

## D.5 Multiple Species Pharmacokinetic Studies

### D.5.a. Balanced excretion studies and metabolic profile following oral administration of [14C]-SND 919 CL2Y to the mouse, rat, rabbit, dog, monkey and pig.

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Upjohn TR 7256-94-036

Sponsor Volume: 1.59

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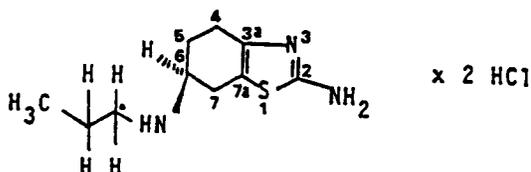
#### Summary:

The excretion and metabolism of [14C]-PPX were evaluated in several species following oral administration. In general between % of administered label was recovered in urine and % in feces. The urinary metabolic profile in mouse, rat, dog, monkey and pig were relatively similar on both a quantitative and qualitative basis. The rabbit had the highest proportion of radioactive metabolites, and a species-specific polar metabolite.

#### Methods:

Substance administered: [14C]-PPX labeled at C1 of the propyl group (Batch 2) or the 2-position of the thiazole ring (Batch 3). Batch 2 was used in all studies except in the rabbit study.

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$C_{10}H_{17}N_3S + 2 HCl$   
\* = labelling with  $^{14}C$

Molecular weight: 284.25 (base)  
357.17 (dihydrochloride)

#### Parameters measured:

Excretion balance (urine, feces) - collect samples at 24 hr intervals for 96 hrs (rat), 168 hrs (mouse, dog, monkey, pig), or 192 hrs (rabbit). Analyze for radioactivity by liquid scintillation spectroscopy.

Metabolic profile - analyzed 0-24 hr urine samples by HPLC with radiometric analysis

Animals:

Species	n (sex)	weight (ca.)	dose (mg/kg, ca.)	dose (MBq, ca.)
mouse	10M, 15F	22 g	0.5	0.09
rat	2M, 2F/dose	208-232 g	0.01, 0.1, 1.4, 10.8 79.1	
rabbit	2M, 2F	2.5 kg	0.5	3.44
dog	4F	kg		
monkey	2M, 2F	kg	0.5	12.95
minipig	2M, 2F		0.5	9.59

\*variation in dog dose due to emesis

Results:

Excretion balance:

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Tab. 1: Mean balances of excretion in % of dose after oral administration  
D.S.a. 1 of  $^{14}\text{C}$ -SND 919 CL 2 Y in 6 animal species

species	N	mg/kg	collection period (h)	urine	feces	sum
mouse	25	0,6		68,42	27,79	96,21
rat	4	0,01		51,87	28,71	80,58
"	4	0,1	"	64,78	38,26	103,04
"	4	1,4	"	48,16	41,15	89,31
"	4	10,8	"	52,14	28,29	80,43
"	4	79,1	"	43,61	25,81	69,42
rabbit	4	0,6		79,25	12,88	92,13
dog*)	4					
monkey	4	0,5		69,07	8,43	77,5
minipig	4	0,5		58,88	12,72	71,5

\*)strong emesis in 3 out of 4 animals

The sponsor suggests that adsorption of [<sup>14</sup>C]-material to surfaces may contribute to low recovery. The fact that the rabbit studies were conducted with material labeled at a different (more metabolically stable) position, and 3 of 4 dogs experienced emesis, hinders species comparisons. In general, % of material was eliminated in the urine, and % was excreted in the feces.

Metabolic Profile:

Generally similar chromatographic profiles are obtained with hr urine specimens from the rat, mouse, dog, minipig, and monkey in that a primary peak, identified as the parent compound, and several are present. However, the major peak in the rat sample elutes approximately 3 min after the major peaks in samples from other species. The sponsor did not indicate how the major peak was identified as pramipexole, or the reason for this discrepancy in elution times for the major peaks.

The chromatograms of the rabbit 0-24 hr urine samples were markedly different from other species examined (e.g. Fig. D.5.a.1; comparison with rat). The largest peak in most of the rabbit chromatograms is not PPX, but appeared much earlier in the elution profile (9-10 min). In addition, numerous smaller peaks were present in these chromatograms. The variation in the position of the radiolabel on the PPX moiety (i.e., ring-labeled material used in the rabbit studies versus side-chain-labeled material in other species) did not appear to contribute to the differences in metabolic profile.

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**D.5.b. Studies on the metabolism of pramipexole including experiments to detect a potential metabolic inversion at the optical active center of the molecule.**

Document #(s): BI Document U95-0494  
Upjohn TR 7256-95-057

Sponsor Volume: 1.60

**Summary:**

These studies were conducted to chromatographically separate and purify pramipexole metabolites in rat and rabbit urine, and determine the propensity for chiral inversion of PPX in rat and human.

**Methods:**

Substance administered: [<sup>14</sup>C]-pramipexole (batch and position of label not identified)

Animals/Dosing: according to the scheme:

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running no.	species	subjects N	treatment			
			dose	regimen	route	MBq/subject
1	rat	3	12 mg/kg	single dose	oral	1.6737
2	rat	2	0.5 mg/kg	single dose	oral	0.743
"	"	2	"	"	"	0.743
3	rabbit	1	1 mg/kg	single dose	oral	3.66
4	rabbit	1	1 mg/kg	single dose	oral	13.16
5	man *)		1.5 mg			
"	(male, subj. 2)	1	t.i.d.	steady state	oral	—
"	man *)		1.5 mg			
"	(female, subj. 9)	1	t.i.d.	steady state	oral	—

\*) samples from study M/27300047, ref. [6]

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Note: the dose in rabbit study is in conflict with the dose stated in the text (0.5 mg/kg)

**Experimental**

Procedures: Samples were subjected to appropriate extraction techniques then analyzed by various methods for metabolite identification

Enantioselective chromatographic techniques were used to determine the occurrence of chiral inversion from the (6S)-(-) configuration to the (6R)-(+ configuration).

**Results:**

Metabolite Identification:

Rabbit urine: 6 chromatographic peaks were separated and tentatively identified.

- M1 - may be a labile conjugate
- M2 - N-dealkylated metabolite
- M3,M4 - hydroxylated metabolites (4 and 5 position of the thiazole ring)
- M5 - parent compound
- M6 - glucuronic acid conjugate

Rat urine: 2 chromatographic peaks were separated and tentatively identified.

- M2 - N-dealkylated metabolite
- M3,M4 - hydroxylated metabolites (4 and 5 position of the thiazole ring)

The "species-specific" polar metabolite suggested in the preceding study was identified as M1. The proportion of total metabolites, M1, and PPX in rat and rabbit urine were as follows:

The proportions of total metabolites, M1 and parent compound in the urines used for metabolite isolation were in % of <sup>14</sup>C activity registered:

species	figure	Total metabolites	Metabolite M 1	Pramipexole
rabbit	1	70.5	12.4	29.5
rabbit	3	96.1	42.6	3.9
rat	4			

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The proposed metabolic pathways are shown in Fig. D.5.b.1.

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Chiral Inversion Studies:

In rat and human urine, very minor peaks that coeluted with the (6R)-(+)  
enantiomer of pramipexole were detected ( $\leq 3\%$  total peak area; Tab. D.5.b.1).

