C.3.c. Segment II in Rats: Reproduction and Teratogenicity

	Main Study	PK Study
Document #(s):	BI Document U88-0068 Upjohn TR 7219-94-074	BI Document U94-0004 Upjohn TR 7256-94-027
Sponsor Volume:	1.43	1.59

Summary:

Pramipexole was administered by gavage at doses of 0, 0.1, 0.5 and 1.5 mg/kg/day to Wistar rats (36/dose) from day 7 to 16 of gestation. Twenty-four rats per group were sacrificed on day 22 for delivery of pups by Caesarean section. The remaining females (12/dose) littered spontaneously and raised the pups to weaning (21 days). A supplementary toxicokinetic study was conducted in 6 pregnant females/dose. Animals were treated on days 7-12, and plasma samples collected at 1, 2, 4 and 24 hrs post-treatment on day 12.

Overt drug-related changes were restlessness and agitation with the middle and high doses, and decreased body weight gain and food intake at the high dose. With respect to reproductive toxicities, a very clear threshold effect was apparent; the MD group was virtually devoid of toxicities, and very pronounced toxicities were evident at the HD. Significant embryolethality 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. No clear drug-related effects on fetal maturation and development, or skeletal/visceral abnormalities were apparent. As in the previous studies, the teratology information is limited by the low number of evaluable pups. One case each of anal atresia (LD), sirenomelia (LD), and cleft vertebra (MD) were noted in pups from PPX-treated dams. Since impairment of body weight development and delayed eye-opening in pups from drug-treated dams did not occur in this study, as they had in the previous Segment I study, it is reasonable to conclude that these impairments were due to drug administration during lactation. Drug exposure in the LD group approximated exposure of humans receiving the projected PPX maintenance dose of 1.5 mg, t.i.d.

Methods:

Dosages: 0.1, 0.5, 1.5 mg/kg/day (Drug Lot: Batch I - main study, Batch IV - PK study; prepared in distilled water) from day 7 to day 16 (inclusive) of gestation in main study, and day 7-12 in PK study.

Low dose is 10 times the expected human dose (at the time of study initiation). The high dose was is somewhat lower than the dose used in a pilot study, in which significant signs of CNS toxicity were observed in dams administered 2.5 mg/kg.

Route of Administration: oral (gavage)

Species/Number: 144 inseminated females for main study, 24 for PK study

Approximate initial weight/age: 230 g / 12 weeks

Parameters monitored/Intervals:

Clinical	-	daily
Body weight	-	days 1, 7-16, and 22 of gestation, and days 1, 6, 13, and 20 of lactation
Food consumption	-	weekly
Plasma Concs	-	1, 2, 4, 24 hrs postdose on day 12

Termination:

Caesarean Section: 24 dams/group were sacrificed on day 22 of gestation. Non-pregnant females were excluded from mean calculations. The number of corpora lutea, fetal viability/loss, and implantation sites were determined. Dams were autopsied. Fetuses were sexed, and examined for skeletal or visceral and cephalic abnormalities.

Spont. delivery: 12 dams/group delivered pups and raised them to weaning. Litter parameters (size, live/still births, gross abnormalities) were recorded, and functional/behavioral/maturational tests were conducted. Pups were sacrificed and autopsied during the fourth week.

Statistics

Statistical comparisons were made by Bartlett's test, one-way ANOVA, Newman-Keuls test for multiple comparisons, and Chi-square and Fisher's exact test.

Results:

Effects on Dams

Mortality: One MD dam died apparently from choking on bedding

Clinical signs:

In the main study, restlessness and agitation at MD and HD, and one HD dam had vaginal bleeding. In the TK study, sedation was noted at the HD, and 5/6 HD dams had vaginal bleeding.

Body Weight Gain:

Significantly decreased in the HD groups from beginning of treatment through gestation (Fig. C.3.c.1).

Food Intake:

Significantly decreased in HD group during week 2 and 3 of gestation. No differences among groups occurred during the lactation period.

Gestation:

21 of 144 females were not pregnant (CON: 7; LD: 3; MD: 8; HD: 3). 25 of 32 pregnant HD dams (18 C-section, 7 spontaneous delivery) had complete resorptions.
In the PK study, all HD dams had complete resorptions.

