



## Review Outline

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## A. PHARMACOLOGY

Volumes: 1.27-1.31 Boehringer-Ingelheim and Upjohn Technical Reports  
1.62 Published articles

The following is a summary of preclinical pharmacology studies on the mechanism of action and efficacy of pramipexole. Most of these studies were reviewed under IND 34,850. The "Neuroprotection" studies (sec. A.3) that were submitted to support a labeling claim were not previously reviewed.

### A.1. Mechanism of Action

#### A.1.a *In vitro* studies

In receptor binding assays, PPX displays high affinity for both D<sub>3</sub> and D<sub>2</sub> cloned receptors (4 to 8-fold higher at D<sub>3</sub>; Tables A.1.a.1, 2). By comparison, the dopamine (DA) agonists bromocriptine and apomorphine are less potent and selective for D<sub>3</sub> and D<sub>2</sub> receptors, whereas pergolide has a higher affinity than PPX for both receptor subtypes (Table A.1.a.2). Consistent with the known receptor-effector coupling schemes for DA receptors, PPX binding to D<sub>2</sub> and D<sub>4</sub> receptors is inhibited by a non-hydrolyzable GTP analog indicating G-protein involvement in the mechanism; D<sub>3</sub> binding is not markedly affected. Autoradiographic studies indicate that the distribution of [<sup>3</sup>H]-PPX binding sites is consistent with receptor subtype mRNA distribution, high in mesolimbic areas (islets of Calleja, nucleus accumbens, olfactory tubercle) that are abundant in D<sub>3</sub> and D<sub>2</sub> receptors, and also in the D<sub>2</sub>-rich caudate nucleus (Table A.1.a.3). Binding sites were less abundant in the ventral tegmental area (VTA) and the substantia nigra (SN). In a Novascreen receptor binding assay, the only other significant binding by PPX was at  $\alpha_2$  receptors (Table A.1.a.4).

In functional studies, PPX (0.1-100  $\mu$ M) decreased cAMP in primary cultures of cerebellar granule cells by a putative D<sub>3</sub> receptor mechanism. PPX inhibited electrically-stimulated DA release in rat striatal slices, presumably via activation of nerve terminal D<sub>2</sub> autoreceptors (i.e., block with haloperidol). PPX did not block synaptosomal uptake of radiolabelled monoamines at concentrations up to 10  $\mu$ M.

A.1.a.1  
Table A.1.a.1 Affinities of Pramipexole to Cloned Dopamine Receptors Expressed in Cultured Human Embryonic Kidney and Chinese Hamster Ovary Cells

Receptor	Radioligand	K <sub>i</sub> * of pramipexole (nM)
Human D <sub>2L</sub> receptor	[ <sup>3</sup> H]pramipexole	3.9†
Human D <sub>2S</sub> receptor	[ <sup>3</sup> H]pramipexole	3.3†
Human D <sub>3</sub> receptor	[ <sup>3</sup> H]pramipexole	0.5†
Human D <sub>4</sub> receptor	[ <sup>3</sup> H]pramipexole	5.1‡

\*K<sub>i</sub>: Values are means of two to four experiments.

† Data from [22]

‡ Data from [23]

Tab. A.1.a.2

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TR No.: 7295-94-011

Table 1. Binding Affinities of Dopamine Agonists for Dopamine Receptor Subtypes.

Compound	Binding at Dopamine Receptors (K <sub>i</sub> ± SEM in nM)			
	D1-Dopamine	D2-Dopamine	D3-Dopamine	D4-Dopamine
Bromocriptine U-43714E	3418 ± 129	27 ± 9	18 ± 2	373 ± 15
Apomorphine U-19542E	491 ± 29	26	17 ± 1	8.2 ± 1
Pergolide U-68326E	1300 ± 132	1 ± 0.2	0.4 ± 0.03	9.3 ± 1
Lisuride U-64047E	62 ± 4	0.3 ± 0.1	2.2 ± 0.2	3.2 ± 0.5
Pramipexole U-98528E	> 2,381	5.3 ± 0.3	1.3 ± 0.2	18 ± 4

Table 2. Binding Affinities of Dopamine Agonists for Serotonin Receptor Subtypes.

Compound	Binding at Serotonin Receptors (K <sub>i</sub> ± SEM)			
	5-HT <sub>1A</sub>	5-HT <sub>1Dα</sub>	5-HT <sub>1Dβ</sub>	5-HT <sub>2</sub>
Bromocriptine	24 ± 9	22 ± 5	708 ± 84	119 ± 37
Apomorphine	103 ± 11	1,399 ± 109	> 4,000	343 ± 51
Pergolide	1.8 ± 0.4	21 ± 6	111 ± 8	26 ± 7
Lisuride	0.2 ± 0.03	7.6 ± 0.8	20 ± 3	5.1 ± 1.1
Pramipexole	> 1,698	2,429 ± 301	> 4,000	> 1,131

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TABLE # A.1.a.3

Densities of D<sub>2</sub> and D<sub>3</sub> Receptors Bound By [<sup>3</sup>H]-PPX in Areas of Rat Brain

The data were derived from quantification of autoradiograms produced from coronal sections.

Structure	Specific Bound (mean±SEM)*		B <sub>max</sub> D <sub>2</sub> Receptor*	B <sub>max</sub> D <sub>3</sub> Receptors*
	right	left		
N. accumbens	42±3 42±9		97±7 97±21	45±3 45±10
Anterior cingulate cortex	13±4 12±5		30±9 28±13	
Sensory cortex	16±3 12±6		37±7 37±18	
Olfactory Tubercle	46±11 47±15		105±25 108±34	49±12 50±16
Islets of Calleja Major	58±14 60±14		133±32 138±32	62±16 64±16
Islets of Calleja Medial	52±8 52±15		120±18 120±35	62±9 62±18
N. caudate	43±8 48±11		99±18 110±25	

\* fmol/mg P based on K<sub>d</sub> = 6.5 nM (D<sub>2</sub>) or 0.37 nM (D<sub>3</sub>).

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A.1.a.4

Table ~~5.1.1~~ Affinities of Pramipexole to Different Receptor Preparations From Brain Homogenates as Estimated by Receptor Binding Assays ([30] unless otherwise indicated).

Receptor	Radioligand	K <sub>i</sub> of pramipexole (nM)
Dopamine D2 receptor	[ <sup>3</sup> H]spiroperidol	1350
Dopamine D2 receptor*	[ <sup>3</sup> H]spiroperidol (high affinity state)	105
Dopamine (D2) receptor†	[ <sup>3</sup> H]pramipexole	2.9
Dopamine D1 receptor‡	[ <sup>3</sup> H]SCH 23390§	>100,000
α <sub>1</sub> -Adrenoceptor	[ <sup>3</sup> H]prazosin	28,700
α <sub>2</sub> -Adrenoceptor	[ <sup>3</sup> H]clonidine	250
Muscarinic receptor	[ <sup>3</sup> H]QNB¶	30,700
Serotonin 5-HT <sub>1</sub> receptor	[ <sup>3</sup> H]serotonin	4,200
Serotonin 5-HT <sub>2</sub> receptor	[ <sup>3</sup> H]spiroperidol	>40,000
Histamine H <sub>1</sub> receptor	[ <sup>3</sup> H]pyrilamine	>10,000
Histamine H <sub>2</sub> receptor	[ <sup>3</sup> H]tiotidine	5,300
β <sub>1</sub> -Adrenoceptor‡	[ <sup>3</sup> H]DH-Alprenolol	>10,000
β <sub>2</sub> -Adrenoceptor‡	[ <sup>3</sup> H]DH-Alprenolol	>10,000
Serotonin-5-HT <sub>1A</sub> ‡	[ <sup>3</sup> H]8-OH-DPAT	3,069
Adenosine-A2‡#	[ <sup>3</sup> H]CGS 21680	>100,000
Benzodiazepine‡	[ <sup>3</sup> H]Flumitrazepam	>10,000
NMDA/MK-801‡#	[ <sup>3</sup> H]MK-801	>10,000
NMDA/Glycine‡	[ <sup>3</sup> H]DCKA	>100,000
AMPA#	[ <sup>3</sup> H]Glutamate	>10,000

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### A.1.b. *In vivo* studies

In behavioral studies, low doses of PPX decreased locomotor activity in mice ( $ED_{50} = 0.084$  mg/kg, p.o.). This is presumably due to activation of  $D_2$  autoreceptors which shut down release of endogenous DA. Higher doses of PPX ( mg/kg, s.c.) stimulated locomotor activity in rats, but did not induce apomorphine-like stereotypic climbing in mice (0.003 - 10 mg/kg, p.o.). Other behavioral effects of PPX that are likely attributed to postsynaptic  $D_2$  receptor activation are yawning in rats ( mg/kg, s.c.), reversal of haloperidol-induced catalepsy in rats ( $ED_{50} = 4.4$  mg/kg, s.c.), dose-dependent induction of gnawing in rats ( mg/kg, s.c.), and stimulation of locomotor activity in reserpinized mice (9 mg/kg, i.p.).

The primary *in vivo* neurochemical effects of PPX appear to result from stimulation of presynaptic  $D_2$  autoreceptors which reduces dopamine turnover. PPX decreased DA synthesis as measured by inhibition of DOPA accumulation in striatum and limbic system after  $\gamma$ -butyrolactone lesions of dopaminergic pathways:

	$ED_{50}$
striatum	0.13 mg/kg, s.c.
limbic forebrain	0.05 mg/kg, s.c.

The effect of PPX in the striatum was blocked by haloperidol indicating a  $D_2$  receptor-mediated action. The more potent, but not statistically significant, effect in the limbic forebrain was suggested to be due to  $D_3$  activation. No pharmacological evidence was provided in support of this contention. A PPX-induced decrease in dopamine release was also demonstrated in  $\alpha$ -methyltyrosine-treated rats ( $ED_{50} = 0.04$  mg/kg, s.c.) and by *in vivo* microdialysis.

Evidence for activation of DA autoreceptors by PPX was also obtained in electrophysiology studies where decreases in the firing rate of nigrostriatal (SNPC) and mesolimbic (VTA) neurons were observed:

	$ED_{50}$
SNPC	0.066 mg/kg, i.v.
VTA	0.082 mg/kg, i.v.

In anterior caudate neurons, PPX stimulated the firing of postsynaptic neurons at higher doses (10 mg/kg, i.v., increased firing by 60%). This was suggested to be a  $D_3$  effect since other  $D_2$ -preferring agonists did not affect firing.

### A.2. Efficacy in Parkinson's Disease Models

PPX ( mg/kg, s.c.) caused contralateral turning in rats with 6-hydroxydopamine (6-OHDA)-induced lesions of the medial forebrain bundle. The  $ED_{50}$  (0.026 mg/kg, s.c.) indicated that PPX was equipotent to apomorphine ( $ED_{50} = 0.03$  mg/kg, s.c.). Maximal effects of PPX occurred between ( ) min, whereas the duration of action for apomorphine was  $\leq 80$  min. The effect was completely blocked by haloperidol, and partially blocked by the  $D_1$  antagonist SCH 23390.

In the MPTP-induced Parkinson's disease model in primates, PPX (            mg/kg, i.m.) dose-dependently reversed parkinson-like symptoms ( $ED_{50} = 0.045$  mg/kg, i.m.). A dose of 0.06 mg/kg relieved virtually all of the symptoms. In a second experiment, an oral dose of 0.075 mg/kg reversed Parkinsonian symptoms for 5-24 hr. Several other Parkinson's agents did not consistently affect symptomology ( $\leq 2$  mg/kg biperiden, p.o.,  $< 300$  mg/kg amantadine, p.o.,  $\leq 2$  mg/kg bromocriptine, p.o.). L-DOPA/carbidopa (15 mg/kg, p.o.) was effective for 2 hrs (Table A.2.a). When tested in combination with the monoamine oxidase inhibitor l-deprenyl (Eldepryl, 0.2 mg/kg, i.m.), the effectiveness of PPX (             $\mu$ g/kg, i.m.) was not potentiated.

Boehringer Ingelheim KG

Tab. A.2.a.