Table ● D.5.b.1

Evaluation of data from stereoselective chromatography of extracts of rat  
urines

Estimation of apparent SND 919 R(+) concentration

measured/calculated:	dimension	rat no1	rat no.2	rat no. 3
concentration				
pramipexole	$\mu\text{g/ml}$	17.935	15.391	41.655
	%	100	100	100
R(+) region standard (background)	dpm/region	484	484	484
R(+) region in vivo urine	dpm/region	685	427	761
R(+) region (net)	dpm/region	201	0	277
S(-) region				
(no background correction possible)	dpm/region	53761	44070	65153
sum: R(+) + S(-)	dpm/2 regions	53962	44070	65430
apparent SND 919 R(+)/R(+) region *)	ng/region	4.8	0	6.6
apparent SND 919 R(+)/ml urine	ng/ml	66.8	0	176
apparent SND 919 R(+) as % of pramipexole concentration	%	0.4	0	0.4

\*) specific activity: 42.371 dpm/ng ; f=0.0236 ng/dpm

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Table ●

Stereoselective separation of extracts from human urine after oral steady  
state administration of 1.5 mg t.i.d. pramipexole (SND 919 S(-) CL2)

subject/ gender	fraction [hours]	pramipexole [ng/ml]	region1 SND 919 R(+)	region2 SND 919 S(-)	ratio [%]
2 (male)	0-4	1860	3962	234497	1.7
	4-8	1270	3273	169776	1.9
	8-12	472	1598	67211	2.4
	intercept *)	8-12	472	491 *)	76575 *)
9 (female)	0-4	899	3619	122147	3.0
	4-8	551	2188	76818	2.9
	8-12	811	1080	119556	0.9
	intercept *)	8-12	811	390 *)	132950 *)

\*)The areas given result from the the linear regression of peak area data of the spiking  
experiments in Figure 20 (peak area ratio vs. ng/ml SND919 R(+) added).  
They were calculated using the intercept of the regression line and a mean value (N=5  
injections) of the SND 919 S(-) peak areas

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## SUMMARY

### Pharmacodynamics

Degeneration of dopaminergic neurons in the substantia nigra is widely accepted as the neuropathological basis of Parkinson's disease (PD). The clinical utility of the dopamine (DA) precursor L-DOPA and dopamine receptor agonists in PD therapy supports this mechanism. However, currently available PD therapies have clinical drawbacks (e.g., on-off fluctuations, delusional side effects) that limit their utility.

Pramipexole (PPX) is a selective dopamine receptor agonist being developed as a replacement therapy for PD. PPX is distinguished from its predecessors by its receptor binding profile: In contrast to other therapeutic DA agonists such as pergolide (Permax) and bromocriptine (Parlodel), PPX preferentially binds to D<sub>3</sub> rather than D<sub>2</sub> receptors when both subtypes are expressed in their agonist conformations. The therapeutic significance of D<sub>3</sub> receptor selectivity is not readily apparent since the role of this subtype in PD is not well-established. However, the sponsor suggests that because of the high D<sub>3</sub> receptor density in mesolimbic regions associated with reward and motivation, PPX may alleviate depression that is often co-morbid with PD. PPX is also distinguished from Permax and Parlodel since the latter compounds have relatively high affinities for 5-HT receptor subtypes, whereas the only non-dopaminergic receptor activity of PPX was detected at  $\alpha_2$  receptors ( $K_i = 250$  nM).

*In vivo* dopaminergic agonist activity of PPX was demonstrated in behavioral, neurochemical and electrophysiological studies. In contrast to the usual locomotor stimulation observed with D<sub>2</sub> agonists and high doses of PPX, low doses of PPX decreased locomotor activity in mice. The dose-dependence may be the result of pre- versus post-synaptic receptor sensitivity. Neurochemical evidence of presynaptic D<sub>2</sub> autoreceptor activity of PPX was a decrease in DA synthesis in striatum and limbic system at doses less than 0.1 mg/kg, s.c., and a decrease in dopamine release in *in vivo* microdialysis studies. In electrophysiology studies, PPX decreased the firing rate of nigrostriatal (SNPC) and mesolimbic (VTA) neurons presumably due to activation of D<sub>2</sub> autoreceptors, but stimulated the firing of postsynaptic anterior caudate neurons, possibly via D<sub>3</sub> receptor activation.

Experimental evidence for the proposed utility of PPX in PD was obtained in the standard animal models. In 6-hydroxydopamine (6-OHDA)-lesioned rats, PPX was approximately equipotent to apomorphine ( $ED_{50} = 0.026$  mg/kg, s.c.) at inducing contralateral turning, but had a longer duration of action. In the MPTP-induced Parkinson's disease model in primates, PPX (0.075 mg/kg, p.o.) reversed parkinsonian symptoms for 5-24 hr. By comparison, the combination of L-DOPA/carbidopa (15 mg/kg, p.o.) was effective for only 2 hrs. Bromocriptine (2 mg/kg) was relatively ineffective in these studies.

Potential neuroprotective effects of PPX were evaluated in three preclinical models of neurodegeneration. Multiple doses of PPX (1 mg/kg, p.o.) beginning shortly after insult prevented degeneration of putative dopaminergic neurons following transient forebrain

ischemia in gerbils or high doses of methamphetamine to mice. PPX also prevented the loss of TH-positive cells due to micromolar concentrations of L-DOPA in primary cultures of rostral mesencephalic tegmentum cells. While these studies are interesting from a mechanistic standpoint and may be considered preliminary evidence of neuroprotection, they are not sufficient to support the sponsor's proposed labeling claim that pramipexole "reduces dopamine-induced neuronal degeneration."

### **Safety Pharmacology**

The safety pharmacology studies of PPX revealed relatively few safety concerns. The most significant issue arose from early clinical studies where hypotensive effects of PPX were identified. Because of concerns that combining PPX with other PD drugs may potentiate this effect, the cardiovascular effects of PPX in combination with Sinemet and Eldepryl were evaluated in rhesus monkeys. The modest bradycardia and blood pressures decreases produced by PPX (0.05 mg/kg, p.o.) were not potentiated by either Sinemet (100 mg/kg L-DOPA/10 mg/kg carbidopa, p.o.) or Eldepryl (0.2 mg/kg, p.o.). Other notable effects of PPX in safety studies were sedation in monkeys, sleep suppression in cats and rats, and emesis in dogs. PPX doses of \_\_\_\_\_ mg/kg lowered basal plasma prolactin levels in male rats. Similar effects of PPX were demonstrated as part of the chronic toxicology studies because of the speculated role of prolactin inhibition in some of the observed histopathological and reproductive changes.

### **Toxicology**

#### Acute Studies

The acute toxicity of PPX was evaluated in mice, rats and dogs following oral and intravenous administration. Animals were observed for 14 days. In rodents, signs of toxicity were exophthalmus, piloerection, irregular breathing, and tremors/convulsions. The ratios of LD<sub>50</sub>s by the p.o. and i.v. routes were approximately 10 in mice (p.o. = 1700, i.v. = 169) and greater than four in rats (p.o. > 800, i.v. = 210). The most prominent toxicity in dogs was emesis at doses of \_\_\_\_\_ mg/kg, i.v., and \_\_\_\_\_ mg/kg, p.o.

#### Chronic Studies

The toxicity and toxicokinetics of PPX administration for one year were evaluated in rats at doses of 0, 0.5, 3.0 and 15.0 mg/kg. The lowest test dose resulted in slight behavioral activation and decreased body weight gain in both sexes. Decreases in cholesterol and triglycerides occurred at all doses in females. Other sporadic clinical chemistry changes were modest elevations in transaminases, alkaline phosphatase and urea, and decreases in serum potassium at the mid and high doses; these were generally more frequent in females than in males. Hematological changes were slight thrombocytopenia (MD, HD) and slight-to-moderate increases in the granulocyte/lymphocyte ratio in females. 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 changes in the glandular epithelial pattern of mammary tissue were also evident in MDF and HDF. The female reproductive changes were attributed to inhibition of prolactin secretion. Eleven animals (4 controls, 7 PPX-treated) were identified with tumors, none of which could be clearly attributed to PPX treatment. The decreases in serum cholesterol, shift in granulocyte/lymphocyte ratios, and ovarian changes were consistent with observations in previous subchronic rat studies

Rat plasma concentrations at the "No Toxic Effect" dose level of 0.5 mg/kg are near the anticipated steady-state C<sub>max</sub> in humans (ng/ml).

