) of vehicle (purified water). All surviving animals were necropsied over a 4-day period (days 707 to 710).

The rats were housed individually in stainless-steel suspended cages in environmentally

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ABLE NO.: 10
ROJECT NO.: 84194

INCIDENCE OF NEOPLASTIC LESIONS
ALL ANIMALS

GROUP 1 VEHICLE CONTROL
GROUP 2 VEHICLE CONTROL
GROUP 3 SKF 101468-A 5 MG/KG/DAY
GROUP 4 SKF 101468-A 15 MG/KG/DAY
GROUP 5 SKF 101468-A 50 MG/KG/DAY

		SEX :	MALE					FEMALE				
		DOSE GROUP :	1	2	3	4	5	1	2	3	4	5
		NO. OF ANIMALS IN DOSE GROUP :	60	60	60	60	60	60	60	60	60	60
LUNG	-TOTAL EXAMINED		60	60	42	50	60	60	60	47	41	60
-0H ALVEOLAR/BRONCHIOLAR CARCINOMA			2	2	0	0	1	1	2	1	0	1
-0B ALVEOLAR/BRONCHIOLAR ADENOMA			22	13	10	25	15	12	7	20	12	6
LYMPH NODE	-TOTAL EXAMINED		0	13	11	10	10	24	21	23	10	16
-0H LYMPHOSARCOMA			1	4	4	3	1	7	4	4	7	0
-0H HISTIOCYTIC SARCOMA			1	2	1	3	0	5	4	5	0	3
LYMPH NODE MESENTERIC	-TOTAL EXAMINED		59	59	29	34	59	59	60	35	34	58
-0B HEMANGIOMA			0	1	0	0	1	0	0	0	0	0
MAMMARY GLAND	-TOTAL EXAMINED		60	59	26	31	60	60	60	32	30	60
-0H ADENOCARCINOMA			0	0	0	0	0	3	0	3	3	0
-0B ADENOMA			0	0	0	0	0	0	0	0	1	0
OVARY	-TOTAL EXAMINED		0	0	0	0	0	60	60	49	51	60
-0B BENIGN LUTEOMA			0	0	0	0	0	0	1	1	0	1
-0B ADENOMA			0	0	0	0	0	2	1	1	3	3
-0B GRANULOSA-THECA CELL TUMOR			0	0	0	0	0	0	1	1	0	1

Table 32

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TABLE NO.: 10
PROJECT NO.: 84194

INCIDENCE OF NEOPLASTIC LESIONS
ALL ANIMALS

GROUP 1	VEHICLE CONTROL		GROUP 4	SKF 101468-A	15 MG/KG/DAY
GROUP 2	VEHICLE CONTROL		GROUP 5	SKF 101468-A	50 MG/KG/DAY
GROUP 3	SKF 101468-A	5 MG/KG/DAY			

		MALE					FEMALE				
SEX :											
DOSE GROUP :		1	2	3	4	5	1	2	3	4	5
NO. OF ANIMALS IN DOSE GROUP :		60	60	60	60	60	60	60	60	60	60
SUBCUTANEOUS TISSUE	-TOTAL EXAMINED	6	3	9	3	7	9	14	12	0	0
-MH UNDIFFERENTIATED SARCOMA		0	1	0	0	0	2	1	1	1	0
-MB LIPOMA		0	0	1	0	0	0	0	0	1	0
-MH LIPOSARCOMA		0	0	0	0	0	0	1	1	0	0
-MH FIBROSARCOMA		0	0	0	0	0	1	0	1	0	1
-MB HEMANGIOMA		0	0	2	0	0	0	0	0	0	0
TESTIS	-TOTAL EXAMINED	60	60	38	39	60	0	0	0	0	0
-MB INTERSTITIAL CELL ADENOMA		0	1	2	2	1	0	0	0	0	0
-MB HEMANGIOMA		0	1	0	0	0	0	0	0	0	0
URINARY BLADDER	-TOTAL EXAMINED	60	60	32	33	60	59	57	32	29	60
-MH LEIOMYOSARCOMA		0	0	1	0	0	1	0	0	0	0
-MB HEMANGIOMA		0	0	0	1	0	0	0	0	0	0
UTERUS	-TOTAL EXAMINED	0	0	0	0	0	60	60	58	57	60
-MH ENDOMETRIAL STROMAL SARCOMA		0	0	0	0	0	4	6	1	6	3
-MB LEIOMYOMA		0	0	0	0	0	3	1	5	4	0
-MB BENIGN ENDOMETRIAL STROMAL POLYP		0	0	0	0	0	3	2	5	6	3
-MH HEMANGIOSARCOMA		0	0	0	0	0	0	1	0	0	1
-MH LEIOMYOSARCOMA		0	0	0	0	0	1	3	2	4	2
-MB GRANULAR CELL TUMOR		0	0	0	0	0	1	0	0	0	0
-MH ADENOCARCINOMA		0	0	0	0	0	0	1	0	0	0
-MB HEMANGIOMA		0	0	0	0	0	0	0	1	1	0

0 -NEOPLASM MB-BENIGN MH-MALIGNANT

Table 33

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TABLE NO.: 8
PROJECT NO.: 84194

INCIDENCE OF HISTOPATHOLOGICAL FINDINGS
ALL ANIMALS

GROUP 1 VEHICLE CONTROL
GROUP 2 VEHICLE CONTROL
GROUP 3 SKF 101468-A 5 MG/KG/DAY
GROUP 4 SKF 101468-A 15 MG/KG/DAY
GROUP 5 SKF 101468-A 50 MG/KG/DAY

	SEX :	MALE					FEMALE				
	DOSE GROUP :	1	2	3	4	5	1	2	3	4	5
	NO. OF ANIMALS IN DOSE GROUP :	60	60	60	60	60	60	60	60	60	60
SUBCUTANEOUS TISSUE	-TOTAL EXAMINED	6	3	9	3	7	9	14	12	8	8
	-CELLULITIS	5	2	4	1	5	0	4	0	0	1
	-EDEMA	1	0	3	2	0	3	2	1	0	2
	-HEMORRHAGE	0	0	0	0	0	1	0	0	1	0
	-ON UNDIFFERENTIATED SARCOMA	0	0	0	0	0	2	1	1	1	0
	-OB LIPOMA	0	0	0	0	0	0	0	0	1	0
	-ON LIPOSARCOMA	0	0	0	0	0	0	1	1	0	0
	-OM FIBROSARCOMA	0	0	0	0	0	1	0	1	0	1
	-OB HEMANGIOMA	0	0	2	0	0	0	0	0	0	0
TESTIS	-TOTAL EXAMINED	60	60	38	39	60	0	0	0	0	0
	-TUBULAR ATROPHY	45	46	24	24	48	0	0	0	0	0
	-MINERALIZATION	2	2	0	1	0	0	0	0	0	0
	-HEMORRHAGE	0	0	1	1	0	0	0	0	0	0
	-ORCHITIS	0	0	1	0	1	0	0	0	0	0
	-AMYLOIDOSIS	0	0	1	0	1	0	0	0	0	0
	-HYPERPLASIA, INTERSTITIAL CELL	2	1	1	1	7	0	0	0	0	0
	-HYPERPLASIA, EPITHELIAL CELL	1	1	0	0	0	0	0	0	0	0
	-GRANULOMA, CAPSULE	0	1	0	0	0	0	0	0	0	0
	-OB INTERSTITIAL CELL ADENOMA	1	1	2	2	4	0	0	0	0	0
	-OB HEMANGIOMA	0	1	0	0	0	0	0	0	0	0
	-CYST	0	0	0	0	1	0	0	0	0	0