Substance	Optimal efficacy from (mg/kg)	Duration of action	Side effects at high dosage
Combination L-dopa (+ ben- serazide and carbidopa)	15 p.o.	h	salivation motor restlessness
Biperidene (Akineton)	up to 2.0		
Amantadine (PK-Merz)	up to 300	unsatisfactory effect	
Bromocriptine (Prävidel)	up to 2.0		
B-HT 920	0.05 p.o.	h	sedation, ataxia
SND 919 Y	0.075 p.o. 0.05 i.m.	h	occasional sali- vation raised reactivity

Tab. 9: Effect of antiparkinsonian drugs and agents under development on MPTP-induced Parkinson's disease in monkeys.

### A.3. Neuroprotection

The sponsor proposes a labeling claim suggesting neuroprotective effects of pramipexole based on data from three preclinical models:

1. Prevention of post-ischemic retrograde degeneration of nigrostriatal dopamine neurons following transient forebrain ischemia (Bilateral Carotid Artery Occlusion Model, BCAA) in gerbils
2. Attenuation of methamphetamine-induced nigrostriatal dopaminergic neurotoxicity in mice
3. Attenuation of L-DOPA neurotoxicity *in vitro*

In the gerbil transient forebrain ischemia study, pramipexole (1 mg/kg, p.o., b.i.d. for 28 days beginning on the day of surgery) attenuated by 40% the loss of tyrosine hydroxylase(TH)-positive neurons in the substantia nigra (Fig. A.3.a.1). A much smaller degree of protection was afforded in the CA1 region of the hippocampus (cresyl-violet staining), and statistically significant in only a preliminary experiment (Fig. A.3.a.2). Details regarding the timing of drug administration relative to the ischemic insult were not provided. In the *in vivo* methamphetamine neurotoxicity study, four daily doses pramipexole (1 mg/kg, p.o.) beginning 1 hr after the last methamphetamine injection (10 mg/kg, i.p., every two hrs for four doses) completely prevented the loss of TH-positive neurons in the substantia nigra of mice five days after methamphetamine dosing (Fig. A.3.a.3). The proposed protective mechanism is prevention by pramipexole of methamphetamine-induced elevations in dopamine turnover which, if not prevented, would lead to tissue damage *via* the generation of oxygen-derived free radicals. In the *in vitro* experiment, nanomolar concentrations of pramipexole prevented the loss of TH-positive cells due to micromolar concentrations of L-DOPA in primary cultures of rostral mesencephalic tegmentum cells (Fig. A.3.a.4). Preliminary evidence suggested the involvement of a heat-sensitive trophic factor in the protective effect of pramipexole.

The gerbil BCAA transient forebrain ischemia model is an acceptable preclinical efficacy screen for drugs proposed in the treatment of stroke. The clinical relevance of the *in vivo* methamphetamine and *in vitro* L-DOPA neurotoxicity models is not established. The results from the ischemia study would provide some preclinical support for a clinical trial proposal, although only one of the two regions examined appeared to be significantly protected. The potential clinical relevance of these findings is further compromised since no mechanistic basis of protection was evaluated. For instance, the observed protection may simply be a consequence of the hypothermic effects of pramipexole, and not related to inhibition of dopamine release. Thus, a labeling claim suggesting that pramipexole "reduces dopamine-induced neuronal degeneration", which has significant and far-reaching clinical implications, is not supported by these preclinical findings.

FIGURE 9. Dose-response for pramipexole's effect on the loss of tyrosine hydroxylase-positive neurons 28 days following a 10-min BCO in the gerbil. Data given as mean  $\pm$  SEM.

A.3.a.1

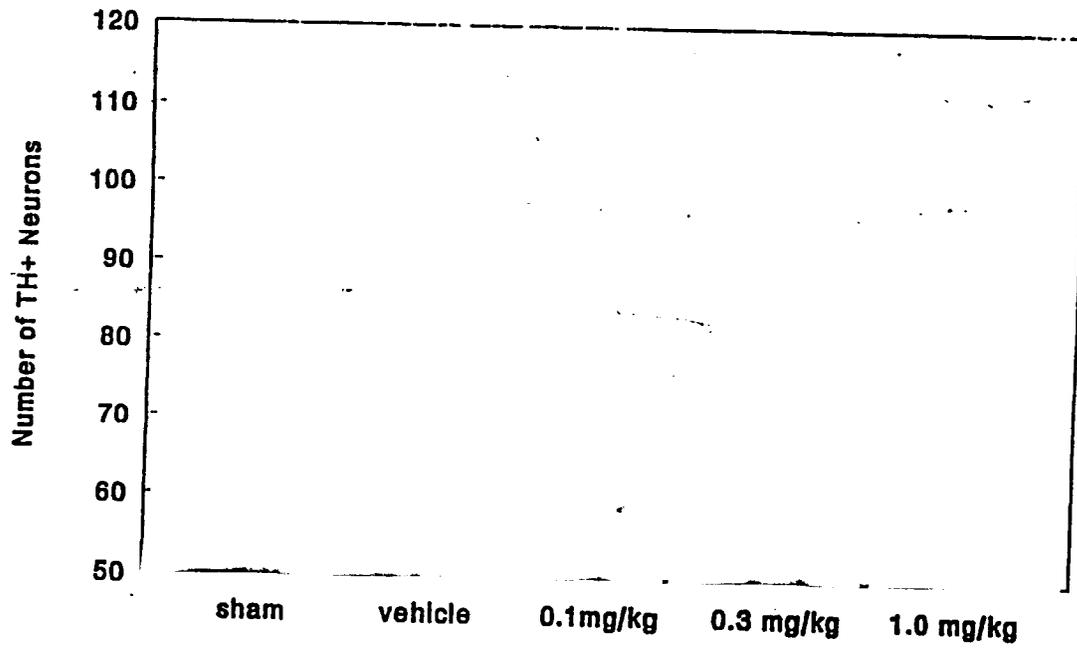


FIGURE 10. Dose-response of pramipexole's effect on neuronal damage in the CA<sub>1</sub> region of the hippocampus 28 days following a 10-min BCO in the gerbil. Data given as mean  $\pm$  SEM.

A.3.a.2

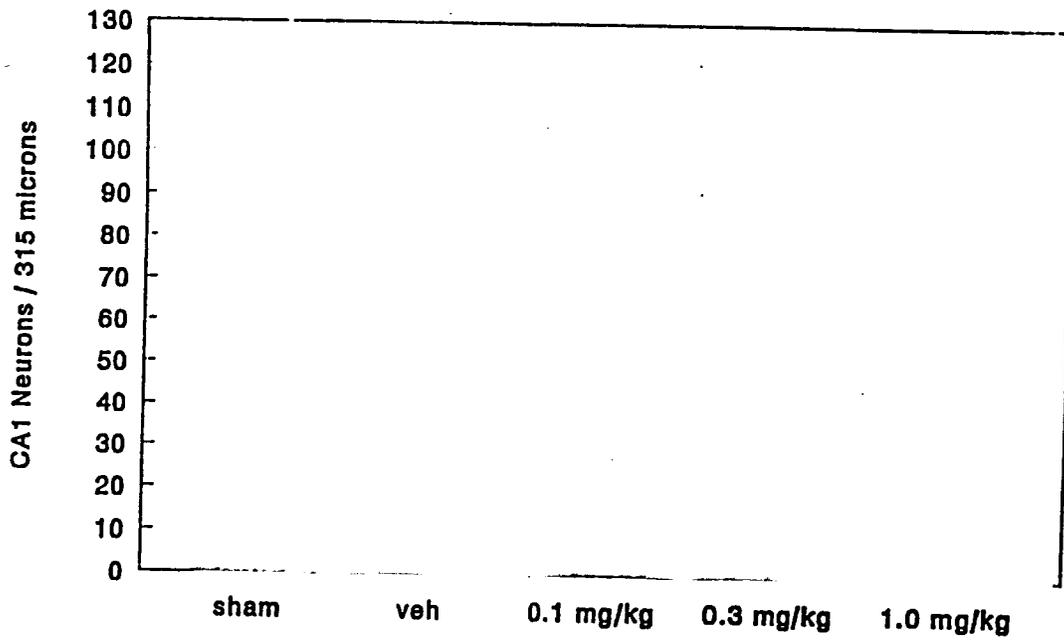
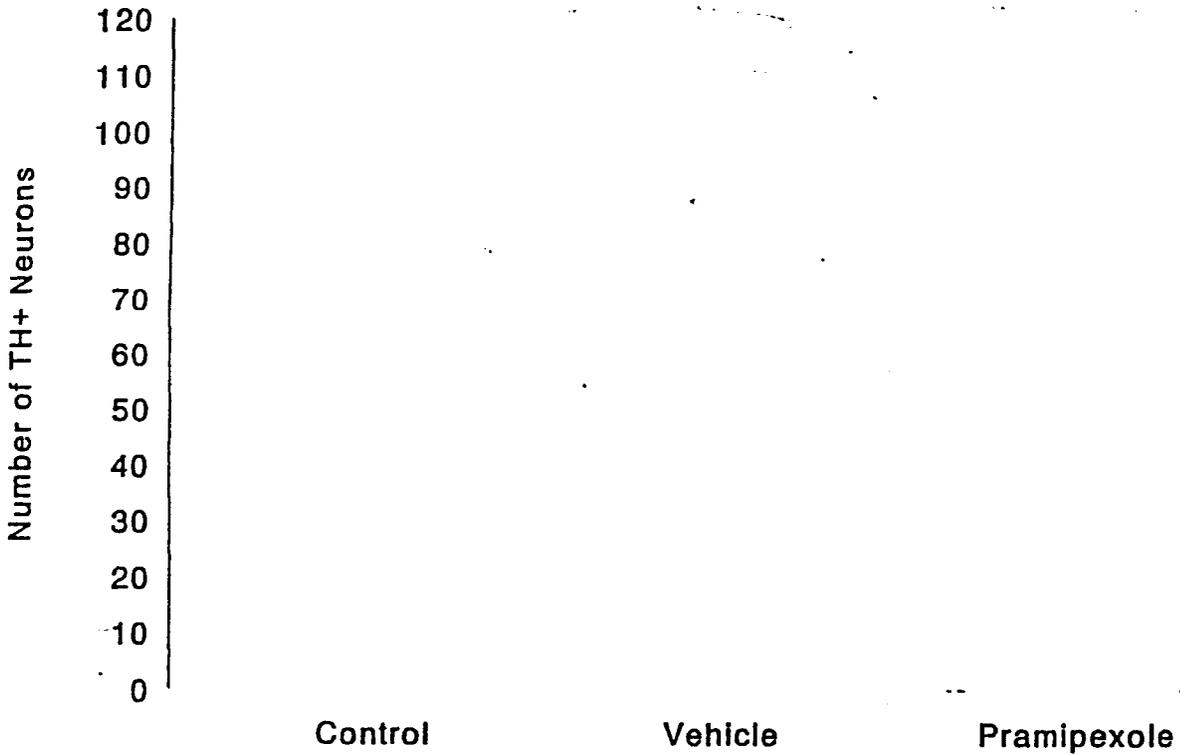


Fig. A.3.a.3

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**Effects of Pramipexole (1 mg/kg)  
on the Loss of Tyrosine Hydroxylase Positive Neurons  
5 Days Following Methamphetamine Treatment in Mice**



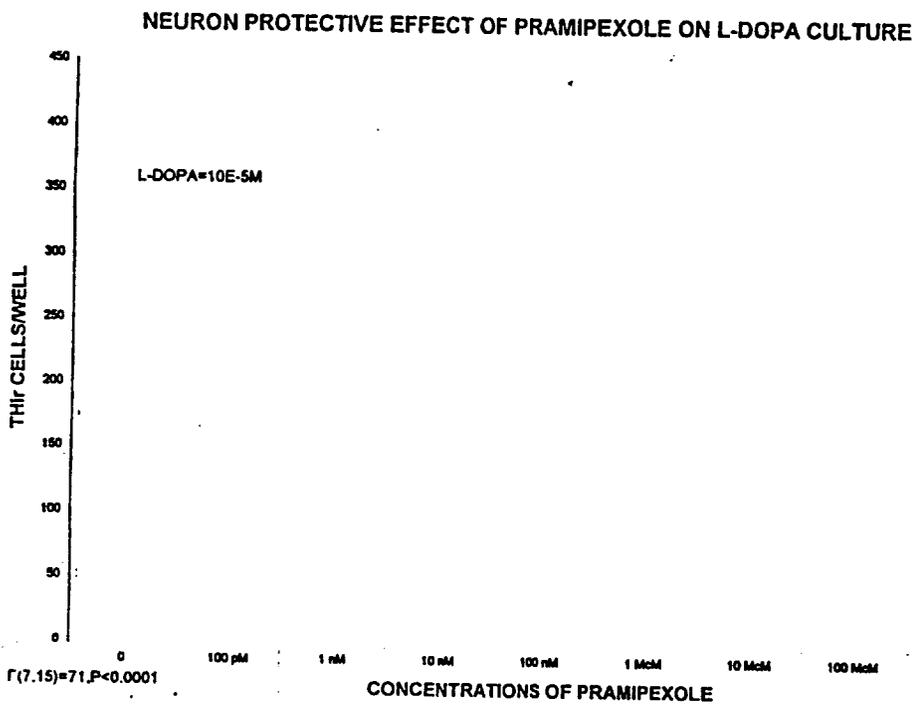
★ p<0.01

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FIGURE 3.a. 1.