The toxicity and toxicokinetics of orally administered PPX (0, 0.1, 0.5 and 2.0 mg/kg/day) for one year were evaluated in rhesus monkeys. Prominent clinical signs of toxicity were behavioral changes (agitation, jumping, swinging, gripping) that diminished over the course of treatment. Body weight and food consumption were not affected by PPX. 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 at various times during the study; however, this effect was only observed in mid-dose males. No treatment-related hematological or urinary changes were evident, and only some modest changes in clinical chemistry occurred. Organ weights were not altered and no histopathological findings were attributed to PPX. One death, a low-dose female, occurred late in the study and did not appear to be drug-related. Plasma concentrations of PPX were approximately 2- (low test dose) to 80-fold (high test dose) higher than the anticipated human steady-state C<sub>max</sub>. Thus, oral administration of mg/kg/day PPX for 52 weeks does not produce significant pathologic effects in monkeys. PPX was also devoid of toxicities in 4-week intravenous toxicity study at doses up to 0.6 mg/kg.

### Reproductive Toxicology

In a rat Segment I study, PPX (mg/kg, p.o.) was given to males for 70 days prior to mating, and females for 14 days prior to mating through gestation (C-section on day 22) or weaning (spontaneous delivery group). The most notable reproductive toxicities in the HD dams were prolongation of estrus in HDF, and reductions in the number of implantations, pregnant females and successful deliveries. Body weight development was impaired in pups of MD and HD dams, possibly because of reduced suckling opportunities or inhibition of prolactin secretion by PPX. Teratogenic effects of pramipexole were not evident, although the data were limited by the low number of evaluable pups. A follow-up Segment I study was conducted which identified PPX-treated females rather than males as the source of infertility. These findings were expected since PPX, like other dopamine agonists, inhibits secretion of prolactin which is necessary for the maintenance of rat pregnancy.

In the rat Segment II study, PPX (mg/kg, p.o.) was administered from day 7 to 16 of gestation. Dams were either sacrificed on day 22 for delivery of pups by Caesarean section, or allowed to raise the pups to weaning (21 days). Significant embryoletality

occurred with the high dose as only 7 of 32 pregnant females had viable offspring. The other 25 pregnancies were classified as complete "early" resorptions. Abnormalities identified in fetuses of drug-treated dams included one case each of anal atresia (LD), sirenomelia and gastroschisis (LD), and a cleft vertebra (MD). Teratology information from the high-dose group was limited by the low number of evaluable pups. Body weight development was not impaired in this study, which suggests that PPX administration during lactation is responsible for this effect.

In contrast to the rat Segment II study, oral administration of PPX (0.1, 1.0, 10 mg/kg) to rabbits from day 6 to 18 of gestation did not produce any embryotoxic, fetotoxic or teratogenic effects.

In a Segment III study, oral administration of PPX (0, 0.1, 0.5 and 1.5 mg/kg/day) to female rats from day 16 of gestation to day 21 of the rearing phase (lactation) caused an impairment of pup development as indicated by a decreased body weight gain during the lactation/rearing phase. As in the Segment I study, reduced suckling opportunities or impaired milk production in the dams may underlie this effect. Fertility of the F<sub>1</sub> offspring was not impaired. No other remarkable drug-related changes in litter parameters or at necropsy of the pups were evident.

Taken together, these data suggest that PPX has toxicological consequences on reproductive and developmental parameters in rats but not rabbits. The prolongation of estrus, impairment of female fertility and early resorptions are likely related to the D<sub>2</sub> receptor-mediated suppression of prolactin, a hormone necessary for the maintenance of rat pregnancy, and may not necessarily be a great human concern. However, a significant consequence of this effect is a low number of evaluable pups from dams treated with the highest dose of PPX (2.5 mg/kg in Segment I study, 1.5 mg/kg in Segment II study), which drastically limits conclusions on the teratogenicity of PPX. Although the Segment III study produced a relatively large sample of pups with no drug-related effects on skeletal, visceral or external abnormalities, these animals were not exposed to drug until after the critical period of organogenesis. Since some relatively rare fetal abnormalities were present in PPX-treated pups in the Segment I and II studies (anal atresia, sireniiform malformation and gastroschisis, and cleft vertebra), teratogenic effects of PPX cannot be discounted in the absence of data from a larger pup population.

The impairment of body weight development in the Segment III study is clearly a PPX-related effect. The sponsor suggests as a basis for this finding that pups are inadequately nursed because of behavioral activation in the dams. Inadequate milk production due to insufficient prolactin levels in the dams is an alternative possibility. However, a direct effect of PPX on pup development cannot be discounted.

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### Genotoxicity

PPX was tested for mutagenic effects in four standard *in vitro* assays (Ames test, SHE cell transformation assay, chromosomal aberrations in CHO cells, V79 gene mutation assay) and the *in vivo* mouse micronucleus test. The testing procedures were generally adequate; where deficiencies existed (i.e., SHE cell assay, micronucleus test), further testing will not be recommended.

No reproducible mutagenic responses to PPX were observed. The only positive signal in any assay was a small, non-reproducible clastogenic effect with S9 activation at the highest test dose (3300 µg/ml) in the CHO chromosomal aberration study.

### Carcinogenicity

In the two-year mouse study, no neoplastic and few non-neoplastic lesions were associated with PPX administration (0.3, 2.0 and 10 mg/kg/day in diet). However, the validity of this study as an assessment of the tumorigenicity of PPX is questionable because of the marked impairment of body weight development ( ) at the intermediate and high test dosages. Plasma exposures at the lowest test level (equal to or less than expected human exposures) were not considered adequate for valid risk assessment.

The rate of premature decedents was higher in PPX-treated animals, primarily because of unscheduled sacrifices due to eczema; a sufficient number of survivors remained at termination. Drug-related clinical observations were increased spontaneous activity at the mid and high dose levels. No statistically significant increases or trends for increases in the incidence of neoplastic lesions in drug-treated animals were apparent according to the sponsor's analysis. A pooled analysis of all mesenchymal/epithelial uterine neoplasms was not presented, although a possible dose-related positive trend was noted (controls: 10%; LD: 10%; MD: 14%; HD: 16%). Unusual histopathological findings in PPX-treated animals were fibro-osseous proliferative lesions in the femurs of females (all dosage groups). This lesion occurred at a relatively high rate in control females (28%), but approximately doubled in incidence in treated animals ( ); similar at the three dosage levels). This type of lesion is known to occur spontaneously in female mice. However, administration of the prostaglandin E analogue misoprostol has also been associated with this lesion in mice, and the issue is addressed in the labeling of that product. A hormonal basis for this effect is suggested, but no experimental evidence was presented. A similar lesion was not observed in long-term studies of PPX in rats or monkeys.

As in the mouse study, a marked impairment of body weight gain in MDF and HDF prevents conclusions regarding the tumorigenicity of PPX (0.3, 2.0 and 8.0 mg/kg/day) in female rats. Body weight gain reductions in males were less than 10%. Mortality occurred in all treatment groups at various times during the study, but no clear drug relationship was evident. An adequate number of survivors remained at termination. The only tumors found at a statistically significant greater incidence in PPX-treated animals were Leydig cell adenomas in MD (44%) and HD (44%) males. A relatively high incidence of these tumors also occurred in control animals (Group 0: 26%, Group 4: 18%). Leydig cell hyperplasia was also increased in MD and HD rats. Changes in females were enlarged corpora lutea (HD

rats), uterine lesions and hemorrhage (MD and HD), alterations in mammary gland patterns from female-like to male/female-like (MD and HD), and diffuse hepatocellular fatty changes (MD and HD). Retinal degeneration occurred in MD and HD males and females.