Table 74

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Table 35

One-Year Oral Toxicity Study of SK&F 101468-A
in the Sprague-Dawley (CD®) Rat

Cumulative Mortality*

	I	II	III	IV
Males	4% (1)	8% (2)	16% (4)	40% (10)
Females	12% (3)	4% (1)	8% (2)	32% (8)
Combined	8% (4)	6% (3)	12% (6)	36% (18)

Monthly Mortality**

Interval Month (Days)	Males I	Males II	Males III	Males IV	Females I	Females II	Females III	Females IV
1 (1- 30)	-	-	-	-	-	-	-	-
2 (31- 60)	-	-	-	-	-	-	-	-
3 (61- 90)	-	-	-	1	-	-	-	1
4 (91-120)	-	-	-	1	-	-	1	-
5 (121-150)	-	-	1	2	-	-	-	1
6 (151-180)	-	1	-	-	-	-	-	1
7 (181-210)	-	-	-	1	1	1	1	2
8 (211-240)	-	-	-	1	1	-	-	1
9 (241-270)	-	-	1	-	-	-	-	1
10 (271-300)	-	1	-	2	-	-	-	-
11 (301-330)	1	-	-	2	-	-	-	1
12 (331-365)	-	-	2	-	1	-	-	-

* Cumulative mortality is expressed as a percentage (with the total number of deaths in parentheses).

** Monthly mortality is indicated as the number of deaths per interval.

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Table 36

One-Year Oral Toxicity Study of SK&F 101468-A
in the Sprague-Dawley (CD®) Rat

Convulsions - Animals and Days of Occurrence

Group	Animal Number	Day(s) of Convulsions	Found Dead or Unscheduled Kill
I	-	-	-
II	R89F-3903	325	-
III	R89M-3917	366	-
	R89M-3924	320	-
	R89F-3937	40	-
IV	R89M-3965	98	Day 98
	R89M-3971	67	Day 67
	R89M-3980	74, 79	Day 132
	R89F-3991	209	-
	R89F-3992	178, 203, 367	-
	R89F-4005	211	Day 211
	R89F-4006	83	Day 83
	R89F-4008	185	Day 185

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Table 37

One-Year Oral Toxicity Study of SK&F 101468-A in the Sprague-Dawley (CD®) Rat

Primary Clinical Observations*

	Males				Females			
mg/kg/d	I 0	II 5	III 50	IV 100	I 0	II 5	III 50	IV 100
Convulsions (clonic, tonic)								
Animals with Occurrences	-	-	8%	12%	-	4%	4%	20%
Number of Occurrences			1x	1-2x		1x	1x	1-3x
Days of Occurrence			320-366	67-98		325	40	83-367
Stereotypic Movements								
Animals with Occurrences	-	-	100%	100%	-		100%	100%
Number of Occurrences			20-52x	9-52x			16-52x	11-52x
Days of Occurrence			7-364	7-364			7-364	7-364
Salivation, Pre-Dose								
Animals with Occurrences	-	4%	28%	20%	-	-	52%	64%
Number of Occurrences		1x	1-11x	1-5x			1-34x	1-20x
Days of Occurrence		364	14-175	14-364			14-364	14-357
Salivation, Post-Dose								
Animals with Occurrences	-	4%	100%	100%	-	4%	100%	100%
Number of Occurrences		1x	20-51x	9-52x		1x	16-52x	11-52x
Days of Occurrence		35	7-364	7-364		35	7-364	7-364
Urine-Wet Fur								
Animals with Occurrences	4%	4%	100%	96%	-	8%	100%	100%
Range of Occurrences	1x	1x	1-29x	1-39x		1x	14-51x	9-52x
Days of Occurrence	209	364	63-357	21-364		168-340	7-364	7-364
Ptosis								
Animals with Occurrences	-	-	96%	96%	-	-	88%	80%
Number of Occurrences			1-31x	1-24x			1-17x	1-19x
Days of Occurrence			14-364	14-364			7-364	14-364
Posture - Abnormal (hunched, low, prostrate, head tilt)								
Animals with Occurrences	-	-	16%	56%	4%	-	96%	84%
Number of Occurrences			1-7x	1-9x	1x		2-19x	1-24x
Days of Occurrence			14-364	63-364	340		7-364	14-364
Ocular Discharge (colorless, clear red, dried red, dried brown, fluid)								
Animals with Occurrences	20%	8%	4%	24%	4%	16%	68%	92%
Number of Occurrences	1-12x	3-11x	1x	1-2x	2x	1-4x	1-15x	2-21x
Days of Occurrence	42-354	126-364	8(	7-357	336-340	28-308	7-357	63-357
Aggression								
Animals with Occurrences	-	4%	24%	48%	4%	-	48%	68%
Number of Occurrences		1x	1-9x	1-4x	1x		1-4x	1-6x
Days of Occurrence		329	7 350	56-259	49		23-210	7-280
Straub Tail								
Animals with Occurrences	-	-	8%	16%	-	-	44%	64%
Number of Occurrences			1x	1-13x			1-20x	1-20x
Days of Occurrence			168-189	98-364			112-364	98-364

* The rats were observed daily for convulsions. Observations for the other clinical signs in this table were recorded at the 52 weekly intervals. These observations occurred with an incidence of 10% or greater in any of the groups.

Table 37

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Table 37(Cont'd)

One-Year Oral Toxicity Study of SK&F 101468-A
in the Sprague-Dawley (CD®) Rat

Additional Clinical Observations (Monthly Monitoring)

	Males				Females			
	I	II	III	IV	I	II	III	IV
Mass								
Animals with Occurrences	-	-	4%	-	16%	8%	12%	4%
Number of Intervals			1		1-2	2-4	2-8	4
Days of Onset			361		340-361	284-340	150-340	284
Abrasion (ear, eye, limb, mouth, neck, snout, tail)								
Animals with Occurrences	8%	4%	20%	24%	-	4%	8%	-
Number of Intervals	1x	1x	1-3x	1-3x		1x	1x	
Days of Occurrence	32	312	284-361	228-361		32	340	
Alopecia								
Animals with Occurrences	-	4%	40%	44%	16%	16%	48%	48%
Number of Intervals		1x	1-8x	1-8x	1-12x	1-7x	1-10x	1-7x
Days of Occurrence		361	32-361	32-361	60-361	60-361	32-361	88-340
Haircoat, Thin								
Animals with Occurrences	-	4%	20%	4%	8%	12%	12%	8%
Number of Intervals		1x	1-3x	1x	1x	1-2x	1-5x	1x
Days of Occurrence		340	116-361	116	172	88-340	32-340	116-312

Table 37 cont.

-- Table 37(i)

Rat LA study

Neoplastic Lesions Occurring with an Incidence >5% in Control or High-Dose Groups*

Lesion	Sex	Control I	Control II	1.5 mg/kg	15 mg/kg	50 mg/kg
Tricho-folliculoma or epithelioma	M	0/70	4/69	1/59	4/60	2/70
	F	0/70	0/70	1/50	0/43	2/70
Fibroma	M	2/70	2/69	4/59	7/60	7/70
	F	2/70	0/70	2/50	4/43	2/70
Fibrosarcoma	M	0/70	2/69	4/59	1/60	1/70
	F	1/70	0/70	1/50	2/43	1/70
Mammary gland adenoma	M	3/61	2/60	3/46	1/43	2/57
	F	39/70	36/70	41/65	32/56	18/69
Mammary gland carcinoma	M	0/61	0/60	1/46	0/43	0/57
	F	6/70	14/70	9/65	9/56	0/69