Dose-dependent neuroprotective effects of pramipexole in an L-dopa toxicity model of THIR cells. Data are mean and S.E. for at least two experiments. Procedures for culture methodology, immunostaining and cell count assessment are described in the Methods. Immediately following plating, RMT cultures were exposed to various concentrations of pramipexole with or without L-dopa (10  $\mu$ M) for 72 hrs and THIR cells were counted.



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**A.4. Other Indications**

The sponsor has submitted several preclinical studies to demonstrate the efficacy of PPX in other indications including anxiety, depression and schizophrenia. These studies are not considered relevant to the present application and were not reviewed.

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## B. SAFETY PHARMACOLOGY

### B.1. Central Nervous System Effects

Aside from the aforementioned behavioral changes, relatively few significant CNS effects were induced by acute treatment with PPX. Ataxia did not occur in mice at PPX doses of  $\text{mg/kg, p.o.}$ , or  $\text{mg/kg, s.c.}$  Doses of  $\text{mg/kg, s.c.}$ , did not lower the threshold for pentylenetetrazol-induced seizures in mice. Monkeys were slightly sedated by doses of  $\text{mg/kg, i.m.}$  and  $300 \text{ } \mu\text{g/kg, p.o.}$  REM and non-REM sleep in rats was suppressed by  $0.3 \text{ mg/kg, p.o.}$  A biphasic effect on sleep was observed in cats; low doses ( $0.1 \text{ mg/kg, p.o.}$ ) increased non-REM sleep initially, followed by reduction in REM and non-REM sleep. Higher doses ( $0.3 \text{ mg/kg}$ ) completely suppressed sleep. PPX decreased body temperature in mice by  $2^\circ\text{C}$  with an  $\text{ED}_{50}$  of  $0.23 \text{ mg/kg, s.c.}$

### B.2. Cardiovascular/Respiratory Effects

In anesthetized cats, PPX  $\text{mg/kg, i.v.}$  caused a slight, transient ( $\text{min}$ ), non-dose-dependent decrease in blood pressure and a slight bradycardia at  $1 \text{ mg/kg}$ , but did not alter respiration. In anesthetized rabbits,  $0.03 \text{ mg/kg, i.v.}$  decreased blood pressure, and  $0.1 \text{ mg/kg}$  decreased both pressure and heart rate. The cardiovascular effects of  $0.3 \text{ mg/kg}$  PPX were blocked with dopamine antagonists. In spontaneously hypertensive rats (SHRs),  $\text{mg/kg, i.v.}$ , PPX lowered blood pressure by a  $\text{D}_2$ -receptor mechanism. Higher doses ( $0.1$ - $1 \text{ mg/kg, i.v.}$ ) increased blood pressure by a peripheral  $\alpha_2$ -receptor mechanism. After oral administration of  $3$  and  $30 \text{ mg/kg}$  PPX to SHRs, a slight bradycardia was the only effect observed. In a study with anesthetized SHRs, PPX ( $\text{mg/kg, i.v.}$ ) decreased blood pressure and the effect could be blocked by either a  $\text{D}_1$  or  $\text{D}_2$  antagonist.

A special series of studies were conducted in rhesus monkeys to evaluate the cardiovascular effects of pramipexole in combination with other drugs used in Parkinson's disease (i.e., Sinemet and Eldepryl;  $\text{mg/kg, p.o.}$ ). In a pilot study, a dose of  $0.05 \text{ mg/kg p.o.}$ , but not  $0.1 \text{ mg/kg}$ , lowered blood pressure for up to 6 hrs. Doses of  $\text{mg/kg}$  produced a non-dose-dependent bradycardia, although the duration of effect increased with dose ( $\text{hr}$ ). In the combination study, slight (non-significant) decreases in heart rate, diastolic blood pressure, and mean arterial blood pressure following  $0.05 \text{ mg/kg}$  PPX, p.o., were not potentiated by either Sinemet ( $100 \text{ mg/kg L-DOPA}/10 \text{ mg/kg carbidopa, p.o.}$ ) or Eldepryl ( $0.2 \text{ mg/kg, p.o.}$ ).

### B.3. Gastrointestinal Effects

Like other dopamine agonists, PPX induced emesis in dogs ( $\text{ED}_{50\text{s}} = 0.0067 \text{ mg/kg, p.o.}$ , and  $0.0052 \text{ mg/kg, s.c.}$ ). The effect was blocked with a dopamine antagonist. PPX inhibited gastrointestinal transit in mice ( $\text{ED}_{50} = 0.033 \text{ mg/kg, p.o.}$ ).

#### B.4. Renal Effects

Conflicting results were obtained in assessments of the renal effects of PPX in rats. In conscious rats, PPX (0.3 mg/kg, p.o.) produced a moderate decrease in urinary volume; electrolyte excretion was not significantly affected. In a study from an independent investigator with anesthetized normotensive (WKY) and spontaneously hypertensive (SHR) rats (Kaneko, et al., J. Auton. Pharmacol., 10(suppl. 1):s53, 1990), PPX (\_\_\_\_\_ mg/kg, i.v.) increased urine volume and Na excretion. The low dose effects of PPX were antagonized by a D<sub>1</sub> antagonist and the high dose effects by a D<sub>2</sub> antagonist. The contrasting effects of PPX in conscious and anesthetized animals may have been due to the anesthesia, PPX dose, strains of rats, or the use of water-loading in the former, but not the latter study. The effectiveness of the D<sub>1</sub> antagonist against PPX in this study suggest that the renal effects of PPX may be mediated by D<sub>1</sub> receptors, and additional biochemical data support this hypothesis.

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**C. TOXICOLOGY**

**C.1. Acute Toxicology**

Conducted by: Boehringer Ingelheim KG  
Department of Experimental Pathology and Toxicology  
D-6507 Ingelheim am Rhein  
West Germany

Sponsor Volume: 1.31

These studies complied with GLP

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**Summary**

Acute toxicology studies were conducted in mice, rats and dogs. Animals were observed for up to 14 days following treatment. The lethality of PPX was approximately 10-fold higher in mice and 4-fold higher in rats by the intravenous versus oral route of administration. Common signs of toxicity were exophthalmus, piloerection, tremors/convulsions, ataxia and hypomotility, nervousness/agitation and hypermotility, and tachypnea or dyspnea. Most deaths following i.v. treatment occurred shortly after dosing, whereas delayed deaths were more common following oral administration. The primary sign at autopsy of animals that died following drug treatment was hemocongestion of large organs. Animals that were sacrificed at the end of the study did not show any consistent pathologies. The dog studies were limited by the pronounced emetic effect of PPX.

**C.1.a. Acute Oral Toxicity in Mice**

Doses: 1400, 2000 mg/kg      n = 10 (5M, 5F)

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Results:

	# deaths	
	1400 mg/kg	2000 mg/kg
0-6 hr	2	3
6-24 hr		3
1-7 days	1	2

Signs: exophthalmos, piloerection, tremors/convulsions, hypomotility

LD<sub>50</sub>: ca. 1700 mg/kg

**C.1.b. Acute Intravenous Toxicity in Mice**

Doses: 100, 125, 160, 200 mg/kg n = 10 (5M, 5F)

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Results:

	# deaths			
	100 mg/kg	125 mg/kg	160 mg/kg	200 mg/kg
0-6 hr	0	0	6	7

Signs: exophthalmos, convulsions, tachypnea

LD<sub>50</sub>s: male: 155, female: 188, m+f: 169

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**C.1.c. Acute Oral Toxicity in Rats**

Doses: Study I: 100, 200 mg/kg n = 10 (5M, 5F)  
 II: 200, 400, 560, 800 mg/kg n = 10 (5M, 5F)

(In study I, animals were housed in plastic cages, and deaths were attributed to choking on bedding. In study II, the animals were housed in suspended cages)

Results:

	# deaths					
	100 mg/kg	200 mg/kg	200 mg/kg	400 mg/kg	560 mg/kg	800 mg/kg
0-6 hr					1	3
6-24 hr						
1-2 days	1	1				
2-7 days	2	4	1		2	2
7-14 days						1

Signs: exophthalmos, chewing, ataxia, automutilation

LD<sub>50</sub>s:

	<u>0-24 hr</u>	<u>&gt; 24 hr</u>
male:	> 800	> 800
female:	> 852	> 548
m + f:	> 957	> 809

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**C.1.d. Acute Intravenous Toxicity in Rats**

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Doses: Study I: 100, 140 mg/kg n = 10 (5M, 5F)  
II: 200, 400, 560, 800 mg/kg n = 10 (5M, 5F)

(In study I, animals were housed in plastic cages, and deaths were attributed to choking on bedding. In study II, the animals were housed in suspended cages)

**Results:**

	# deaths				
	100 mg/kg	140 mg/kg	140 mg/kg	180 mg/kg	225 mg/kg
0-6 hr					7
6-24 hr		1			
1-2 days	2	1			
2-5 days	2	3			

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Signs: exophthalmos, dyspnea, convulsions, ataxia, hypomotility  
Autopsy: pulmonary hemocongestion  
LD<sub>50</sub>: ca. 210 mg/kg

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**5. Acute Oral Toxicity in Beagles**

Doses: 0.001, 0.01, 0.1, 1.0 mg/kg; n = 1/sex/dose

Results: 0.01-1 mg/kg caused emesis; 1 mg/kg caused mydriasis and decreased food intake

**6. Acute Intravenous Toxicity in Beagles**

Doses: 1, 3, 5, 10 µg/kg; n = 1/sex/dose

Results: 3 µg/kg caused salivation; doses ≥ 3 µg/kg in females and ≥ 5 µg/kg in males caused emesis

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## C.2. Chronic Toxicology

### C.2.a. 52-Week Toxicity Study in the Rat

Conducted by: Boehringer Ingelheim KG  
Dept. Exp. Pathol. and Toxicol.  
D-6507 Ingelheim am Rhein  
Germany

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Document #'s: BI Document 90-0557  
Upjohn TR 7219-94-067

Sponsor Volumes: 1.34-1.36

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This study complied with GLP

#### Significant notation:

The sponsor has attached an Amendment (no. 2) to the original report. The basis for the amendment was a discrepancy in the terminology used by histopathologists evaluating the data from the 52-week rat chronic toxicity study and the 2-year rat carcinogenicity study. Briefly, mammary gland changes were observed in female rats from the mid- (3.0 or 2.0 mg/kg) and high- (15.0 or 8.0 mg/kg) dose groups of both studies. In the 52-week study, the changes were described as an "increase in mammary gland acini (control: 13/20, low dose: 6/20, mid dose: 17/19, high dose: 20/20) with concurrent hypertrophy and/or hyperplasia of the glandular epithelium (controls 0/20, low dose: 0/10, mid dose: 8/19, high dose: 19/20)...". In the 2-year rat carcinogenicity study, these changes were designated as a "change in the normal glandular growth pattern", according to the work of Cardy ("Sexual dimorphism of the normal rat mammary gland", *Vet Pathol*, 28:139-145, 1991). Consequently, the histopathology slides from the 52-week rat study were re-evaluated using the terminology of Cardy. Diagnoses that were originally described as "increase in glandular acini" were changed to "proliferation of glandular epithelium". Original diagnoses of "hypertrophy" or "hyperplasia" were changed to "mixed tubuloalveolar/lobuloalveolar pattern" or "lobuloalveolar pattern." According to the redefinition, only one case of mammary gland hyperplasia (alveolar) was identified in the 52-week rat study (1 LDF).