The proposed basis for the neoplastic and nonneoplastic lesions in reproductive and endocrine structures is PPX-induced inhibition of prolactin secretion, which was demonstrated at week 60 and 69 (ca. 10-fold decrease in females, 100-fold decrease in males). Reductions in serum prolactin in males purportedly trigger an elevation in LH production and release leading to the Leydig cell adenomas and hyperplasia. Direct evidence for a PPX effect on serum LH was not provided. Nonetheless, the finding is suggested to be of questionable relevance to humans given the high background incidence of this tumor in rats (as demonstrated in this experiment), and since several widely-used compounds also produce Leydig cell tumors in rats but are not known to do so in humans (cimetidine, hydralazine, vidarabine, israpidine). The female reproductive changes (corpora lutea enlargement, uterine lesions, and mammary gland pattern changes) were also observed in the one year rat study. The sponsor contends that the potential human relevance of these findings is questionable because of the divergent influences of prolactin on female hormone levels in rats and humans. However, the effect of PPX on estrogen and progesterone was not shown in any study. Thus, the proposed mechanisms for the pathological changes in rodents are plausible and supported in the literature by studies with other dopamine agonists, but direct support for these mechanisms specifically in the case of PPX is not present in this submission.

Retinal degeneration was the most notable non-neoplastic finding of this study. Follow-up studies to address this issue and were independently reviewed by an FDA consultant (Dr. Tim O'Neill). Briefly, the sponsor has provided evidence that treatment with PPX (25 mg/kg, p.o.) for 13 weeks in combination with constant light exposure produces retinal degeneration in non-pigmented albino rats, but not in pigmented Brown-Norway rats. Retinal degeneration was not observed after long-term treatment of minipigs and monkeys. Thus, pigmentation may protect against the retinotoxic effects of PPX. The proposed mechanism for this effect is inhibition of retinal disk-shedding, which occurs universally in vertebrates. This leaves open the possibility that a similar effect could occur in humans.

#### Pharmacokinetics/ADME

The absorption, distribution, metabolism, and excretion of pramipexole were evaluated in several species (mouse, rat, rabbit, monkey, human, dog, and minipig) following oral and intravenous administration of single or multiple doses. Only the single dose pharmacokinetic studies conducted in species used for animal toxicology (rat, monkey, rabbit) studies are reviewed here. Human studies are included for comparative purposes. Toxicokinetic studies were reviewed as part of the main study review.

The analytical methodologies included the use of radiolabeled [ $^{14}\text{C}$ ]-PPX, HPLC with electrochemical detection, radioimmunoassay, or GC with chemical ionization mass spectroscopy. The former two methods were used for the majority of analyses, and provided adequate sensitivity.

In most test species, PPX is absorbed rapidly and nearly completely following oral administration. Peak plasma concentrations are generally reached within 2 hrs of treatment, except for the rabbit ( $t_{max} = 6$  hrs.). The calculated bioavailabilities in all species exceeded 69%, and in humans this value was greater than 90%. After intravenous administration, the systemic clearance of PPX was high, and terminal half-lives were relatively short (<4 hrs), except for rabbits and humans ( $t_{1/2} =$  hr). The excretion and metabolism of [ $^{14}C$ ]-PPX were evaluated in several species following oral administration. In general, between % of administered label was recovered in urine and % in feces. Renal excretion of PPX is the primary route of elimination in humans (ca. 90%;  $Cl_{ren} = 409$  ml/min). Except for the rabbit, the major urinary fraction is unchanged parent compound. Significant biliary excretion of radioactivity was detected in cannulated rats. The plasma concentration:time profile of PPX in monkeys suggested enterohepatic circulation of the drug. The degree of biotransformation is low to moderate in most species, and the urinary metabolic profile in mouse, rat, dog, monkey and pig were relatively similar. Biotransformation in rabbits differed from the other species on both a qualitative and quantitative basis. The rabbit had the highest proportion of radioactive metabolites, and a species-specific polar metabolite. Plasma protein binding of PPX was relatively low in all species (dog, pig, monkey, man). PPX did not undergo chiral inversion *in vivo* in rats or humans.

The tissue distribution of PPX was evaluated in rat. A similar pattern was observed following i.v. or oral administration with highest concentrations detected in the lacrimal, salivary, and adrenal glands, kidney, pancreas, bone, liver and lung. At 24 hrs, radioactivity was still present in liver, kidney, and adrenals. Brain levels of radioactivity peaked at 2 hrs after i.v. PPX, and were detectable for up to 6 hrs. In a steady-state pharmacokinetic study of PPX, the highest levels of PPX were observed in brain tissue when compared to plasma and CSF. In a study of pregnant Wistar rats, [ $^{14}C$ ]-PPX and/or its metabolites rapidly crossed the blood:placental and blood:brain barriers after i.v. or oral administration (1.0 mg/kg). PPX Concentrations of label tended to be higher in the placenta than in maternal blood or in the fetus. Highest fetal tissue concentrations of radiolabel were detected in the liver. In both dams and fetuses, brain levels were higher than plasma levels for up to 6 hrs postdose. In lactating rats, [ $^{14}C$ ]-PPX and/or its metabolites were excreted into rat milk following oral administration of 0.5 mg/kg; higher levels of radioactivity were detected in milk than in plasma. Most of the radioactivity appeared to be metabolites of pramipexole.

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## EVALUATION

The following issues were identified in nonclinical studies as pertinent to the human use and labeling of pramipexole:

### 1. Carcinogenicity/Mutagenicity:

The carcinogenicity study in mice cannot be considered adequate for risk assessment purposes because of the marked impairment of body weight development (37-45%) at the intermediate (2.0 mg/kg/day) and high (10.0 mg/kg/day) dose levels, and inadequate plasma levels at the lowest test dose (0.3 mg/kg/day). Because of the potent effects of PPX on body weight development, it is likely that similar difficulties with study interpretation would be encountered at doses levels between 0.3 and 2.0 mg/kg. Thus, additional studies will not be recommended. The Carcinogenicity Assessment Committee will be consulted for a resolution.

A similar problem was encountered in the carcinogenicity study of female rats where body weight gain was reduced by % at the intermediate and high dose levels. In males, which received adequate exposures based on toxicity and plasma level determinations, the only tumor that occurred at clearly higher incidence in PPX-treated animals were Leydig cell adenomas (CON: 22%, 0.3 mg/kg: 34%, 2 mg/kg: 44%, 10 mg/kg: 44%). PPX did not appear to reduce the latency of tumor appearance. Because of the high background incidence of this tumor type in rats, and the possibility that species-specific hormonal mechanisms may be involved in their appearance, the significance of these findings in humans is questionable. The sponsor has included a description of the findings in the proposed labeling, which is an acceptable means of addressing this issue.

PPX did not cause significant mutagenicity in an appropriate test battery at acceptable concentrations.