* Only taking into account routinely processed tissues

Lesion	Sex	Control I	Control II	1.5 mg/kg	15 mg/kg	50 mg/kg
Liver cell adenoma	M	2/70	3/70	1/70	3/70	5/70
	F	0/70	1/70	0/70	4/70	1/70
Liver cell carcinoma	M	1/70	1/70	1/70	0/70	1/70
	F	0/70	0/70	0/70	0/70	0/70
Islet cell adenoma	M	7/70	5/70	3/53	1/52	2/70
	F	2/70	4/70	0/48	0/35	2/70
Islet cell carcinoma	M	3/70	0/70	2/53	0/52	2/70
	F	1/70	0/70	0/48	0/35	0/70
Interstitial cell tumor, testis	M	2/70	3/70	2/70	12/70	32/70
Pheochromo-cytoma	M	8/70	4/70	3/55	5/56	2/70
	F	1/70	4/70	0/55	0/52	0/70
Pituitary adenoma	M	39/70	38/68	46/69	29/70	12/70
	F	50/70	60/70	58/70	54/70	40/70
Pituitary carcinoma	M	0/70	0/68	0/69	1/70	0/70
	F	1/70	3/70	1/70	0/70	0/70
C-cell adenoma	M	5/70	5/70	0/54	2/51	6/69
	F	4/70	4/70	2/49	2/36	4/70
C-cell carcinoma	M	0/70	3/70	4/54	1/51	3/69
	F	3/70	4/70	1/49	1/36	5/70

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in the Sprague-Dawley (CD®) Rat