#### Summary:

Pramipexole was administered in the diet to Wistar rats (Chbb:THOM) for 52 weeks at dose of 0, 0.5, 3.0 and 15.0 mg/kg. Toxicology dosage groups were composed of 20 rats/sex; a satellite group of 7 rats/sex/dose were used for toxicokinetic analysis. Administration of the lowest test dose resulted in slight behavioral activation in both sexes, and decreased body weight gain, cholesterol and triglycerides in females. Body weight gain was reduced in both sexes by the mid and high doses. The female clinical chemistry changes were more evident at the mid and high doses. In addition, sporadic, modest elevations in transaminases, alkaline phosphatase and urea, and decreases in serum potassium occurred at the mid and high doses,

generally more frequently in females than in males. Slight thrombocytopenia (MD, HD) and slight-to-moderate increase in the granulocyte/lymphocyte ratio were evident in females. Organ weight changes were reduced liver weights in HDM, and reduced thymus weights in MDF and HDF. Ovarian weights were increased at all dosage levels, and enlarged corpora lutea were observed at the mid and high dose. Histopathological changes in the uteri (dilatation, serous contents, pyometra) and mammary glands (the above-mentioned glandular pattern changes) were also evident in MDF and HDF. Leydig cell hyperplasia occurred only in PPX-treated males, but the incidence was highest at the low dose level. Eleven animals (4 control, 7 PPX-treated) were identified with tumors, none of which could be clearly attributed to PPX treatment. One control and one LDM had Leydig cell adenoma.

Toxicokinetic analyses suggested that PPX concentrations tended to increase dose-proportionally in females, but were greater than dose-proportional in males. This resulted in higher plasma levels in HDM compared to HDF at week 26 and 52.

The "No Toxic Effect" level was considered as 0.5 mg/kg. The plasma levels measured at 1 hr after the start of the light phase during week 26 and 52 at this dose (4.0 - 6.5 ng/ml) approximate the steady-state C<sub>max</sub> in humans administered the projected maintenance dose of 1.5 mg PPX, t.i.d (5.5-7.2 ng/ml).

**Methods:**

Dosages: 0.5, 3.0, 15.0 mg/kg (Batch II)

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Route of Administration: Drug-in-diet

Species/Strain/Number: Rat/Wistar (Chbb:THOM)

80 males, 80 females for toxicology  
21 males, 21 females for plasma toxicokinetics

Mean initial weights: males: 296.1g  
females: 193.8g

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Dosages/Group Designation:

Group	Dosage (mg/kg/day)	Number of animals	
		males	females
0 (control)	0	20	20
1 (low dose=LD)	0.5	20	20
2 (middle dose=MD)	3.0	20	20
3 (high dose=HD)	15.0	20	20
4 (for determination	0.5	7	7
5 of plasma con-	3.0	7	7
6 centrations only)	15.0	7	7

Parameters monitored/Intervals:

Clinical - daily  
 Body weight - weekly  
 Food consumption - weekly  
 Water consumption - weekly (weeks 14, 26, 36, 48)  
 Spontaneous Activity - during weeks 1, 17, 34, 49 for a 22-hr period  
 Ophthalmology - weeks -1 & 51 (groups 0 and 3); weeks 12 & 25 (3 only)  
 Hematology - weeks -2, 6, 13, 27, 39, 52

Erythrocytes  
 Haemoglobin  
 Haematocrit  
 MCV, MCH, MCHC  
 Reticulocytes

Leucocytes  
 Differential blood count  
 Thrombocytes  
 Thromboplastin time (TPT)

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Clinical Chemistry - weeks -2, 6, 13, 27, 39, 52

GPT  
 GOT  
 Alkaline phosphatase  
 Bilirubin  
 Cholesterol  
 Triglycerides  
 Urea  
 Creatinine

Glucose  
 Sodium  
 Potassium  
 Calcium  
 Chloride  
 Inorganic phosphate  
 Protein  
 Protein electrophoresis

Urinalysis - weeks 12, 25, 38, 42, 50 (groups 0 and 3)  
 weeks 38, 42 (groups 1 and 2)

Specific gravity  
 pH  
 Protein  
 Glucose

Blood  
 Ketone bodies  
 Bilirubin  
 Urobilinogen  
 Nitrite  
 Examination of  
 sediment

Plasma Conc - weeks 1, 26, 52 at hrs 1 and 8

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Organ Weights - termination

Brain  
 Pituitary gland  
 Salivary glands (Glandula submaxillaris et  
 sublingualis major)  
 (Parotid gland if required)  
 Thyroid gland  
 Thymus  
 Heart

Lungs  
 Liver  
 Spleen  
 Kidneys  
 Adrenal glands  
 Gonads  
 Prostate

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Histopathology

Tongue  
 Cervical lymph nodes  
 Both pinnae with ear tattoo  
 Trachea and larynx (latter not sectioned)  
 Oesophagus  
 Aorta  
 Sternum  
 Pancreas  
 Stomach  
 Small intestine (duodenum, jejunum, ileum)  
 Large intestine (caecum, colon, rectum)  
 Mesenteric lymph nodes  
 Urinary bladder  
 Seminal vesicles  
 Uterus (incl. cervix uteri and vagina)  
 Mammary tissue  
 Skin  
 Skeletal muscle (M. semimembranosus)  
 Femur with stifle joint  
 Sciatic nerve  
 Spinal cord  
 Injection site (parenteral studies)

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Results:  
 Mortality:

Group	0		1		2		3	
	m	f	m	f	m	f	m	f
Died	0	2	2	0	0	0	2	1
Sacrificed	1	0	0	0	0	0	1	0
Total	1	2	2	0	0	0	3	1
*	5	10	10	0	0	0	15	5

No deaths could be directly attributed to the drug. Six animals (2 CON, 1 LD, 3 HD) died under anesthesia for blood sampling, and 1 LDM had abscess-forming pneumonia. One sarcoma-bearing control male, and one cachectic HDM were sacrificed.

Clinical: increased activity - MD, HD (both sexes); effect more evident in females (wk 1, 17, 34, 49)

Body Weight Gain:

MDM - sig. reduction - weeks 10, 11  
 HDM - sig. reduction - weeks 1-32, and 39  
 All F groups - sig. reduction throughout study

Food Intake: During week 1, food intake was decreased in MDM, HDM, and all treated females. The animals recovered during week 2, and some significant increases were recorded over the course of the study in MDM and all female groups. The diet delivered the targeted drug dose generally within 2%.

Water Intake: No drug-related effects

Ophthalmology: No drug-related oculo-toxic effects were apparent. Cataracts and corneal abnormalities (opacity, calcium deposits) appeared to be spontaneous lesions.

Hematology:

Significant mean changes were noted on various parameters over the course of the study, but few clearly dose- or time-related effects were evident.

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granulocyte/lymphocyte ratio-	-	gradual increase in F
decrease RBC	-	dose-dep. in F (wk 13) MDF (wk 6) HDF (wk 6, 39, 52)
decrease Hb	-	MDF (wk 6, 13) HDF (wk 13)
decrease Hct	-	dose dep in all F (wk 13) MDF (wk 6) HDF (wk 52)
decrease platelets	-	MDF (all times except wk 39) HDF (all times)
decrease lymphocytes	-	LDF (wk 6) MDF (wk 6, 27, 52) HDF (wk 6, 27, 39, 52)

To assess the relative magnitude and frequency of thrombocytopenia in individual female rats, the occurrence of platelet count decreases on the order of 20% and 40% were noted:

	Control	LD	MD	HD
week 6	-	1, 0	1, 0	2, 0
" 13	-	-	1, 0	-
" 27	1, 0	2, 0	5, 1	6, 0
" 39	0, 1	7, 0	5, 0	9, 1
" 52	2, 2	7, 0	8, 1	5, 4

first number = # of animals with 20% reduction; second number = # of animals with 40% reduction

As shown, the number of female rats with reduced platelet counts tended to increase with time and dose. However, there was not a clear worsening in all animals with time.

In males, no clear dose or time dependent effect on reduction of platelet counts was evident. Generally, 2-4 animals in each group including controls had marginal reductions in platelets. However, platelets in one MD male were reduced by 67% at week 52.

#### Clinical Chemistry:

The most dramatic, clearly dose-related effect was decreased cholesterol in females. The mean elevations in SGPT and SGOT in females were significant ( $p < 0.05$ ), but marginal; only sporadic instances of significant individual elevations (2X control values) were recorded. By week 52, no elevations were evident in PPX-treated female rats. At week 52, SGPT and SGOT levels were elevated in 1 LD and 1 HD male rats, and SGPT was elevated 1 MD male. Serum bilirubin levels fluctuated in all dosage groups throughout the study.

increased SGPT	-	MDF (wks 6-27) HDF (wks 6-39) MDM (wk 6) LDM, MDM (wk 27)
increased SGOT	-	MDF, HDF (wks 6-52)
increased AP	-	MDF, HDF (wks 6-52)
decreased cholesterol	-	dose-dep decrease in females, marked at HD; (wk 6-52) no changes in males
decreased triglycerides	-	all females, not dose-dep (largest effect in MD); (wk 6-52) MDM, HDM - small effect
increased urea	-	MDF, HDF (wk 6-52) MDM (wk 13, 27, 39)
decreased K	-	dose-dep decrease in females (wk 6- 52); HDM (wk 6-52)

#### Protein Analysis:

The noted changes were generally within the normal range. Individual variations that were outside of the normal range at week 52 were elevated  $\gamma$ -globulin in 1 LDM and increased  $\alpha_2$ -globulin in 1 HDF.

decreased total protein	-	MDF, HDF (wk 39 & 52) MDM (wk 39)
decreased albumin	-	MDF, HDF (wk 6-52)

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	-	HDM (wk 52)
increased globulin	-	MDF, HDF (wk 6-52)
decreased globulin	-	MDM (wk 39)
increased $\gamma$ -globulin	-	MDF, HDF (wk 6-52) HDM (wk 13, 27, 52) MDM (wk 27)
decreased $\gamma$ -globulin	-	LDM (wk 39)
decreased $\alpha_1$ -globulin	-	LDF (wk 52) MDF (wk 13, 39, 52) HDF (wk 39, 52)
decreased $\alpha_2$ -globulin	-	MDM (wk 27) HDM (wk 27, 39)
increased $\alpha_2$ -globulin	-	LDM (wk 39)
increased $\beta$ -globulin	-	MDF, HDF (wk 39, 52)
Urinalysis:		
blood/RBCs	-	MDF, HDF (also found in some controls, LDM, MDM)

Organ Weights:

↓ liver	-	HDM
↓ thymus	-	MDF, HDF
↑ ovary	-	LDF, MDF, HDF

Gross Pathology:

Premature decedents: - no drug-related findings

Survivors:

uterine dilatation	-	2/20 LDF 5/20 MDF 6/19 HDF
ovarian size increase	-	14/20 MDF 18/19 HDF
thymus, small	-	3/19 HDF

Histopathology:

Males:

Leydig cell, hyperplasia - 10 LDM  
7 MDM  
2 HDM

adenoma - 1 ConM  
1 LDM

kidney, pyelonephritis - 2 MDM, 1 HDM

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Females:

corpora lutea, enlarged - 2 CON  
0 LD  
18 MD  
19 HD

pyometra - 1 MD  
5 HD

adrenals, decreased lipids/  
birefringent substances 3 HD

kidney, pyelonephritis 1 HDF

uterus, dilatation - similar incidence rate ( % in all  
groups)

squamous metaplasia - 4 HDF

glandular cystic metaplasia - 1 MDF  
1 HDF

pyometra - 1 MDF  
5 HDF

bladder, squam. metaplasia - 1 HDF

mammary gland - see discussion of amendment

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Neoplasia:

A total of eleven animals were diagnosed with tumors during the study. The tumors are known to occur spontaneously in Wistar rats. No clear pattern of frequency or distribution was identified; thus, the tumors were not clearly related to PPX administration (Tab. C.2.a.1).