### 2. Reproductive Toxicology

The reproductive toxicology studies do not adequately support the sponsor's proposed labeling of PPX in Pregnancy Category B. Three major concerns arose from the studies that are incongruent with the proposed labeling statements:

- a. PPX is clearly embryotoxic in rats at doses of mg/kg/day
- b. Because of embryotoxicity, teratology information is severely limited by the low number of evaluable pups from HD dams
- c. A developmental parameter, body weight gain, was significantly impaired in pups from HD dams in both Segment I and III studies

The data that forms the basis for statements a & b can be summarized as follows:

Study	Delivery	# Dams w/ Viable Pups		# Viable Pups/Group	
		Other Groups	HD Group	Other Groups	HD Group
Seg I <sup>a</sup>	C-Section	35/36	1/12	146-174	10
	Spontaneous	34/34	5/12	158-160	75
Seg I <sup>b</sup>	C-Section	46/48	6/48	308-330	31-53
Seg II	C-Section	58/72	4/24	222-250	51
	Spontaneous	32/36	3/11	133-138	39

Other Groups = controls, LD and MD combined; <sup>a</sup> = initial study, <sup>b</sup> = follow-up study

The impairment of rat pregnancy (i.e., low number of implantations, high number of early resorptions) by mg/kg/day PPX was predictable based on the pharmacological effect of PPX as a dopamine agonist. The sponsor has demonstrated that PPX, like other dopamine agonists, inhibits prolactin secretion in rats. Prolactin is well-established as a factor necessary for the maintenance of rat pregnancy. In fact, dopaminergic inhibition of prolactin secretion is the basis for the proposed ICH guidelines stating that **the rat is an inappropriate model for reproductive toxicology studies of dopamine agonists**. Thus, the reproductive toxicology studies could theoretically be considered deficient since it is comprised of only of one Segment II study in a single "appropriate" species, the rabbit. The rabbit is also problematic since it is unique among all species with regard to pramipexole pharmacokinetics. Although the reproductive impairments in the rat were expected, the data cannot be completely disregarded because of design flaws on the part of the sponsor. The rat studies clearly demonstrated impaired fertility and harm to the fetuses, and thus the drug should be placed in Pregnancy category C according to CFR 21, Part 201.57.

The limitations on the teratogenicity study which arose as a consequence of embryoletality should also be mentioned in labeling, particularly in light of the occurrence of some rather rare malformations in fetuses of the Segment II study (LD: one case of atresia ani, one case of sirenomelia and gastroschisis; MD: one case of cleft vertebra), which may also merit a labeling statement.

(Note: The background incidence of cleft vertebra varies markedly depending the type of affected vertebra. Fetal incidences are 0.638% for cleft thoracic vertebra, and 0.033% for cleft lumbar vertebra - MARTA Glossary of Fetal Alterations).

Body weight development was significantly impaired in pups from MD and HD dams in the Segment III study, which is not consistent with the sponsor's labeling statement that pups from PPX-treated dams develop normally. Body weight development in pups from HD dams in the Segment I study also appeared reduced, but could not be statistically evaluated because of the low pup number. A slight (insignificant) delay in eye-opening was also observed in the Segment I study. The sponsor's assertion that this impairment of body weight development is due to a drug

effect in the dam, and not a direct effect on pup development is plausible, and may be correct, but no experimental support is provided. The sponsor suggests that the basis for this finding is that PPX stimulates the dams and thereby reduces suckling opportunities by the pups; inhibition of lactation is another possibility. Irrespective of the mechanism, the question of whether the drug is affecting the dams or the pups could be addressed in a cross-fostering study. This study will not be required for approval, but until evidence is presented that argues against a direct effect of PPX on pup development, the labeling should accurately reflect the submitted findings.

Although the sponsor does not suggest inhibition of lactation as a basis for impaired pup development, the label states that PPX decreased lactation in rats. No reports addressing this effect were found in the submission. The sponsor needs to identify the pertinent reports that support this statement so that they can be reviewed.

Pharmacokinetic studies indicated that drug exposures in the rat and rabbit LD groups in the Segment II studies approximated the exposures of humans receiving the expected PPX maintenance dose of 1.5 mg, t.i.d.

### 3. Other Toxicology

#### a. Retinal degeneration

The sponsor has addressed the issue of retinal degeneration by submission of the original findings from the rat 2-year carcinogenicity study to an Expert Scientific Panel for review, and by conducting experiments suggested by the panel aimed at characterizing a potential mechanism of degeneration and the influence of pigmentation on the development of retinal lesions in two strains of rats. These studies were reviewed under IND 34,850, and were also reviewed by an FDA consult, Dr. Tim O'Neill (see Appendix for Dr. O'Neill's review).

The sponsor's conclusion from these studies was that PPX inhibits retinal disk-shedding, a mechanism of disk turnover. Inhibition of this mechanism leads to degeneration of the retinal epithelium and photoreceptor cells. Degeneration occurred only in albino rats, and not in pigmented Brown-Norway rats suggesting that pigmentation may protect against this toxicity. This finding tends to reduce this concern with respect to human exposure. However, as concluded by the Expert Panel, disk-shedding is a universal vertebrate phenomenon. Thus, humans may still be at risk for this toxicity, albeit less sensitive due to the presence of pigmentation.

With respect to the sponsor's contention that retinal degeneration is a species/strain-specific effect, Dr. O'Neill raised the question of how closely the other species (monkeys, swine) were evaluated for retinotoxicity. The sponsor did not conduct ultrastructural or quantitative (e.g., thickness of cell layers) analyses in these species, which would have provided a more sensitive measure of toxicity. The sponsor emphasizes the point that the original findings in the rat carcinogenicity study was made during week 76, a long latency period. The monkey study was 52 weeks, and the minipig study was only 13 weeks, which may have been too short to create the

lesion. More sensitive indices of toxicity may have identified early stages of degeneration. The monkey and minipig studies were conducted several years before the 2-year rat study, so there was no reasonable basis to monitor for this lesion. However, a retrospective analysis of the tissues from these studies could have provided firmer support for the statement that retinal degeneration does not occur in these species. Thus, the sponsor's label will require modification to more accurately describe the studies in other species and their limitations. In view of the limitations, the possibility that pramipexole may damage human retinas cannot be discounted, and patients should be periodically monitored.

The proposed labeling statement that there is a long latency period for the effect is misleading since the sponsor has demonstrated that the effect can be produced in rats in 13 weeks.

The sponsor claims in the submission that the retinal lesions in Wistar rats are not indicative of retinotoxicity. Dr. O'Neill disagreed with this conclusion, and recommended (oral communication) the inclusion of a labeling statement to this effect.

b. Fibro-osseous proliferative lesions in mice

An unusual histopathological finding from the 2-year mouse study was the development of a bone lesion in female mice described as a fibro-osseous proliferative lesion. The spontaneous occurrence of this lesion was relatively high (28%), but its incidence rate approximately doubled in drug-treated mice. A dose-related trend was not evident. Similar lesions in mice have been described in the literature as arising spontaneously in aging females of certain strains (Sass and Montali, Lab Animal Sci, 30:907, 1980), and have also been observed following treatment with estrogen (Silberberg and Silberberg, Gerontology, 16:201, 1970), or the prostaglandin E1 analogue misoprostol (Dodd and Port, Vet Pathol., 24:545, 1987). The speculated mechanism for this PPX effect is an estrogen:progesterone imbalance, which is consistent with the positive effect of estrogens on bone deposition (i.e., osteoporosis therapy). No hormonal data was presented to support this mechanism.

Based on the literature descriptions, the toxicological consequence of this lesion is an invasion of marrow space by new bone and fibrous structures due to enhanced osteoblastic activity. This may be expected to reduce hematopoiesis, but this effect did not occur in this study. The potential significance of this lesion in humans is difficult to assess because of the paucity of information in the literature. The occurrence of this lesion in female mice following misoprostol appears in the labeling. Thus, inclusion of these findings in the PPX product labeling should also be considered.

4. "Neuroprotection" claim

The sponsor cites studies in which PPX protects against neurodegeneration in response to ischemia, methamphetamine (*in vivo*), and L-DOPA (*in vitro*). Only the ischemia model is generally recognized as a clinically relevant model, and in the best

case scenario this study would be acceptable evidence of efficacy to support a clinical trial for stroke. However, this data is inadequate to support a labeling claim with such widespread implications.

5. Application Deficiency

The sponsor's INDICATIONS AND USAGE statement does not specify that PPX is for use as an adjunct to any other Parkinson's disease medications (e.g. L-DOPA). Therefore, toxicology studies on the possible long-term, reproductive, or carcinogenic/mutagenic effects of PPX in combination with any other PD medications were not requested by the agency. A short-term cardiovascular study of PPX in combination with Sinemet or Eldepryl in monkeys was requested prior to the initiation of Phase I studies in patients stabilized on these drugs; no significant interactive effects occurred. Since combination therapies are frequently used in PD, and PPX may become part of that approach, the fact that the toxicological consequences of such interactions have not been evaluated in animals should appear in the label.