Male Body Weight Graph

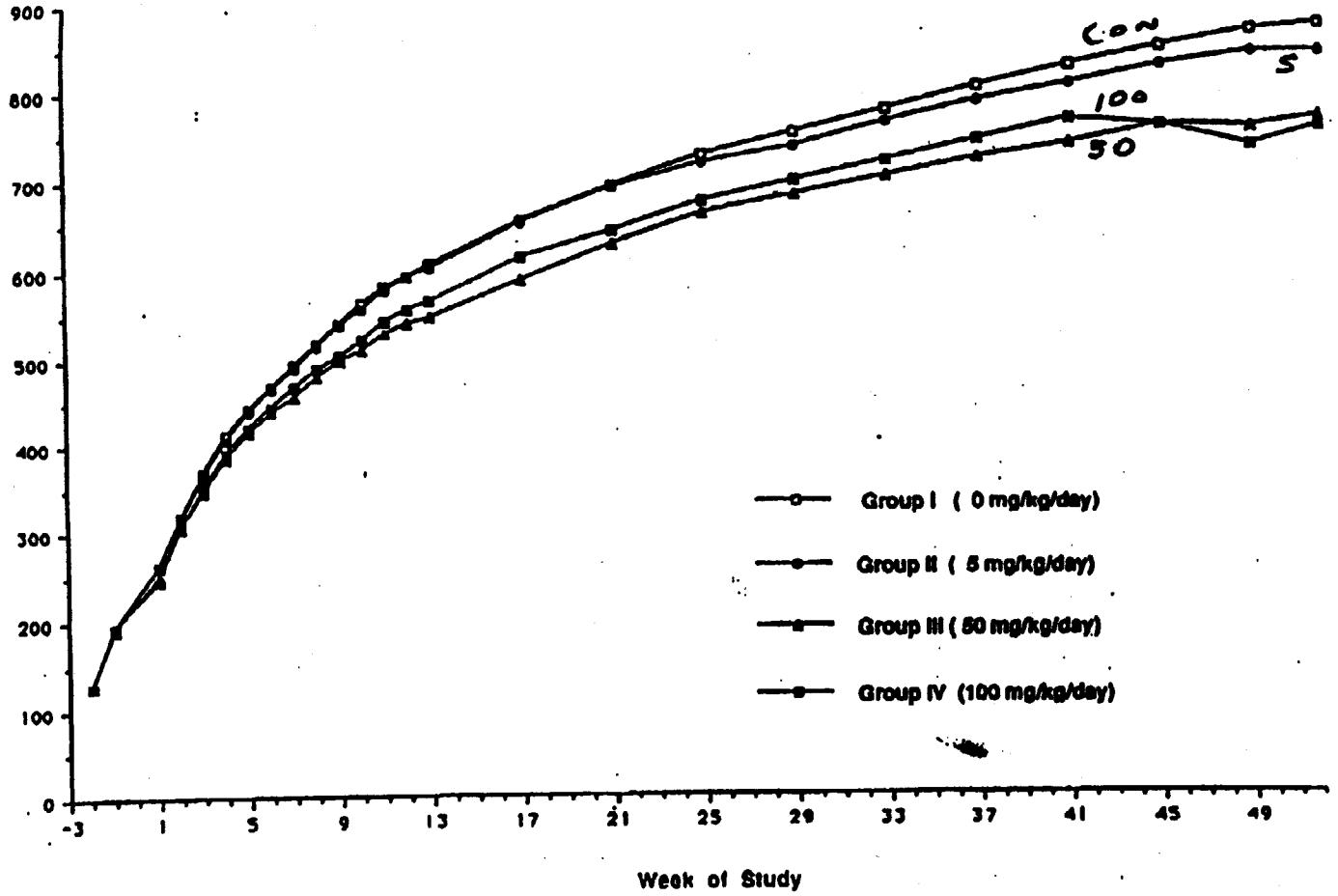

Fig 26

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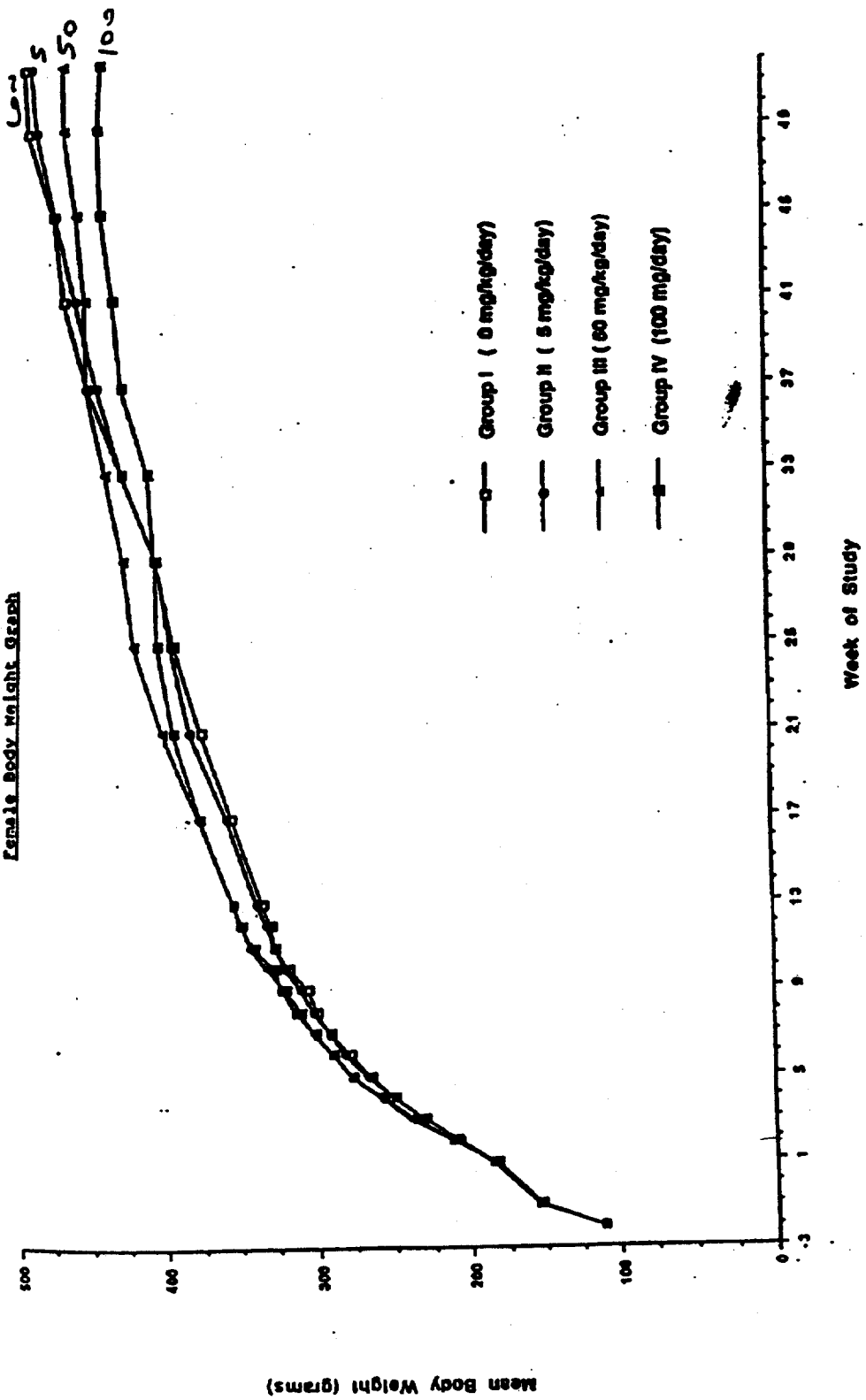
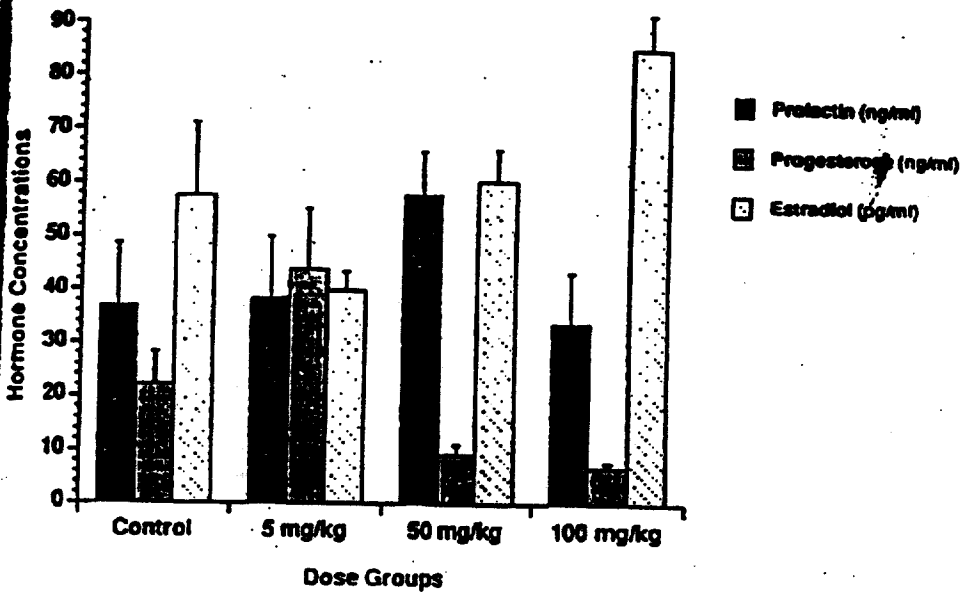


Fig 27

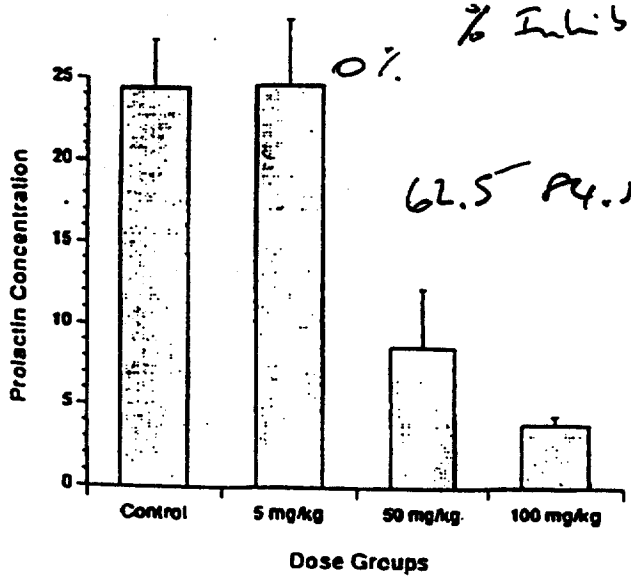
44.

Figure 5
One-Year Oral Toxicity Study of Sk&F 101468-A
in the Sprague-Dawley (CD®) Rat

Plasma Hormone Concentrations in Female Sprague-Dawley Rats After 1 Year of SK&F 101468-A Treatment



Plasma Prolactin Concentration in Male Sprague-Dawley Rats after 1-Year SK&F 101468-A Treatment



% Inhib
0%
62.5 84.1 %

Fig 28

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Table 11 (Cont'd)

FINDINGS	GROUP	NUMERIC INCIDENCE OF MICROSCOPIC OBSERVATIONS							
		MALES				FEMALES			
		0.0 mg/kg SK&F 101468	5.0 mg/kg SK&F 101468	50.0 mg/kg SK&F 101468	100.0 mg/kg SK&F 101468	0.0 mg/kg SK&F 101468	5.0 mg/kg SK&F 101468	50.0 mg/kg SK&F 101468	100.0 mg/kg SK&F 101468
KIDNEY:		(25)	(5)	(5)	(25)	(25)	(3)	(3)	(25)
Cast, proteinaceous, tubular, multifocal				1	1	2		1	1
Nephropathy, chronic progressive		25	4	3	21	20	3	1	20
Inflammatory cell infiltrate, mononuclear, interstitial, multifocal, Bilateral						1			
Pyelonephritis, acute, multifocal, Bilateral						1			
Cyst, NOS		1						1	1
Dilation, pelvic		2	1	1					
LIVER:		(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
No abnormality detected		7	4	5	1	12	3	3	4
Congestion, acute		[redacted]							
Telangiectasis			3				1		
Inflammatory cell infiltrate, granulomatous		1	5	1	1		2	1	
Inflammatory cell infiltrate, mononuclear, periportal		3	4	1	2	7	13	13	3
Vacuolation, hepatocellular		[redacted]				[redacted]			

Figures in parentheses represent the number of animals from which this tissue was examined.
The absence of a numeral indicates that the lesion specified was not identified
(B) = benign, (M) = malignant

C09033 (Study Completed)

Table 38

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Table 11 (Cont'd)

18-FEB-93
6
PLACEX V8.20d

FINDINGS	GROUP	NUMERIC INCIDENCE OF MICROSCOPIC OBSERVATIONS							
		MALES				FEMALES			
		0.0 mg/kg SK&F 101468	15.0 mg/kg SK&F 101468	50.0 mg/kg SK&F 101468	100.0 mg/kg SK&F 101468	0.0 mg/kg SK&F 101468	15.0 mg/kg SK&F 101468	50.0 mg/kg SK&F 101468	100.0 mg/kg SK&F 101468
LIVER:		(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Degeneration, cystic		2	4	3	1			1	1
Necrosis, hepatocellular, multifocal			1	1	2				3
Hypertrophy, hepatocellular, centrilobular									
Basophilic cell focus									
Eosinophilic cell focus									
Mixed cell focus									
Clear cell focus									
Biliary hyperplasia									
Fibrosis, peribiliary									
MYELOMONOCYTIC LEUKEMIA (M)		4	9	3	1	4	1		
LUNG:		1				1	1		
No abnormality detected		(25)	(2)	(4)	(25)	(25)	(2)	(3)	(25)
Congestion, acute		4		1	1	6		1	3
Hemorrhage, acute									
Edema, acute		1		4		1		1	1

Figures in parentheses represent the number of animals from which this tissue was examined.
The absence of a numeral indicates that the lesion specified was not identified
(B) = benign, (M) = malignant

C0903J (Study Completed)