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Incidence of tumor types, listed according to animal number and primary site per group and sex (G 59 - SND 919 CL 2Y, feed, rat, 52 weeks)

Study group Sex*	0		1		2		3	
	m	f	m	f	m	f	m	f
Heart Neurilemmoma*								316
Testis Leydig cell adenoma	008**u		116u**					
Pituitary gland Adenoma nos.		058 064 065		159				
Cervical lymph node(s) Malignant lymphoma						204		
Thymus Malignant lymphoma						204		
Skin/subcutis Fibrosarcoma Sarcoma nos.								369
Brain Granular cell tumor								161

\*m = male, f = female

+ = according to the classification of Alison et al.<sup>1</sup>

\*\*u = unilateral

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Plasma Concentrations:

In females, plasma concentrations of PPX were proportional to dose. In males, plasma concentrations were greater than dose-proportional. The plasma concentrations of PPX in males 1 hr after dosing appeared to be much higher during week 26 and 52 compared to week 1. Plasma concentrations in males 8 hrs after dosing were highest during week 26 compared to weeks 52 and 1. In females, time-related differences were not marked (Tab. C.2.a.2).

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Table 2. Rat Mean Plasma Pramipexole Concentrations (ng/mL)  
C. Z. a. 2 in the 52-Week Toxicity Study\*

1 Hour After Start of Light Phase					
Sex	Dose (mg/kg)	Week			Mean
		1	26	52	
Female	0.5	4.79	6.45	5.04	5.43
	3	22.45	36.24	25.25	27.98
	15	176.77	189.74	163.49	176.67
Male	0.5	1.87	4.10	4.02	3.33
	3	13.33	32.74	37.23	27.77
	15	174.46	385.39	332.98	297.61
8 Hours After Start of Light Phase					
Female	0.5	2.37	5.09	2.72	3.39
	3	11.74	21.65	10.18	14.52
	15	39.72	99.93	56.29	65.31
Male	0.5	1.41	2.65	2.53	2.20
	3	12.08	21.82	22.20	18.70
	15	87.15	219.94	136.04	147.71

\* [18] arithmetic means

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**C.2.b. 52-Week Chronic Toxicity Study in Rhesus Monkey**

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Document #(s): BI Document U90-0513  
Upjohn TR 7219-94-065

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Sponsor Volumes: 1.39-1.41

This study complied with GLP

**Summary:**

Pramipexole was administered by gavage at doses of 0, 0.1, 0.5 and 2.0 mg/kg/day to rhesus monkeys (4/sex/dose) for 52 weeks. Few notable drug-related toxicities were apparent, but dosing was limited to 2.0 mg/kg because of drug-induced injurious behavior in the animals during the early phase of the study. The most significant drug-related effect was bradycardia with increased R-R and Q-T intervals recorded during weeks 29/30, 36/37, and 47/48 at 1.5 to 6 hrs post-dose; however, this effect was only observed in mid-dose males. One death, a low-dose female, occurred late in the study; death did not appear to be drug-related. Behavioral changes (agitation, jumping, swinging, gripping) occurred early in the study but diminished over the course of treatment. Body weight and food consumption were not affected by PPX. There were no treatment-related hematological or urinary changes, and only some modest changes in clinical chemistry were noted. Organ weights were not altered and no histopathological findings were attributed to PPX. Plasma concentrations of PPX were measured 2, 4, 6 and 24 hrs after drug treatment during weeks 1, 26, and 50. Monkey plasma concentrations 2 hrs after dosing were approximately 2- (low test dose) to 80-fold (high test dose) higher than the human C<sub>max</sub>, following the projected human PPX maintenance dose of 1.5 mg, t.i.d. (5 ng/ml). Thus, oral administration of mg/kg/day PPX for 52 weeks does not produce significant pathologic effects in monkeys.

**Methods:**

Dosages: 0.1, 0.5, 2.0 mg/kg/day (Batch II)

Low dose is two times the expected human dose (at the time of study initiation). The high dose was selected as the highest tolerable dose based on a range-finding study.

Route of Administration: oral (gastric intubation after feeding)

Species/Number: Rhesus monkeys (16 males, 16 females)

Mean initial weights:

males:                    kg  
females:                 kg

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**Toxicokinetic Analyses:**

Blood was sampled during weeks 1, 26 and 50 at 2, 4, 6 and 24 hrs after drug administration. (Note: The analysis was by an RIA method rather than the HPLC/EC method used in rodent studies.)

**Parameters monitored/Intervals:**

Clinical	-	daily
Body weight	-	weekly
Food consumption	-	weekly
Fecal Occult Blood	-	predose and wks 13, 26, 40 and 52
Hematology	-	predose and wks 6, 13, 26, 39 and 52

- hemoglobin concentration (Hb)
- mean cell volume (MCV)
- red blood cell count (RBC) and derived indices:
  - mean cell hemoglobin (MCH)
  - packed cell volume (PCV)
  - mean cell hemoglobin concentration (MCHC)
- thrombin time (TT)
- prothrombin time (PT)
- partial thromboplastin time (PTT)
- total and differential white blood cell count (WBC)
- reticulocytes
- platelets
- blood sedimentation rate

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With the exception of thrombin time (TT), prothrombin time (PT), and partial thromboplastin time (PTT), all the above parameter were examined on blood collected in EDTA anticoagulant.

Thrombin time (TT), prothrombin time (PT), and partial thromboplastin time (PTT) was determined on blood collected into trisodium citrate (0.11 mol/l, ratio 1:9).

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Clinical chemistry - predose and wks 6, 13, 26, 39 and 52

- glutamate oxalacetate transaminase (GOT) ✓
- glutamate lactate dehydrogenase (GLDH) ✓
- glutamate pyruvate transaminase (GPT) ✓
- alkaline phosphatase (AP) ✓
- leucine aminopeptidase (LAP) ✓
- creatin kinase (CK) ✓
- $\gamma$ -glutamyl transferase ( $\gamma$ -GT) ✓
- sodium ( $\text{Na}^+$ ) ✓
- calcium ( $\text{Ca}^{++}$ ) ✓
- potassium ( $\text{K}^+$ ) ✓
- chloride ( $\text{Cl}^-$ ) ✓
- phosphorus ( $\text{P}^{---}$ ) ✓
- total protein (TP) ✓
- total bilirubin ✓
- urea
- creatinine ✓
- albumin ✓
- albumin/globulin ratio (A/G ratio) ✓
- glucose ✓
- cholesterol ✓
- triglycerides ✓
- butyryl cholinesterase

In addition, protein electrophoresis (albumin, alpha 1, alpha 2, beta, and gamma) was performed.

Urinalysis - predose and wks 6, 13, 26, 39 and 52

The following measurements were performed:

- pH
- volume
- specific gravity

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The following semiquantitative estimations were made:

- protein
- blood
- glucose
- ketones
- bilirubin
- urobilinogen
- reducing substances
- microscopy of centrifuged deposits: epithelial cells, leucocytes, erythrocytes, organic components, casts, and inorganic components

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Plasma Conc - wks 1, 26, 50  
 Ophthalmology - predose and wks 13, 26, 39 and 52  
 ECG, BP - months 7, 9 and 12 (1.5, 3, 6 and 24 hrs after dosing)  
 Myelogram - from sternum  
 Histopathology - on the following tissues:

Samples of the following tissues (with exception of the eyes which were fixed in Davidson's fluid) were preserved in 10 per cent neutral buffered formalin:

- adrenals
- aorta (arch and anterior abdominal)
- bone marrow
- brain (cerebral, cortex, thalamus, midbrain, medulla, cerebellum)
- cecum
- colon
- duodenum
- epididymides
- esophagus
- eyes (and optic nerve)
- gall bladder
- Harder's gland
- heart
- ileum
- jejunum
- kidneys
- liver
- lungs (with mainstem bronchi)
- lymph nodes (mandibular and mesenteric)
- ovaries
- pancreas
- pituitary
- prostate
- rectum

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PROJECT NO. 059-018

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- rib and bone marrow (beyond the requirements of the study protocol)
- salivary gland (submaxillary)
- sciatic nerve
- seminal vesicle
- skeletal muscle
- skin and mammary gland
- spinal cord (cervical)
- spleen
- sternum and bone marrow
- stomach
- testes
- thymus
- thyroids (with parathyroids)
- tongue
- trachea
- urinary bladder
- uterus
- all unusual lesions

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The above tissues were transferred to the study sponsor for further processing and examination.

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Histopathology was conducted by the sponsor.

#### Statistics

Body weight, food consumption and organ weight comparisons were made by one-way ANOVA and Newman-Keuls test for multiple comparisons. Hematology, clinical chemistry, ECG, BP, and organ/body weight ratios were compared by one-way ANOVA based on ranks, and Newman-Keuls test for multiple comparisons. Plasma concentration data were evaluated by ANOVA to assess the dose-proportionality relationship.

#### Results:

**Mortality:** One LDF died on day 364 of study. Signs of a persistent bacterial infection (chronic purulent pericarditis and pleuritis), but no signs of systemic toxicity were present.

#### Clinical Signs:

Dose-related increases in agitation occurred during the first months of the study. The effect was most prominent the night of the first administration as indicated by the presence of wounds caused by thrashing in cages. In the following weeks, the behavioral changes (jumping, swinging, gripping) that started 3-4 hrs after treatment and persisted for several hours diminished, such that during the final months of treatment these behaviors did not occur.

#### Body Weight Gain:

No statistically significant changes were observed (Fig. C.2.b.1).

#### Food Intake:

Occasional statistically significant effects were observed, but no clear drug-related trends (Fig. C.2.b.2).

#### Ophthalmology:

According to the sponsor, no treatment-related ocular changes occurred, but a number of notations appeared in the Pathologists report (i.e., visible cribrosum plate, pale optic disc). These findings were discussed with Dr. Tony Carreras of HFD-540 who concurred with the sponsor's conclusion.