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## LABELING

### CLINICAL PHARMACOLOGY

#### Para. 2, Sent. 2:

"Animal studies additionally suggest that pramipexole affect dopaminergic mesolimbic pathways thought to promote motivation."

This statement is very speculative should be deleted.

#### Para. 3 and 4:

These paragraphs address the potential neuroprotective effects of PPX in *in vivo* (transient forebrain ischemia, methamphetamine neurotoxicity) and *in vitro* (L-DOPA neurotoxicity) models of neurodegeneration. Only the ischemia model is generally accepted as clinically relevant, but it is not sufficient to support a labeling claim with such widespread implications.

### PRECAUTIONS

Include a statement to this effect:

"The toxicological consequences (long-term, reproduction, carcinogenicity/mutagenicity) of using pramipexole in combination with other Parkinson's disease medications have not been evaluated in animals."

### Carcinogenesis, Mutagenesis, Impairment of Fertility

#### Para. 1:

Control group B is indicated as having 60 animals. The number should be 50.

#### Para. 2:

The findings and significance of Leydig cell adenomas and hyperplasia in rats should be simplified since there is no experimental support for an effect of PPX on LH secretion or LH receptor number:

"These findings are of questionable significance in humans because of their high background incidence in rats, the absence of similar changes in mice treated with PPX for 2 years, and the probable involvement of endocrine mechanisms that are not relevant to humans."

#### Para. 4:

Para. 4:

The statements regarding the impact of PPX on fertility in rats and *potential* impact on fertility in humans should be reworded:

"In rat fertility studies, doses of 2.5 mg/kg/day pramipexole prolonged estrus cycles and inhibited nidation. These effects were associated with reductions of serum prolactin, a hormone necessary for implantation and maintenance of pregnancy in rats."

**Pregnancy**

The sponsor proposes that pramipexole should be placed in Pregnancy category B. Several factors argue against this proposal. The reproductive toxicology studies demonstrated a clear embryotoxic effect in rats at doses of 2.5 mg/kg/day. Because of the high rate of embryo loss, teratology information was very limited. The sponsor's contention that teratogenic effects were not observed is also disputable since 3 fetuses in the Segment II study (2 LD, 1 MD) had rare malformations. These facts are not clearly stated in the proposed labeling. In addition, the sponsor's statement that pups of pramipexole-treated dams developed normally, specifically referring to the Segment II study, is misleading since impairments of body weight development were observed in both the Segment I and Segment III studies. Therefore, the data presented only support a Pregnancy category C labeling:

*"Pregnancy Category C.* There are no adequate and well-controlled studies in pregnant women. Pramipexole should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Administration of 2.5 mg/kg (28 times the human dose) pramipexole to rats during gestation inhibited nidation. Administration of 1.5 mg/kg (17 times the human dose) to rats on days 7-16 post-coitum (p.c.) resulted in early resorption of embryos. Because of the pregnancy impairment and embryoletality, limited teratogenicity data from the highest test dosages of pramipexole (            mg/kg) were obtained. Rare malformations were observed in fetuses from dams treated on days            post-coitum. Of 250 fetuses from dams treated with 0.1 mg/kg pramipexole, one fetus with atresia ani, and one fetus with sirenomelia and gastroschisis were found. Of 222 fetuses from dams treated with 0.5 mg/kg pramipexole, one case of cleft vertebra was noted. Body weight development was impaired in pups from dams treated with 2.5 mg/kg pramipexole through gestation and weaning, or 1.5 mg/kg from day 16 p.c. through weaning. Other developmental parameters including fertility were normal in F<sub>1</sub> pups.

Administration of up to 10 mg/kg/day to rabbits on days 6 through 18 p.c. did not result in any embryotoxic, fetotoxic, or teratogenic effects."

## Nursing Mothers

Experimental support for the statement that PPX decreased lactation in rats is not in the section of the Integrated Summary (Vol. 1.26, p. 5.1/126) indicated in the labeling statement. This statement should be deleted.

## ANIMAL TOXICOLOGY

### 1. Retinotoxicity

#### 1st Para.:

Modify as follows:

"Pramipexole was retinotoxic in albino rats in a 2-year rat carcinogenicity study. The incidence of lesions was dose dependent in animals receiving 2 or 8 mg/kg/day. The first retinal lesions were observed during week 76 of the study."

#### 2nd Para.:

Modify as follows:

"Pramipexole was not retinotoxic in a 2-year carcinogenicity study in mice treated with similar or higher doses (0.3, 2, or 10 mg/kg)."

#### 3rd Para.:

Modify as follows:

"Limited evaluation of the retina in other long-term animal studies did not reveal signs of retinotoxicity in monkeys that received pramipexole (0.1, 0.5, or 2.0 mg/kg/day) for 12 months, or minipigs that received pramipexole (0.3, 1, or 5 mg/kg/day) for 13 weeks."

#### 4th Para.:

Modify as follows:

"Investigative studies demonstrated that pramipexole reduced the rate of disk shedding from the retinal photoreceptor cells of albino rats. In a comparative study, retinal degeneration occurred in albino rats after 13 weeks of treatment with 25 mg/kg/day pramipexole and constant light (100 lux), but not in pigmented rats exposed to the same dose and even higher light intensities."

Thus, the retina of albino rats may be uniquely sensitive to the damaging effects of pramipexole. The potential significance of this effect in humans has not been established, but cannot be disregarded since retinal disk shedding is a universal vertebrate mechanism."

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ANIMAL TOXICOLOGY (cont.)

2. Fibro-osseous proliferative lesion

"An increased incidence of fibro-osseous proliferative lesions occurred in the femurs of female mice treated for 2 years with 0.3, 2.0 or 10 mg/kg. Lesions occurred at a lower rate in control animals. Similar lesions were not observed in male mice, or rats and monkeys of either sex that were treated chronically with pramipexole. The significance of this lesion to humans is not known."

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## RECOMMENDATIONS

1. The NDA is approvable.
2. Labeling recommendations are in the preceding section. A major point of contention is the sponsor's proposed labeling of pramipexole in Pregnancy category B. The submitted data support category C labeling. The labeling could be amended if negative toxicological findings in appropriately designed Segment II and cross-fostering studies are obtained. An appropriate segment II design would involve either administration of pramipexole to rats on days 8-16 (after the prolactin-dependent stage on days 6-7), or the use of another species (hamster, mouse).

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Thomas D. Steele, Ph.D.  
Pharmacologist/Toxicologist

Original NDA 20667

cc.: /Division File, HFD-120  
/G. Fitzgerald, Ph.D.  
/J. Feeney, M.D.  
/J. Purvis  
/T.D. Steele, Ph.D.

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Pharmacology/Toxicology Review  
IND 34,850, Amendment No. 107  
12/14/95

**Drug:** Pramipexole (SND 919)                      **Reviewer:** Thomas D. Steele, Ph.D.

**Sponsor:** The Upjohn Co.

**Indication:** Parkinson's Disease

**Contents of Submission:**

1. "Retinal Degeneration in Albino Rats" - a discussion of toxicological findings and scientific citations to support the safe use of pramipexole in humans
2. Expert Scientific Panel Report on retinal degeneration in the 2-year pramipexole carcinogenicity study
3. Two technical reports:
  - TR 7219-95-043: "SND 919 CL 2 Y: Influence on the Rod Outer Segment Disk-Shedding Mechanism in the Retina of Albino Rats"
  - TR 7219-95-048: "SND 919 CL 2 Y: Influence on Retinal Degeneration in the Albino Rat with and without Light as a Co-factor"
4. Draft protocol: "U-98528E: 21-Day Drug-in-Diet Toxicokinetic Study with Pramipexole in Male and Female Rats"

This review will primarily address the technical reports on Pramipexole-induced retinal degeneration.