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10-FEB-93
13
PLACES V8.304

Table 13 (Cont'd)

FINDINGS	GROUP	NUMERIC INCIDENCE OF MICROSCOPIC OBSERVATIONS — MALES 0.0 mg/kg SK&F 101468	MALES 5.0 mg/kg SK&F 101468	MALES 50.0 mg/kg SK&F 101468	MALES 100.0 mg/kg SK&F 101468			FEMALES 0.0 mg/kg SK&F 101468	FEMALES 5.0 mg/kg SK&F 101468	FEMALES 50.0 mg/kg SK&F 101468	FEMALES 100.0 mg/kg SK&F 101468		
STOMACH:		(25)	(25)	(24)	(25)			(25)	(25)	(25)	(25)		
Gastritis				1							1		
Myositis			1					[illegible]	2				
Serositis				1	1			1					
Hyperplasia, epithelial, cardia											1		
TESTIS/EPIDIDYMIS:		(25)	(25)	(25)	(25)								
No abnormality detected		16	12	11	8								
Congestion, acute					1								
Inflammatory cell infiltrate, lymphocytic, epididymal		8	7	7	9								
Atrophy, seminiferous tubules			1		2								
Degeneration, epididymal, epithelial, unilateral					1								
Aspermia/hypospermia, testicular/epididymal			1		3								
Hyperplasia, Leydig cell		[illegible]	[illegible]	[illegible]	[illegible]								
Sperm granuloma/spermatocele/mesenteric granuloma			3	2									

Figures in parentheses represent the number of animals from which this tissue was examined.
The absence of a numeral indicates that the lesion specified was not identified
(B) = benign, (M) = malignant

G89033 (Study Completed)

Table 38 cont

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Table 13 (Cont'd)

10-FEB-93
15
PLACES V0.3B4

NUMERIC INCIDENCE OF NICROSCOPIC OBSERVATIONS

FINDINGS	MALES				FEMALES			
GROUP	0.0 mg/kg DIET (10)	15.0 mg/kg DIET (10)	150.0 mg/kg DIET (10)	100.0 mg/kg DIET (10)	0.0 mg/kg DIET (10)	15.0 mg/kg DIET (10)	150.0 mg/kg DIET (10)	100.0 mg/kg DIET (10)
URINARY BLADDER:								
No abnormality detected	(25) 25	(2) 2	(13) 13	(25) 24	(23) 22	(1) 1	(2) 2	(25) 24
Congestion, acute								
Hemorrhage, acute				1				1
Cystitis, ulcerative/necrotizing				1	2	(25) 15	(23) 7	(25) 0
UTERUS:							2	
No abnormality detected					(25) 15	(25) 7	(23) 2	(25) 0
Atrophy								
Dilation, luminal:								
inflammatory cell: infiltrate, neutrophilic					4	5	4	3
Squamous cell metaplasia								
Endometrial cystic change					4	5	4	3
Cystic endometrial hyperplasia								
Endometrial hyperplasia					1	1	6	

Figures in parentheses represent the number of animals from which this tissue was examined.
The absence of a numeral indicates that the lesion specified was not identified
(B) = benign, (M) = malignant

(Study Completed)

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Appendix 4

One-Year Oral Toxicity Study of SK&F 101468-A
in the Sprague-Dawley (CD®) Rat

List of Tissues Collected

Gross pathology

Adrenal Glands
Animal Identification (tail, etc.)
Aorta, thoracic
Brain
Cecum
Cervix
Colon
Duodenum
Epididymides
Esophagus
Eyes
Femurs
Gall Bladder
Heart
Harderian Gland
Ileum
Jejunum
Kidneys
Larynx
Liver, left lateral lobe
Liver, right lateral lobe
Lung
Lymph Nodes, mandibular
Lymph Nodes, mesenteric
Macroscopic Lesions
Mammary Gland
Nasal Cavity
Ovaries
Pancreas
Parathyroids
Pituitary
Preputial Clitoral Gland
Prostate
Rectum
Rib (CCJ)
Salivary Glands, mandibular, sublingual, parotid (both sets)
Sciatic Nerve
Seminal Vesicles
Skeletal Muscle
Skin
Skull, base; nasal turbinates, ear canals and accessory tissues
Spinal Cord, lumbar
Spleen
Sternebrae
Stomach
Testes
Thymus
Thyroid
Tongue
Trachea
Urinary Bladder
Uterus
Vagina

Table 39

445

Appendix 5

One-Year Oral Toxicity Study of SK&F 101468-A
in the Sprague-Dawley (CD®) Rat

List of Tissues Processed

Microscopic pathology

Adrenal Glands
Aorta
Bone Marrow (Sternebrae)
Brain (3 levels)
Colon
Duodenum
Esophagus
Eye
Heart
Ileum
Jejunum
Kidneys
Liver, left lateral lobe
Liver, right lateral lobe
Lung, right caudal lobe
Lung, left lobe
Lymph Node, mesenteric
Lymph Node, mandibular
Macroscopic Lesions
Mammary Gland
Ovaries
Pancreas
Parathyroid Gland
Pituitary
Prostate
Salivary Gland, parotid
Salivary Gland, sublingual
Salivary Gland, mandibular
Seminal Vesicle
Skeletal Muscle (Sternal)
Skin
Spinal Cord, lumbar
Spleen
Sternebrae
Stomach
Testes/Epididymides
Thymus
Thyroid Gland
Trachea
Urinary Bladder
Uterus

Table 39 cont

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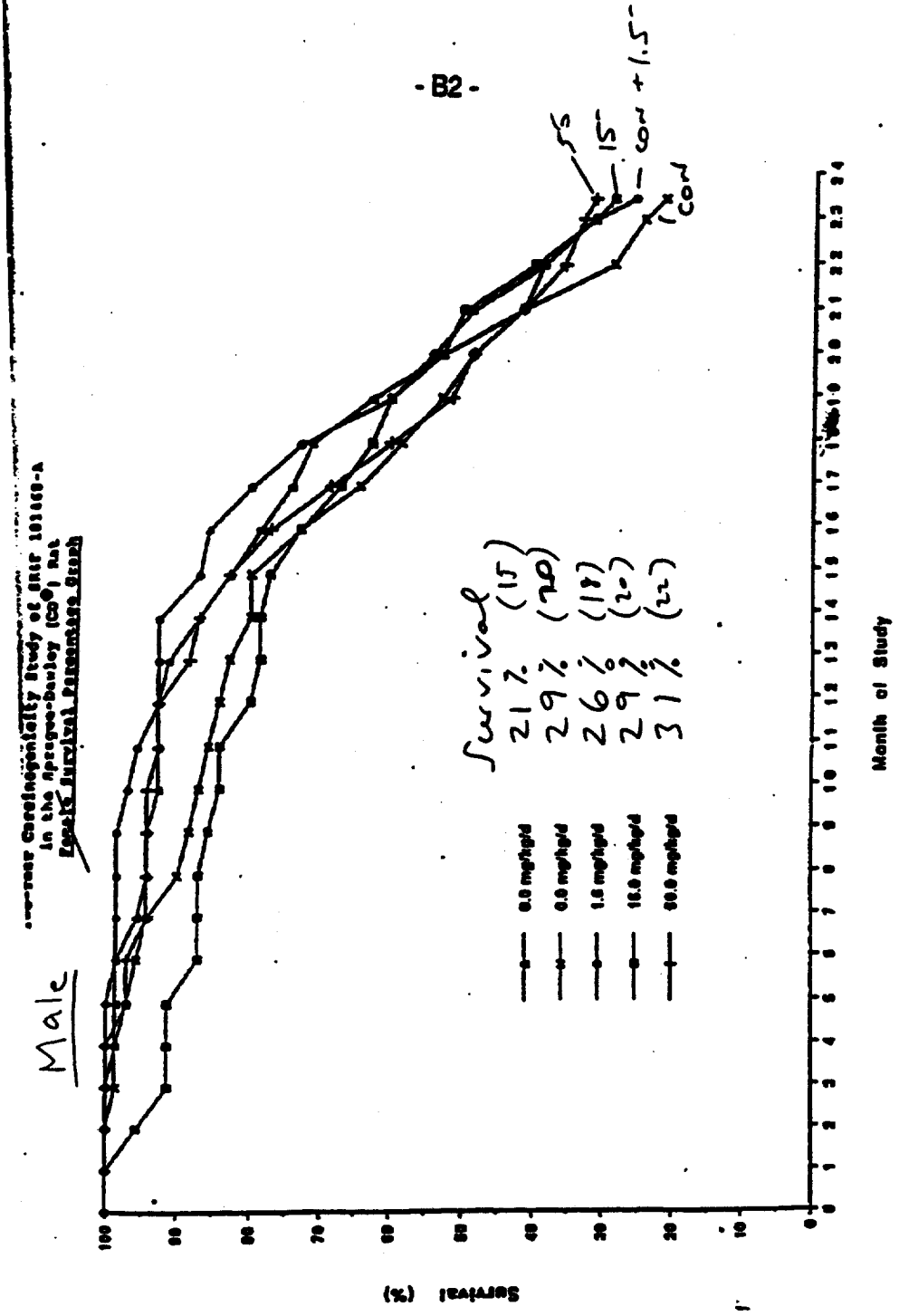