Fig. C.2.b.1

Figure 1

Project No. 059-018

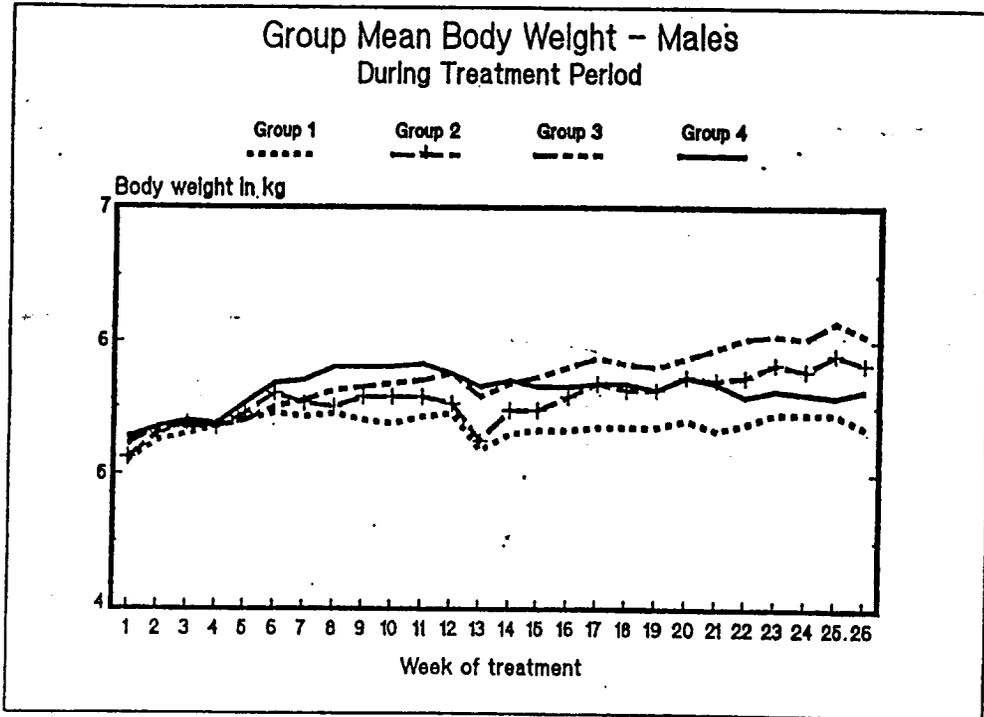
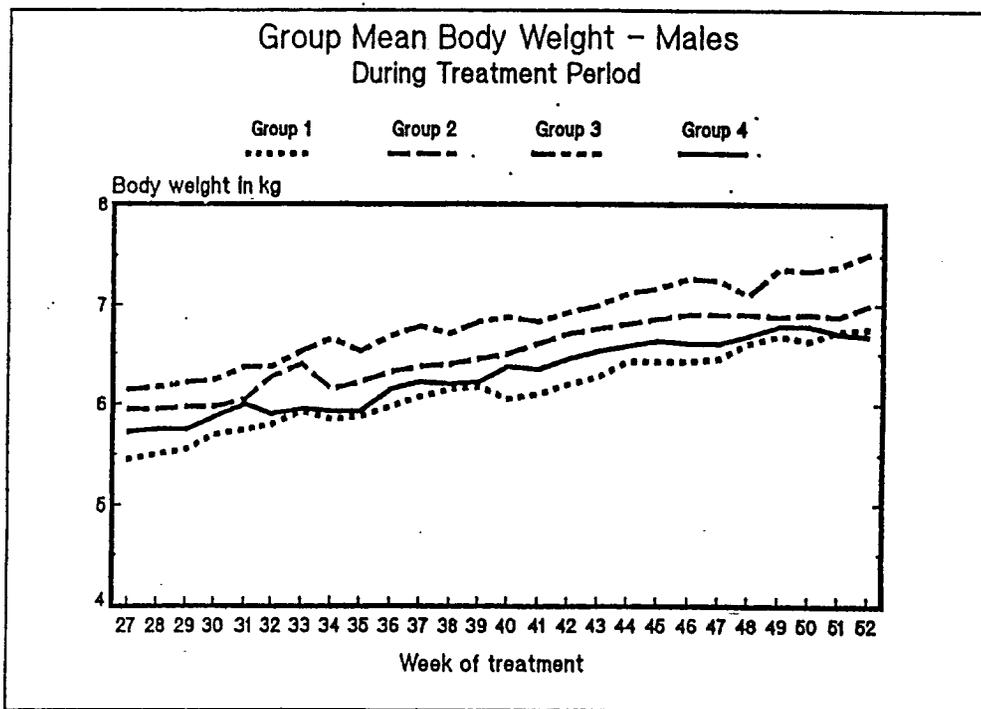


Figure 1 (cont.)

Project No. 059-018



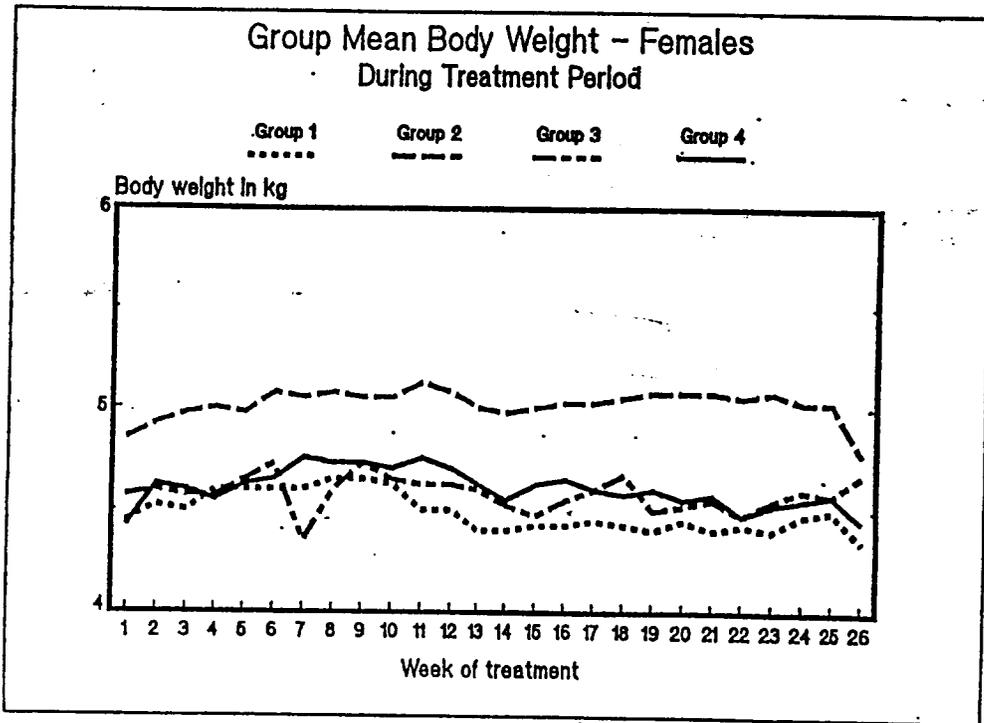
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Figure C.2.b.1 (cont.)

Figure 2

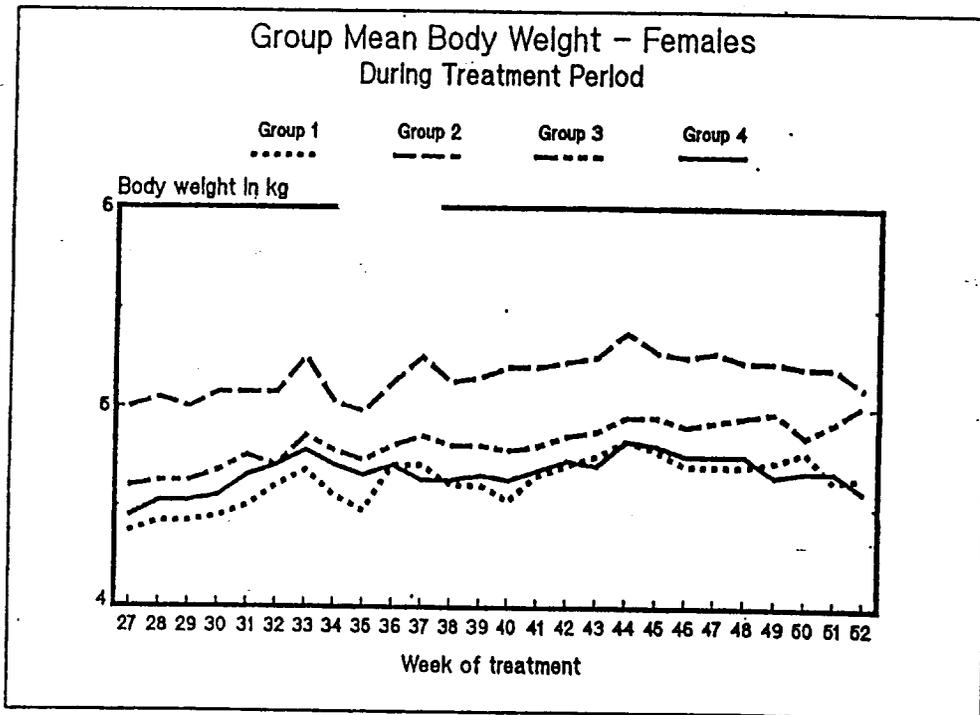
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Figure 2 (cont.)

Project No. 059-018



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Figure C.2.b.2.

Figure 3

Project No. 059-018

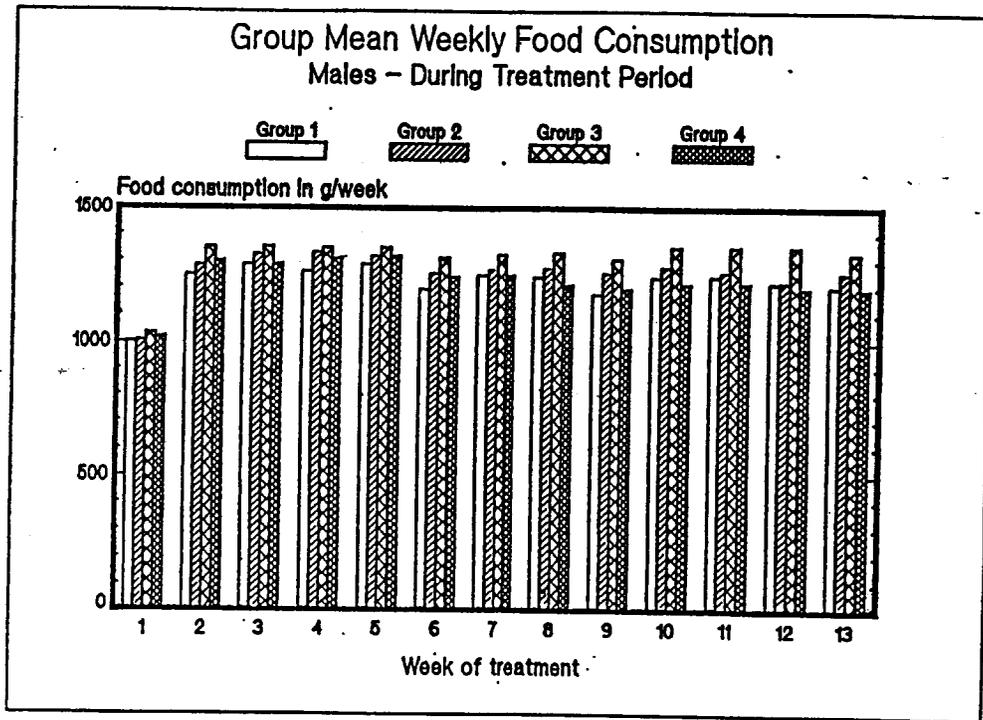
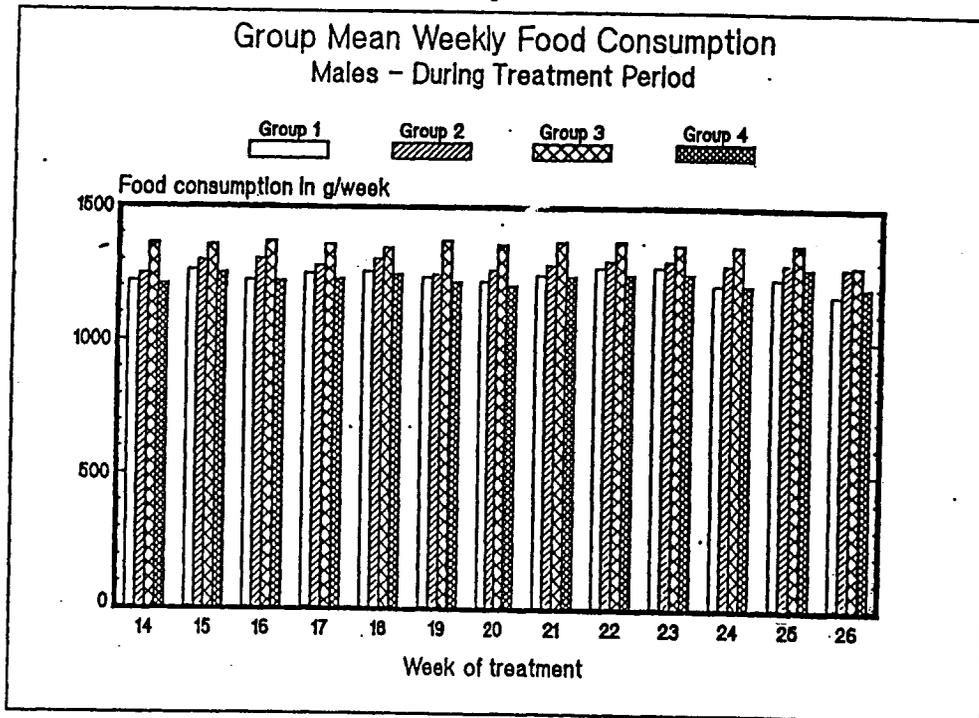


Figure 3 (cont.)