**Rationale:**

Pramipexole (PRAM) is a D2 dopamine receptor agonist proposed for use in Parkinson's disease. An unexpected finding in the 2-year rodent carcinogenicity study was the development of retinal degeneration in rats treated with the middle (2.0 mg/kg/day, in diet) and high (8.0 mg/kg/day, in diet) doses of PRAM. The effect was dose-dependent (males: MD = 51%, HD = 90%; females: MD = 21%, HD = 77%). The degeneration was characterized microscopically by a bilateral loss of photoreceptor (PR) cells. In about two-thirds of the affected animals, the lesion was characterized as "mild"; it was restricted to the upper hemisphere of the eye, and the peripheral (outer) portion of the retina. In the "severe" form (remaining one-third of affected animals), PR cell loss occurred in both hemispheres, the entire retina was lesioned, and vessels penetrated the retinal pigment epithelium. These lesions were first observed in premature decedents at week 76 of the two-year study. The "No Effect" dose level for retinal degeneration

in the two-year rat carcinogenicity study was 0.3 mg/kg/day. In a parallel mouse two year study, a low incidence (8-28%) of retinal degeneration was observed in all dosage groups (0, 0.3, 2.0, 10 mg/kg/d, in diet; no dose-dependence).

Histopathological findings indicated that the outer portion of the retina, which contains the PR cells and the retinal epithelium, is affected most consistently and severely by PRAM. Thus, the speculated mechanism by which PRAM induces retinal degeneration should occur in this region rather than the inner (interplexiform and amacrine) cell layer where dopaminergic cells are found. One such mechanism is the PR cell "disk-shedding and renewal system", wherein the older, outermost disks of the rods and cones are shed and phagocytized in retinal pigment epithelial cells and replaced by new disks within the PR cell. The activity of this mechanism leads to the appearance of "phagosomes" containing digested disks in the retinal pigment epithelium. If the shedding/renewal system is disrupted, old disks are not shed and phagocytized, and degeneration of the retinal epithelium and PR cells may result. The disk-shedding/renewal system follows a circadian rhythm in rats and peaks shortly after the onset of light. Retinal melatonin appears to be a primary activator of disk-shedding, and its synthesis is inhibited by dopamine agonists and constant light. Therefore, the sponsor proposes that disruption of this disk-shedding/renewal system by PRAM is a possible mechanism of retinal degeneration. In addition, the sponsor contends that PRAM-induced retinal degeneration is a strain-specific effect and occurs only in albino (non-pigmented) rats, which are known to be susceptible to damage by light alone. Thus, pigmentation can be viewed as protective, and degeneration should not occur in pigmented animals, including humans.

These two hypotheses were the focal points of the experiments presented in this submission. The first experiment (TR 7219-95-043) was a combination of a model development/mechanistic study in which an inhibitory effect of PRAM (25 mg/kg, p.o. daily for 30 days) on disk-shedding in a "sensitive" albino strain (Wistar) of rats was established. In the second series of studies (TR 7219-95-048), the role of pigmentation in retinal degeneration was addressed by comparing the effects of daily treatments with PRAM (25 mg/kg, p.o. for 30 days), with light as a cofactor, on microscopic retinal changes in non-pigmented (Wistar) and pigmented (Brown Norwegian) rats. Based on the results of these studies, the sponsor concludes that PRAM-induced retinal degeneration is due to a direct D<sub>2</sub> receptor-mediated inhibitory effect on disk-shedding/renewal in non-pigmented animals, and is therefore not a safety risk in pigmented species (i.e., humans).

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TR 7219-95-043: "SND 919 CL 2 Y: Influence on the Rod Outer Segment Disk-Shedding Mechanism in the Retina of Albino Rats"

**Study Design:**

Results of two individual studies, a preliminary (I09) and a main study (I22), were presented.

Subjects: Male Wistar rats  
I09: N = 25; 15 control, 10 PRAM  
I22: N = 50; 25 control, 25 PRAM

Treatments:

PRAM animals were treated daily by gavage with 25 mg/kg for 30 days. The time of drug administration was 7-8 am (this was not reported in the submission; the information was obtained via a teleconference with the firm on 12/8/95). Animals were housed under "strict barrier conditions". The light intensity varied between 12-60 lux. The light cycle reported in the document text was 12:12 hr, but diagrams of the experimental design indicated "lights out" at 8 pm and "lights on" at 6 am.

Necropsy:

Animals were sacrificed on days 30/31 at the following time points:

8 pm, 5 am, 8 am, 10 am, 12 pm

These times were selected to establish the circadian pattern of disk-shedding. In the preliminary study, 3 control and 2 PRAM animals were sacrificed at each point. In the main study, five animals from each group were sacrificed at each time point.

Parameters:

*Morphology:* Eyes were removed, marked to distinguish upper and lower hemispheres, and sectioned for light and transmission electron microscopy. Toluidine blue-stained sections for light microscopy were evaluated for histopathological changes. Ultrathin EM sections were stained with uranyl acetate and lead citrate, and analyzed quantitatively for the number of phagosomes within the retinal pigment epithelial (RPE) cell layer.

*Other Observations:* Body weight and food intake were measured once weekly.

## Results:

### Morphology:

Light microscopy did not reveal any significant treatment-related changes. Quantitative EM analysis of phagosome numbers in RPE cells of control animals demonstrated a circadian pattern of disk-shedding. A small increase in phagosome number at 5 am was followed by a sharp rise at 8 am, 2 hrs after light onset. The peak number of phagosomes was approximately eight-fold greater than that at any other time point.

Pramipexole markedly blunted the peak rise in phagosome number at 8 am. The average reduction in phagosome number in PRAM-treated animals was 69% (combination of data from both studies). No significant differences in phagosome number were evident at any other time point.

A separate evaluation of phagosome numbers in the individual hemispheres did not reveal a striking hemispheric pattern of drug effect. The peak in phagosome number in both hemispheres was blunted by pramipexole, although statistical significance was achieved only in the upper hemisphere.

### Other Observations:

As observed in the previously reported 13-week toxicity study, PRAM significantly reduced body weight throughout the study. The effect is probably secondary to a reduction of food intake, although PRAM-treated animals in the main study (I22) consumed significantly more food. These data were not elaborated since they were addressed in previous studies.

TR 7219-95-048: "SND 919 CL 2 Y: Influence on Retinal Degeneration in the Albino Rat with and without Light as a Co-factor"

### Study Design:

Results of two individual studies, a preliminary (I10) and a main study (I31), were presented.

### Subjects:

I10: N = 96 (48 M, 48 F) Wistar rats  
N = 20 (10 M, 10 F) Brown Norway rats

I31: N = 64 (32 M, 32 F) Wistar rats  
N = 64 (32 M, 32 F) Brown Norway rats

### Treatments:

I10: This study was primarily to determine the appropriate light intensities for the main study. The Wistar rats were divided into three groups exposed to light intensities of either 60, 100, or 200 lux. The Brown Norway rats were exposed to 500 lux light. The groups were subdivided into control

and PRAM (25 mg/kg, p.o.) treatment groups. Animals were treated once daily (7-8 am) until the day before necropsy. The longest treatment duration was 13 weeks. Interim sacrifices were conducted at week 3, 5, and 9 (2M/2F) for Wistar rats, and week -1, 5, and 9 (1M/1F) for Brown Norway rats.

*I31:* Wistar rats were exposed to a light intensity of 100 lux, and Brown Norway rats were exposed to 500 lux. Thirty-two rats of each strain (16M/16F) received either PRAM (25 mg/kg, p.o.) or no drug once daily (7-8 am) until the day before necropsy. Eight rats (4M/4F) from each group were sacrificed at weeks 3, 5, 9, and 13.