Fig 29

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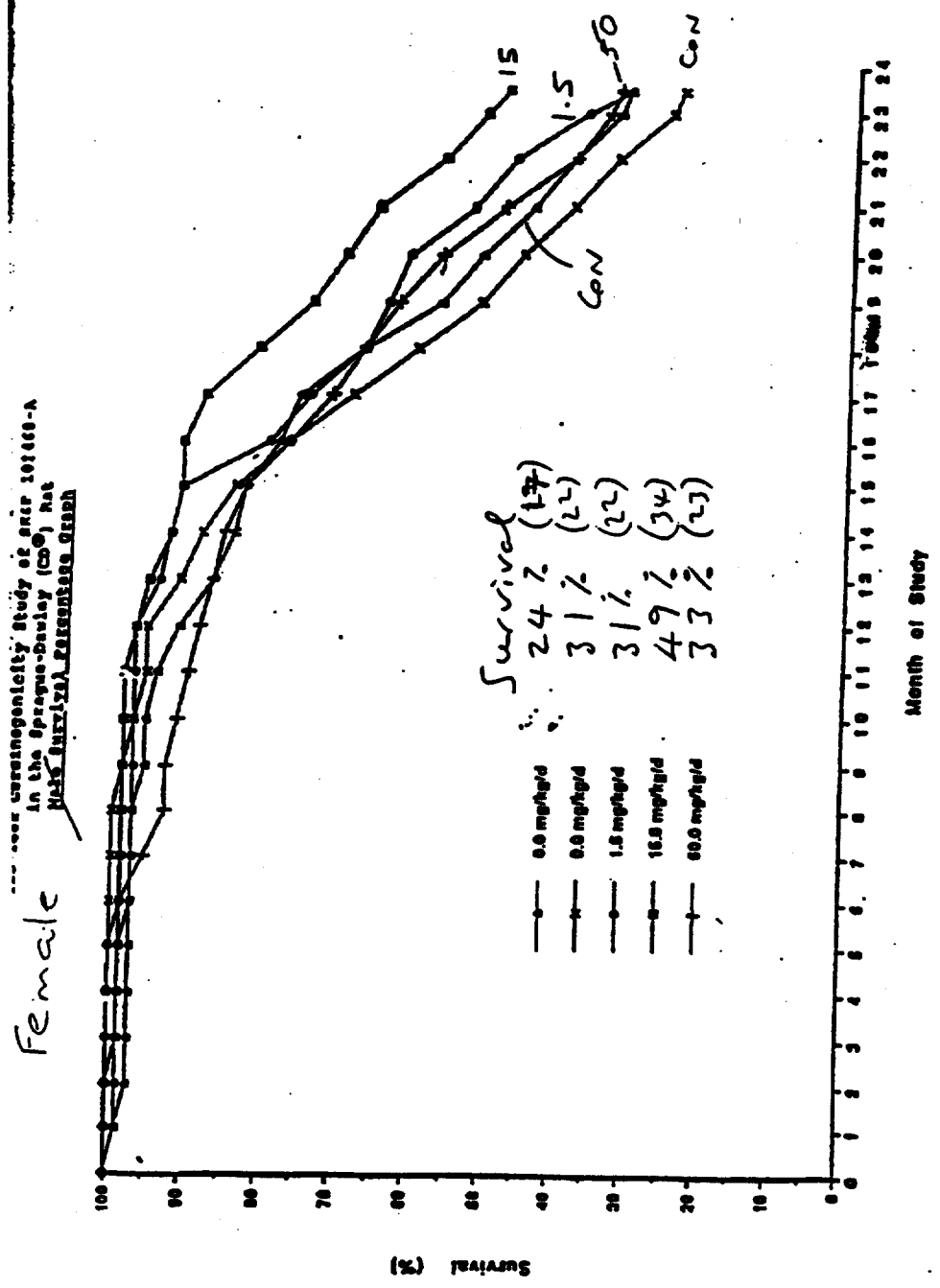