Project No. 059-018



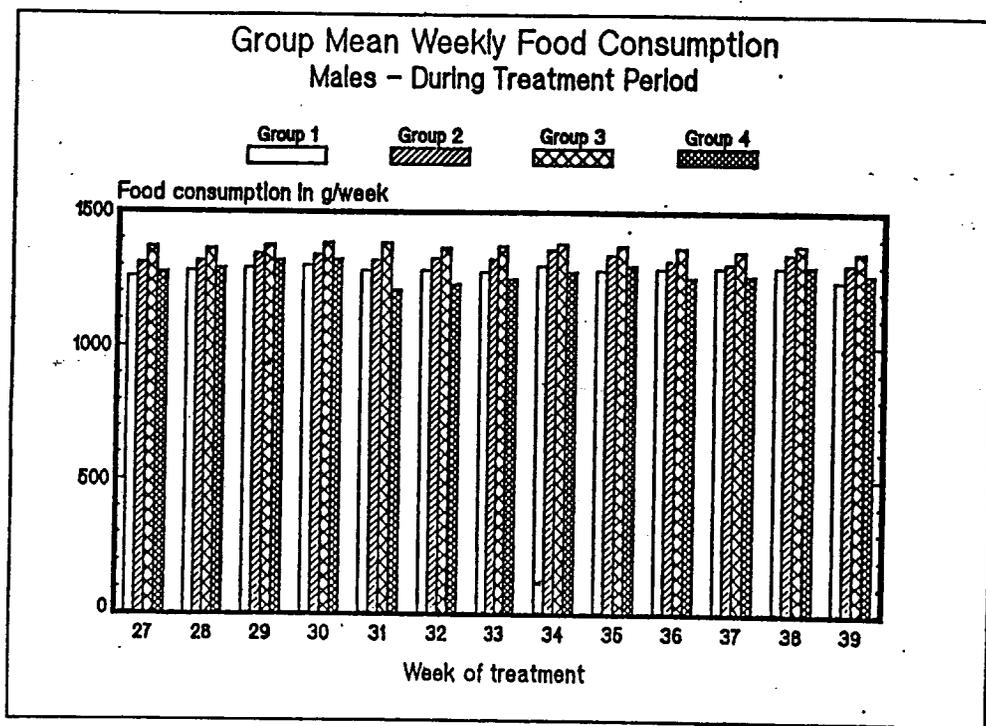
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Fig. C.2.b.2. (cont.)

Figure 3 (cont.)

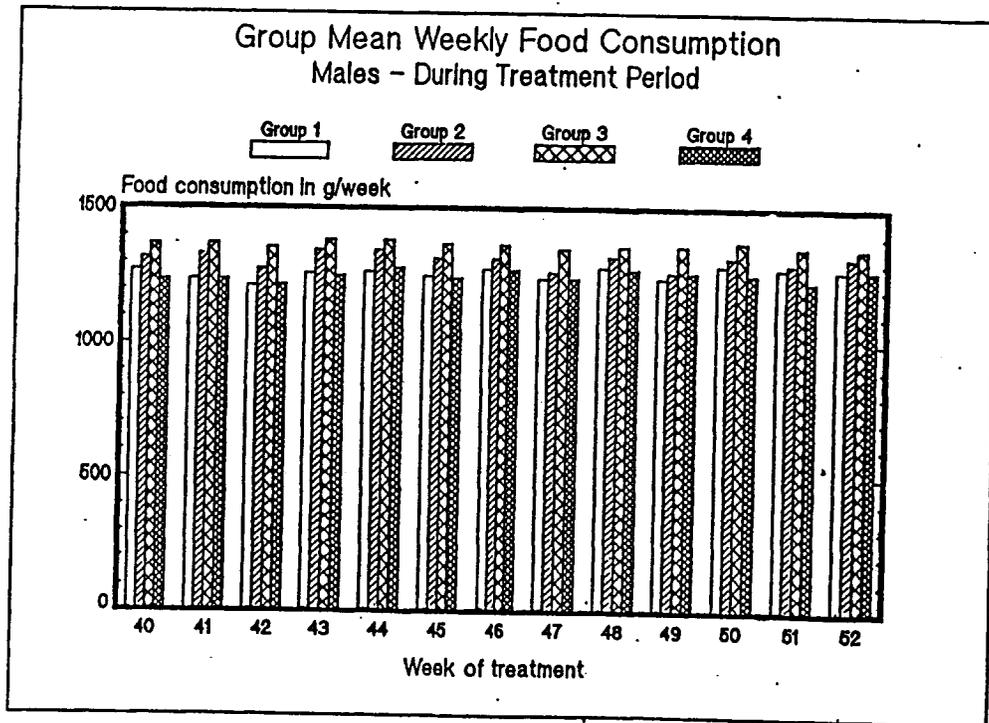
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Figure 3 (cont.)

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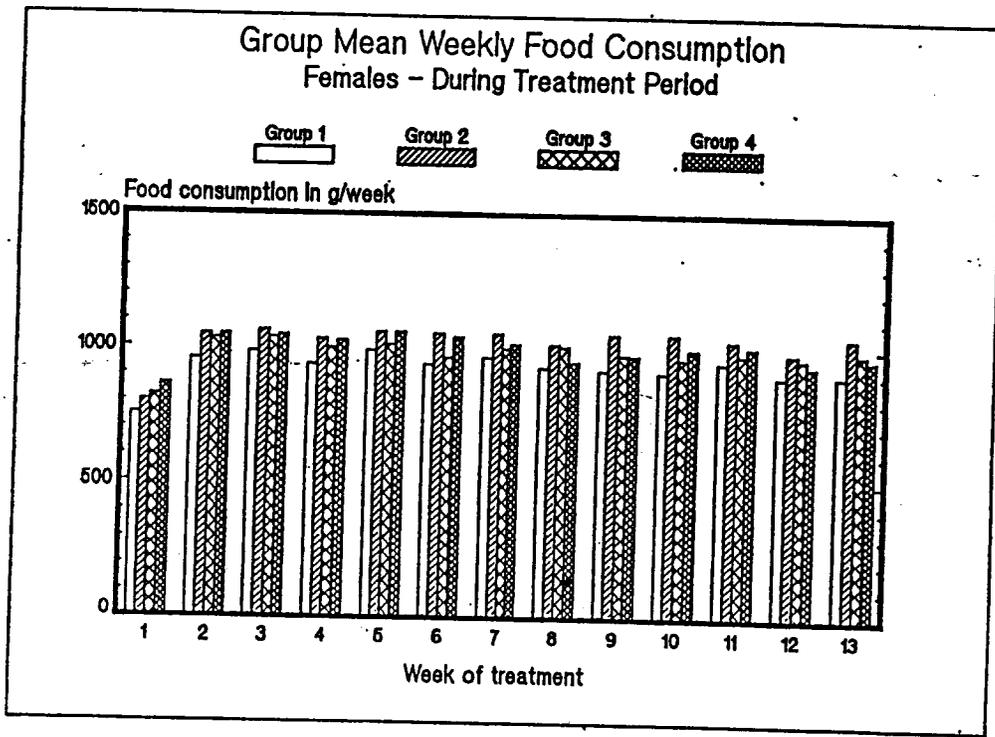


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Figure C.2.b.2. (cont.)

Figure 4

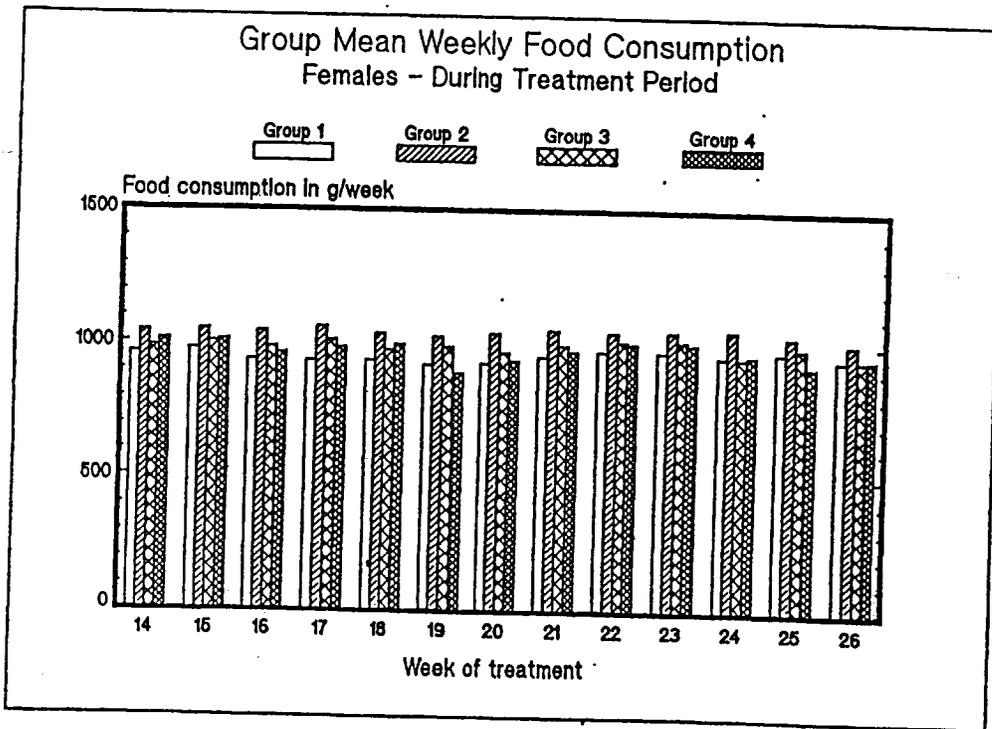
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Figure 4 (cont.)

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Figure C.2.b.2. (cont.)

Figure 4 (cont.)

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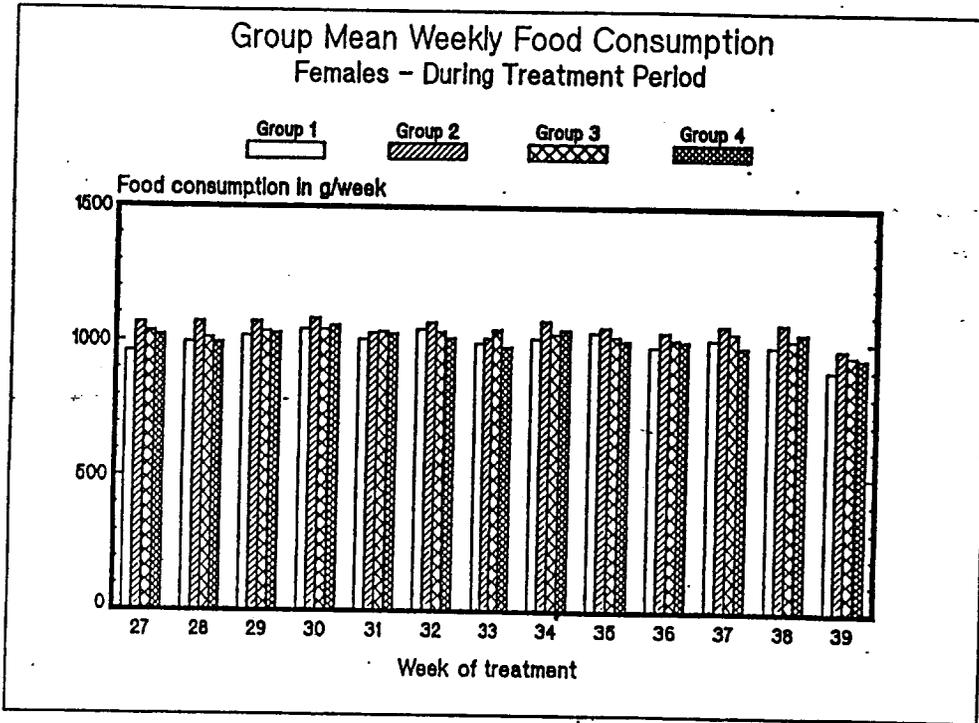
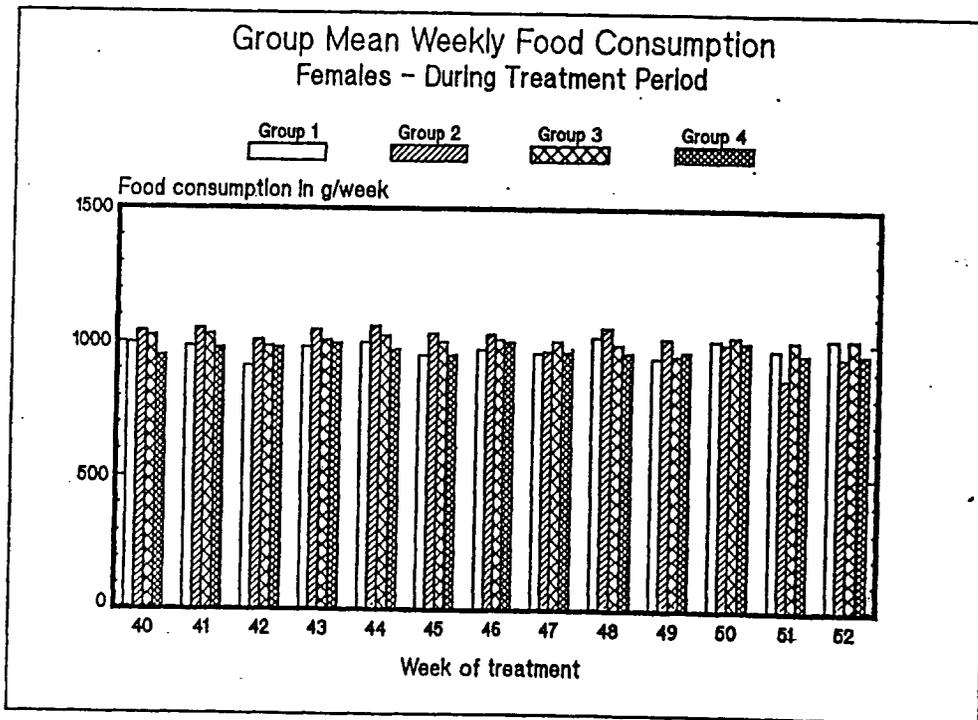


Figure 4 (cont.)