Animals were housed under "strict barrier conditions". The lights were on 24 hr/day.

Necropsy:

Animals were sacrificed, eyes were marked to distinguish upper and lower hemispheres, removed and processed for light microscopy. H/E-stained sections were evaluated for histopathological changes to the retinae.

Other Observations:

Body weight and food intake were measured once weekly.

**Results:**

Morphology:

I10: Continuous exposure of albino Wistar rats to light for 3 months resulted in intensity-dependent retinal damage as assessed by loss of photoreceptor cell nuclei. PRAM caused more pronounced retinal degeneration, and shortened the latency for onset of degeneration compared to control animals. The effect was clearest in the 100 lux treatment groups; reduction in 100 lux/control animals were first apparent at week 5, whereas 100 lux/PRAM animals showed PR cell loss at week 3. All albino rats exposed to 200 lux light for 13 weeks were blind. Hence, the 100 lux intensity level was most appropriate for the main study.

None of the control or PRAM-treated Brown Norway rats exposed continuously to 500 lux light for 13 weeks showed any signs of retinal damage.

I31: Continuous exposure of untreated albino Wistar rats to 100 lux light resulted in PR cell degeneration. The severity of degeneration increased with exposure duration. PRAM treatment shortened the latency and increased the severity of damage. Both the upper and lower hemispheres of the eyes were lesioned.

None of the control or PRAM-treated Brown Norway rats exposed continuously to 500 lux light for 13 weeks showed any signs of retinal damage.

#### Other Observations:

Body weight and food intake data were collected but not evaluated by the sponsor. As a part of this review, these data were assessed to determine if there were strain differences in response to the drug other than retinal degeneration. Some interesting trends were noted, such as a PRAM-induced reduction of body weight gain in the Wistar, but not Brown Norway, rat. While such a finding suggests the possibility of strain differences in drug exposures/metabolism, the fact that the two strains were never subjected to similar light intensities prevents the evaluation of this hypothesis.

#### **Summary and Conclusions:**

The major finding of these studies was that daily treatment of albino Wistar rats, but not pigmented Brown Norway rats, with pramipexole (25 mg/kg, p.o.) produces retinal degeneration characterized by loss of photoreceptor cells. Duration and intensity of light exposure are critical co-factors for degeneration; lesion severity is increased and onset latency is shortened with increasing intensity of light. A drug effect can be observed with a treatment duration as short as 3 weeks in Wistar rats exposed to constant 100 lux light.

The proposed mechanism of PRAM-induced retinal degeneration is an inhibition of the circadian-controlled disk-shedding/renewal system, which is necessary for a constant supply of "fresh" disks in the PR cell. A reduction in disk-shedding is reflected by a decrease in phagosome number in the retinal epithelial cell layer. The disk-shedding/renewal mechanism is apparently activated shortly after light onset by melatonin. The biosynthesis of melatonin in vertebrate retina is inhibited by D<sub>2</sub> receptor activation. Thus, stimulation of D<sub>2</sub> receptors by pramipexole prevents melatonin-induced activation of disk-shedding leading to retinal degeneration.

The data presented support the sponsor's contention that: 1) pramipexole-induced inhibition of disk-shedding/renewal is a possible mechanism of degeneration, and 2) non-pigmented animals are more susceptible to the damaging effects of pramipexole than pigmented animals. The latter hypothesis is the basis for the sponsor's assertion that the risk of retinal damage in humans is minimized by pigmentation. However, some outstanding deficiencies in experimental support for this hypothesis remain:

1. A primary assertion is that pramipexole-induced retinal degeneration is due to D<sub>2</sub> receptor activation, and therefore may be a universal phenomenon for this class of drugs. However, there are no pharmacological data showing that the effect is blocked by D<sub>2</sub> antagonists or, more importantly, replicated by other DA agonists. A single citation that the MAO-A inhibitor clorgyline disrupts retinal rhythmicity is cited to support dopaminergic involvement. However, it is well-known that DA is metabolized by MAO-B; thus, the effect of clorgyline may have been due to elevation of retinal 5-HT rather than DA. In addition, the sponsor cites a study in which the D<sub>2</sub> agonist bromocriptine protected against light-induced retinal damage. If a clinically-important, positive control such as L-DOPA had been included in these studies and shown to

replicate the effect of pramipexole, than the retinal damage safety concerns would have been reduced.

2. Little explanation is provided for how pigmentation protects against PRAM-induced retinal damage. There is a very brief mention of the role of melanin in light absorption, but how the proposed mechanism (i.e., disk-shedding) is differentially affected in pigmented and non-pigmented animals is not examined. This is an important point, as indicated by the sponsor's Expert Panel, since disk-shedding is a universal vertebrate phenomenon, and if "alteration of this mechanism is the critical step in the development of retinal lesions in the albino rat given pramipexole, the Panel cannot exclude the possibility that a comparable retinal degeneration could occur in human beings chronically treated with the drug."
3. Although the "Pigmented versus Non-pigmented Rat" study provides evidence to support the hypothesis those strain differences in susceptibility to retinal damage exist, the question of whether this is a selectivity or sensitivity issue remains. A superficial perusal of the body weight and food intake data in TR7219-95-049 suggested the possibility of strain differences in response to pramipexole on these parameters, although the high light intensity the Brown Norway rats were exposed to could not be discounted as a factor. Thus, exposure level or mechanistic comparisons between the Wistar and Brown Norway strains would have been useful.
4. Also along the lines of species sensitivity, the argument is raised that pigmented animals in other long-term toxicity studies that were exposed to higher doses of PRAM than anticipated human exposure, did not show signs of degeneration. Results of a one-year monkey study (0.1, 0.5, 2 mg/kg/d, p.o.) and a 13-week minipig study (0.3, 1, and 5 mg/kg/d, p.o.) are cited. It is noted that in the 2-year rat carcinogenicity study, microscopic retinal changes were first observed at week 76. Using the argument that pigmentation is protective, degenerative changes in pigmented animals may not necessarily be expected until exposure durations greater than those conducted in the monkey or minipig studies are achieved.
5. The finding that albino mice in the 2-year carcinogenicity study did not incur dose-dependent retinal degeneration also argues against pigmentation as the sole or primary determining factor for susceptibility to PRAM-induced retinal damage.

Additional general comments on the submission are:

1. The sponsor repeatedly attempts to distinguish the retinal degenerative effects of pramipexole from retinotoxicity based on the following findings from the 2-year rat carcinogenicity study where the effects were first observed:
  - Higher doses of PRAM in toxicity studies of shorter duration did not cause photoreceptor cell damage in albino rats
  - The latency time for degeneration was greater than 52 weeks
  - The hemispheric pattern of degeneration differentiates it from retinotoxicity

which usually involves the entire retina

The data provided in this submission completely contradicts these arguments. Microscopic degenerative effects of 25 mg/kg PRAM (in combination with constant light) were observed as early as three weeks after treatment initiation. Even under normal lighting conditions, mechanistic endpoints (i.e., disk shedding) were affected after 30 days of treatment. The upper and lower hemispheres appear to be equally sensitive to the retinal degenerative effects of PRAM using the markers employed in these studies. Although this is primarily a semantic issue, the data for PRAM presented in this submission meet the sponsor's own criteria for a retinotoxic agent.

2. The "No Effect" level of PRAM for retinal degeneration in the 2-year rat carcinogenicity study was 0.3 mg/kg/d, and the expected human dose is 0.1 mg/kg/d. This represents a three-fold "safety margin". However, the target patient population (Parkinson's disease) is elderly, may have preexisting visual disturbances, and has a compromised dopaminergic system. Receptor supersensitivity is a well-established phenomenon in animals depleted of dopamine, and the possibility exists that D2 receptors in the retina of PD patients may have an exaggerated response to agonist administration. These factors may result in a narrowing of the safety margin for PRAM.

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