Female

Fig 30

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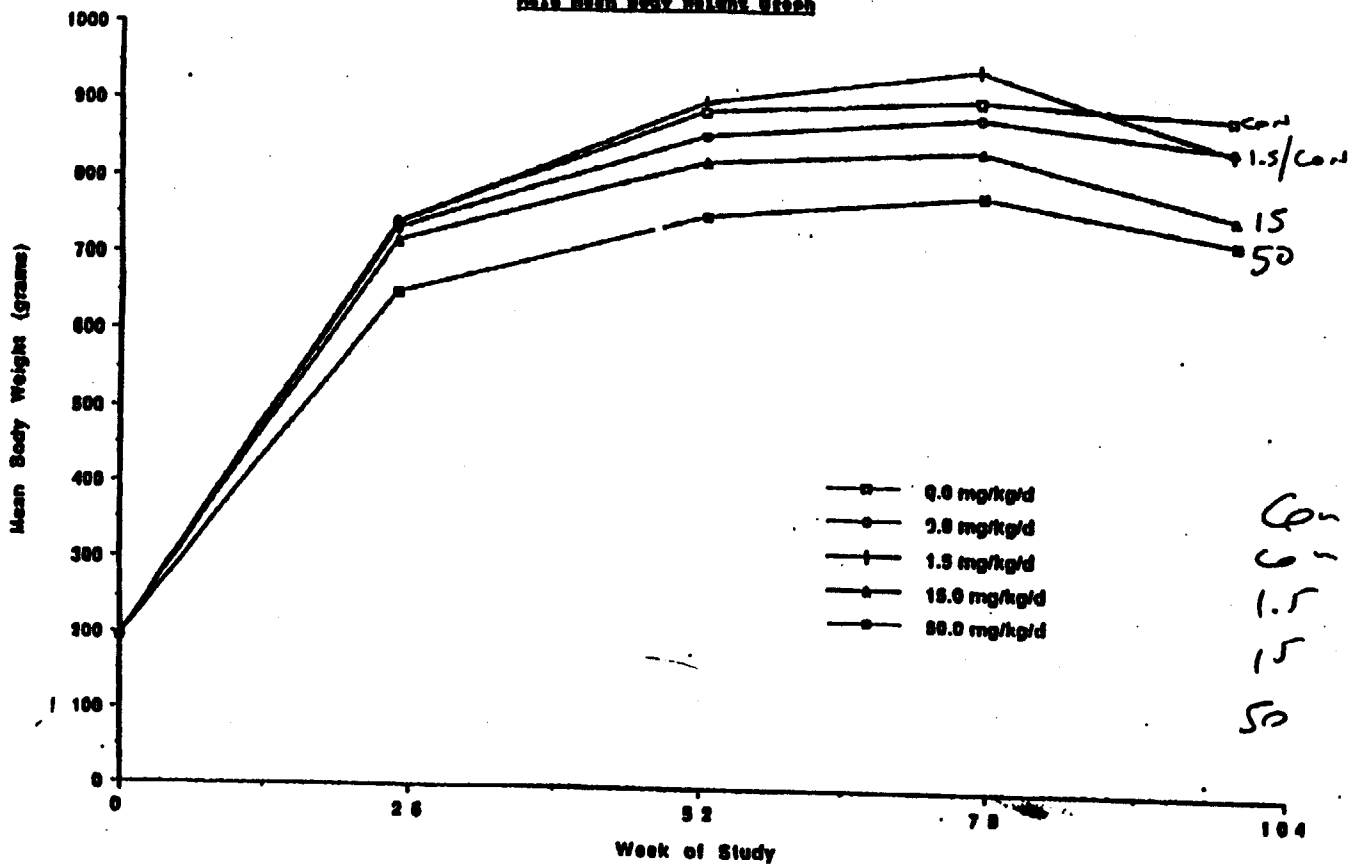
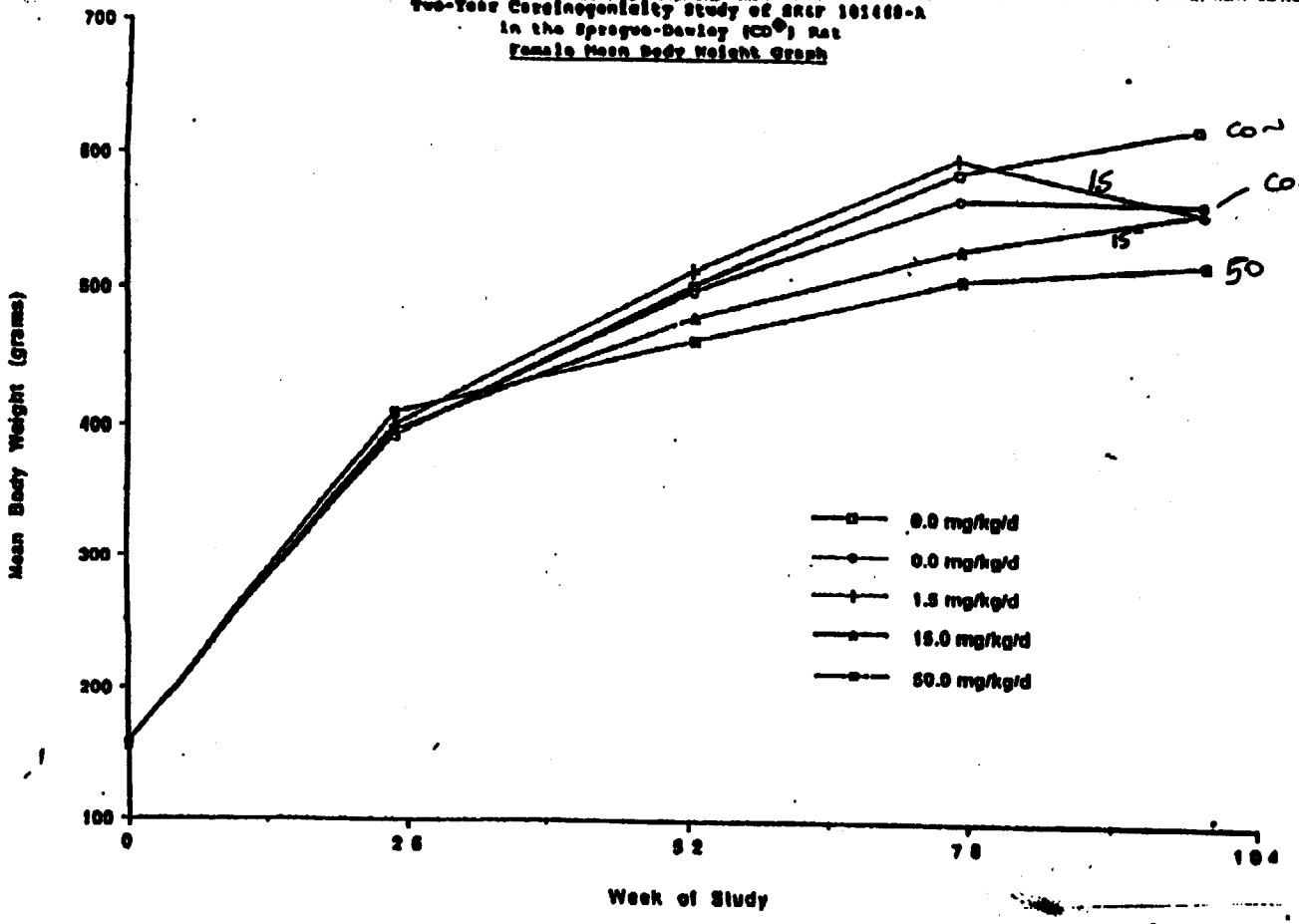
Figure 4
Two-Year Carcinogenicity Study of SMAP 101468-A
in the Sprague-Dawley (CD®) Rat
Male Mean Body Weight Graph



Body wt.
B3's

	start	E1	Tgain
Con	197	901	357
Con	197	847	330
1.5	197	872	342
15	194	754	287
50	195	721	272

Fig 31



	Body wt		
	start	End	% gain
Con	156	620	297
Con	156	565	262
1.5	157	546	248
15	154	561	264
50	155	529	241

Fig 32

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Table 2

1-Year Oral Toxicity Study of SK&F 101468-A
in Cynomolgus Monkeys (G90009)

Summary of Clinical Observations Related to Drug Treatment

Clinical Signs	Males*				Females*			
	I	II	III	IV	I	II	III	IV
Stereotypic Behavior/ Abnormal Activity								
Animals with Sign	-	-	-	3	-	-	-	3
Total Incidents	-	-	-	5	-	-	-	6
Incidents per Animal	-	-	-	1-2	-	-	-	1-4
Days of Occurrence	-	-	-	7-9	-	-	-	5-9
(inc excessive grooming + formication)				239,240				
Salivation, Post-dose or During Dosing								
Animals with Sign	-	1	3	4	-	-	3	4
Total Incidents	-	1	19	245	-	-	45	265
Incidents per Animal	-	1	3-8	1-240	-	-	3-37	1-257
Days of Occurrence	-	307	59-371	20-371	-	-	3-369	8-371
Salivation, Pre-dose								
Animals with Sign	-	1	4	2	-	-	1	1
Total Incidents	-	1	6	5	-	-	1	1
Incidents per Animal	-	1	1-2	2-3	-	-	1	1
Days of Occurrence	-	33	55-364	2-93	-	-	232	64
Emesis								
Animals with Sign	3	4	3	2	1	1	1	4
Total Incidents	6	51	39	130	7	1	144	65
Incidents per Animal	1-3	1-46	7-17	3-127	7	1	144	1-43
Days of Occurrence	48-343	6-352	9-349	1-370	60-196	162	1-371	10-335