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Hematology:

Group variations:

There were occasional statistically significant events, but no clear drug-related trends and variances were within a normal range.

- increase PT - MDF (wk 39)
- decrease PT - MDF (wk 52)
- increase PTT - LDM (wk 26)  
MDM (wks 6, 13, 26, 39)  
HDM (wks 26, 39)  
MDF (wks 39, 52)

Individual variations:

- decrease RBC (RBC range) - 2 0F (wk 52) APPEARS THIS WAY  
ON ORIGINAL  
1 LDM (wks 26, 52)  
1 MDM (wk 26)  
1 HDM, 1 HDF (wk 52)
- decrease WBC (range = /L) - 2 0F (wk 52)  
1 LDM (wk 0, 26)  
1 LDF (wk 0, 13, 26, 39, 52)  
1 HDM (wk 0, 6, 39)
- increase WBC - 1 LDM (wk 6)
- increase Baso % ( $\geq 4$ ) - 1 MDM (wk 6) APPEARS THIS WAY  
ON ORIGINAL  
1 MDF (wk 6)  
1 HDF (wk 6)
- increase Eos % ( $\geq 10$ -m, 8-f) - 1 LDF (wk 6), 1 LDF (wk 26)
- decrease Lymphos % ( $\leq 25$ ) - 1 0F (wk 26)  
1 LDF (wk 52)  
1 MDM (wk 6)  
1 MDF (wk 6, 26)  
1 MDF (wk 26)  
1 HDF (wk 13)

Clinical Chemistry

Group variations:

There were occasional statistically significant events, but no clear drug-related trends.

Individual variations:

Listed are variations that occurred outside of a reference range (in parentheses) in drug-treated animals only.

increase LDH - 1 HDF (wk 13)  
(3x con & predose)

APPEARS THIS WAY  
ON ORIGINAL

increase AP - 1 LDM (wks 13, 26)  
(2x pre & >1000) - 1 LDF (wk 52)

increase BUN - 4 LDM (wk 52)  
(2x con & predose) 3 MDM (wk 52)  
2 HDM (wk 52)  
(no corresponding increases in creatinine)

APPEARS THIS WAY  
ON ORIGINAL

increase CPK - At week 52, several PPX-treated and control animals had levels that were more than 3x higher than predose levels.

Urinalysis:

Hemoglobin was detected most frequently and in relatively greater amounts in HDF at most time points. However, at week 52 no Hb was detected in samples from this group.

Cardiovascular Measurements:

The most significant cardiovascular effect was bradycardia with a corresponding increase in R-R interval. The effect was most evident in animals of the mid-dose group. During week 29/30, heart rate was reduced at hrs post-dose in MDM, but the effect was statistically significant only at 6 hr. Significant bradycardia occurred at 1.5 hr in MDM during week 36/37 and 47/48, and 6 hr post-dose in MDF during week 36/37.

Significant effects on blood pressure were decreases in systolic, diastolic, and mean arterial pressures at 6 hr postdose in HDF during weeks 29/30, and an increase in mean arterial pressure in LDF at 1.5 hr postdose during weeks 47/48. Significant pressure elevations were detected in females of all dosage groups at 24 hrs postdose in weeks 36/37, but this may have been the result of subnormal pressures in control animals.

Other significant effects on ECG recordings were:

increase Q-T -	MDM, 3 hr	(wk 29/30)
	MDM, 1.5 hr	(wk 36/37)
	L,M&HDM, 1.5 hr	(wk 47/48)
decrease QRS -	HDF, 3 hr	(wk 29/30)
	HDM, 6 hr	(wk 47/48)

Although predose recordings were not taken, the noted cardiovascular effects appeared to be drug-related since readings were normal 24 hrs postdose.

**Organ Weights:**

No clear drug-related changes occurred. There was a nearly significant increase in testes weight in MDM, and a nearly significant decrease in adrenal weights in HDF.

**Histopathology:**

Findings observed only in drug-treated animals were:

testes:	reduced spermiogenesis	-	1 LDM
			2 HDM
epididymis:	reduced sperm number	-	2 LDM
			2 HDM
spleen:	focal fibrosis	-	1 MDM
	reduced follicle size	-	1 LDM

The male reproductive effects were attributed to sexual immaturity.

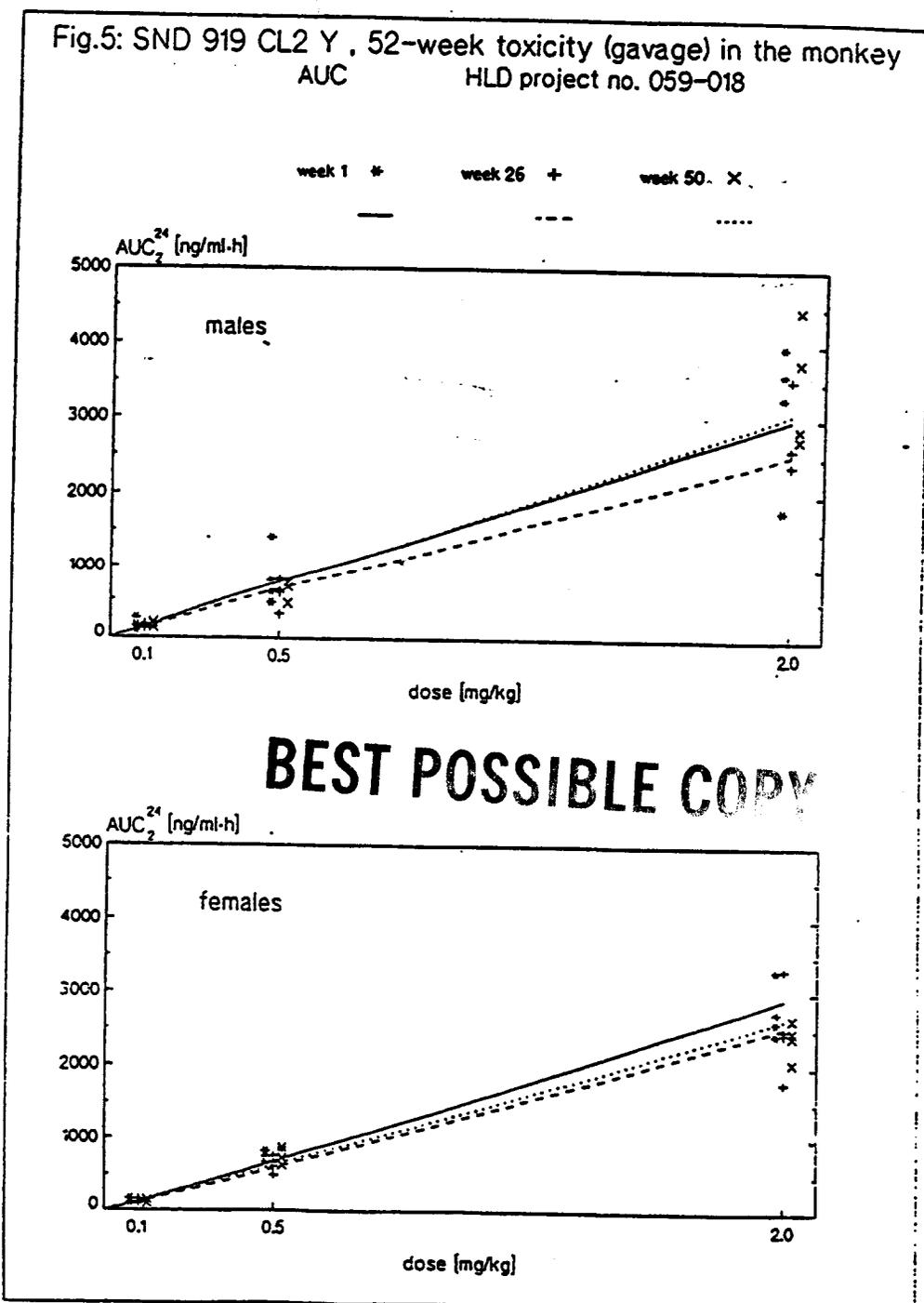
**Myelogram:**

No drug-related changes in the erythropoietic or granulopoietic system were evident.

**Plasma Concentrations:**

PRAM concentrations in monkey plasma were determined by RIA at 2, 4, 6 and 24 hrs postdose during weeks 1, 26 and 50. Increases in plasma concentrations and AUCs were approximately dose-proportional. ANOVA indicated that significantly higher concentrations were present in males at the 6 and 24 hr time points, and also according to AUC. There were no suggestions of drug accumulation (Fig. C.2.b.3, Tab. C.2.b.1).

Figure C.2.b.3



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Tab. ●: 919 CL 2 Y / rhesus monkey / 52-week oral toxicity /  
 project no. 059-018  
 Mean plasma concentrations [ng/ml]

HOURS	SEX	DOSE	WEEK			MEAN
			1	26	50	
2	Female	0.1	12.325	15.525	10.963	12.938
		0.5	60.613	98.746	73.575	77.644
		2	199.100	397.000	235.237	277.112
	MALE	0.1	8.637	19.450	9.250	12.446
		0.5	66.258	85.096	55.213	68.856
		2	209.375	421.800	316.875	316.017
4	FEMALE	0.1	11.212	10.088	9.000	10.100
		0.5	68.563	64.150	63.350	65.354
		2	221.517	275.062	208.700	235.093
	MALE	0.1	12.633	15.075	15.238	14.315
		0.5	66.138	62.100	58.325	62.188
		2	223.000	288.437	297.312	269.583
6	FEMALE	0.1	8.000	6.762	7.112	7.292
		0.5	53.058	37.721	52.338	47.706
		2	202.500	146.562	159.187	169.417
	MALE	0.1	11.450	9.200	11.875	10.842
		0.5	54.183	35.638	43.754	44.525
		2	238.042	161.250	238.538	212.610
24	FEMALE	0.1	0.775	0.637	1.087	0.833
		0.5	3.900	3.238	4.058	3.732
		2	12.563	12.400	18.042	14.335
	MALE	0.1	2.325	1.187	1.212	1.575
		0.5	8.575	3.175	4.262	5.337
		2	14.896	19.904	18.800	17.867

Tab. ●: 919 CL 2 Y / rhesus monkey / 52-week oral toxicity /  
 proj. no. 059-018  
 Mean AUC (2 - 24 hours) data [ng/ml·h]

SEX	DOSE	WEEK			Mean
		1	26	50	
Female	0.1	121.725	109.063	109.875	113.554
	0.5	763.421	633.392	760.175	718.996
	2	2780.195	2524.350	2406.887	2570.478
Male	0.1	169.329	152.287	169.387	163.668
	0.5	817.542	594.246	647.767	686.518
	2	3159.355	2790.312	2466.375	2818.021

△△

Tab. C.2.b.1.

Plasma  
Concs

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AUC