* 4 animals/group/sex

Table 40

Figure 1
1-Year Oral Toxicity Study of SK&F 101468-A
in Cynomolgus Monkeys

Body Weight - Males

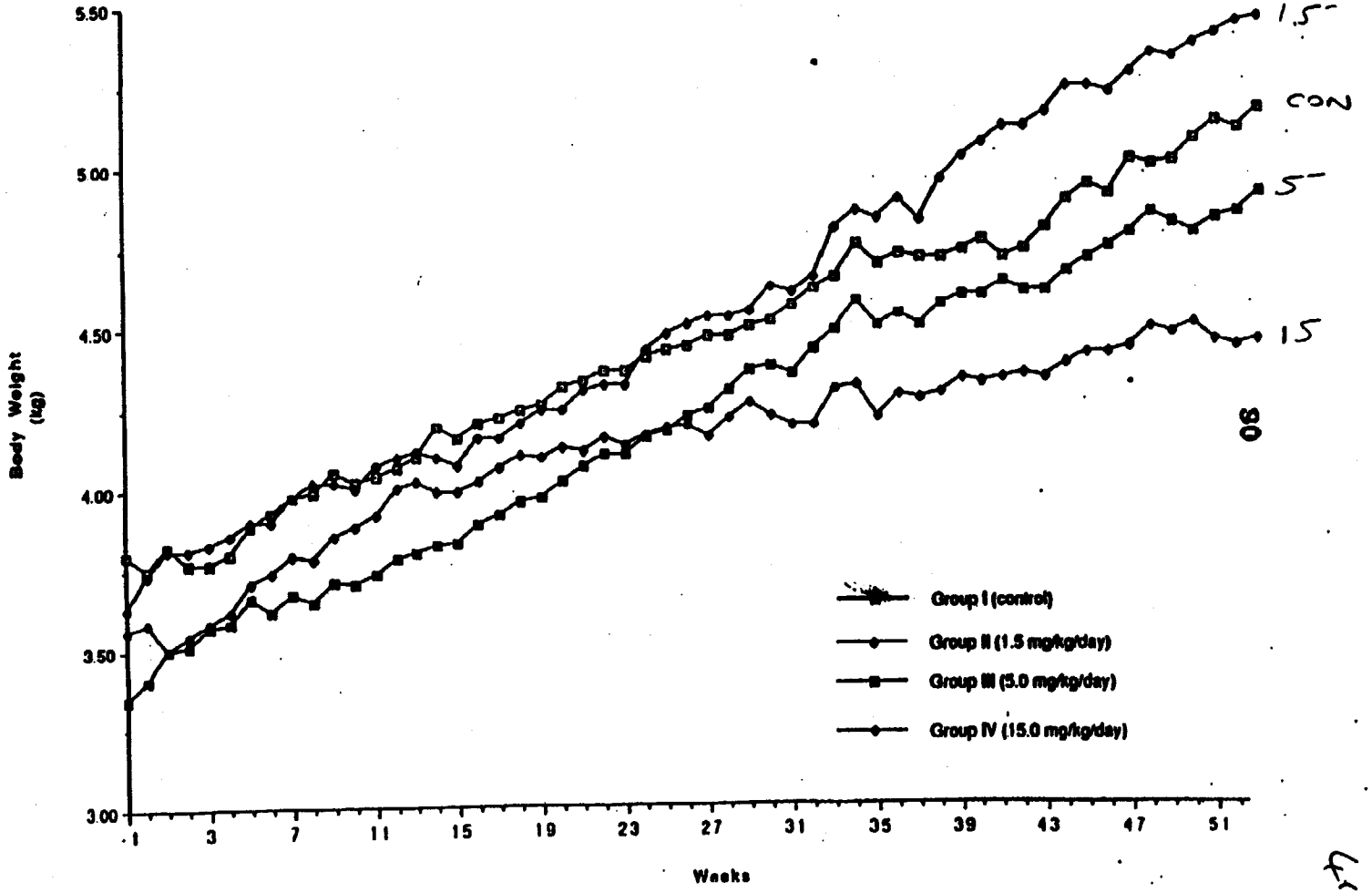

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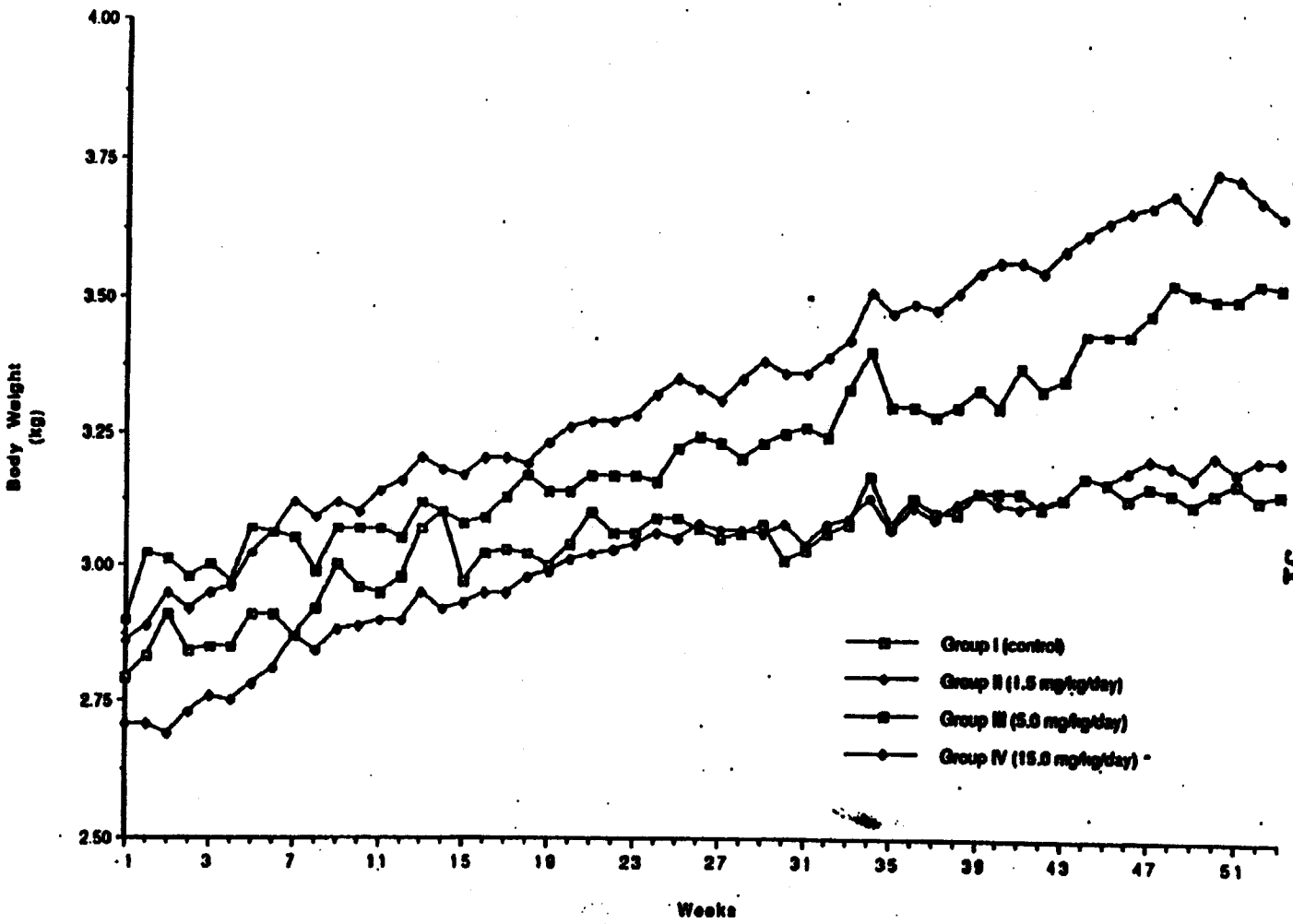
Figure 2

1-Year Oral Toxicity Study of SK&F 101468-A
in Cynomolgus Monkeys

Body Weight - Females



15
5
1.5
0
91

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Fig 7U