Necropsy: No pathologies were found in the dams.

Effects on fetuses and pups

C-section group: The total litter resorption by 18 of 22 pregnant dams in the HD group resulted in some significant differences in mean litter values. There were no significant differences in mean fetal or placental weights, or in the incidence of external, skeletal, visceral or cephalic abnormalities (Tab. C.3.c.1-3), although some rare malformations were noted in pups of PPX-treated dams (one case of anal atresia at LD, one case of sirenomelia with gastroschisis at LD, and one case of cleft vertebra at MD).

Spontaneous delivery group: The mean number of live pups per dam was significantly reduced in the HD group because 7 of 10 pregnant animals had total resorptions (Tab. C.3.c.4-5). There was a dose-dependent trend, but no significant increase in pup body weight development through weaning (Fig. C.3.c.2). No functional, behavioral or maturational impairments, or structural abnormalities were evident in the pups (Tab. C.3.c.6-7).

Plasma Concentrations (Satellite Study)

Except for 24 hrs after the 0.1 mg/kg dose, PPX was detectable in plasma. The highest concentrations were noted 1 hr after dosing. Increases in AUC were generally dose proportional. PK parameters ($C_{1\,hr}$, AUC_{1-24}) associated with the lowest test dose approximated those of humans receiving the projected PPX maintenance dose of 1.5 mg, t.i.d. ($Cmax_{ss}$ = ng/ml, AUC_{0-8} =)

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Fig. C.3.c.1.

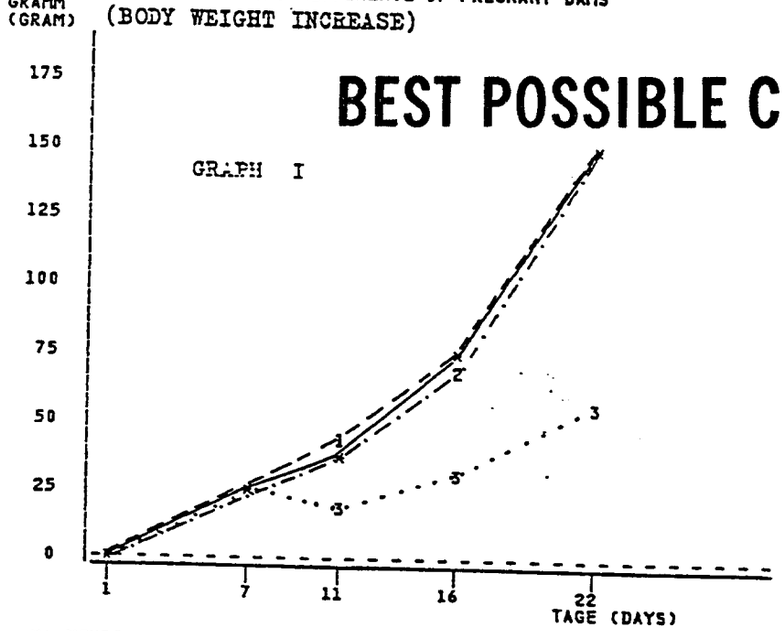
SND 919 CL2Y RATTEN / RATS

SEGM. 2

KOERPERGEWICHT TRAECHTIGER MUTTERTIERE (G) KAISERSCHNITT-GRUPPE
BODYWEIGHTS OF PREGNANT DAMS (G) CAESAREAN SECTION GROUP
MITTELWERTE / MEAN VALUES

		N	1	7	11	16	22
KONTROLLE CONTROL	MW	19	222.4	246.8	262.7	296.1	373.8
	SD		10.7	12.9	13.3	15.7	27.3
0.1 MG/KG	MW	21	224.3	250.6	267.7	300.5	376.3
	SD		8.2	9.5	10.0	12.3	24.7
0.5 MG/KG	MW	18	222.7	247.1	257.4	291.1	373.2
	SD		8.6	13.3	14.6	13.9	21.7
1.5 MG/KG	MW	22	222.6	246.6	238.8 ***	254.1 ***	280.5 ***
	SD		8.9	14.2	16.0	22.9	50.0

MITTLERE GEWICHTSDIFFERENZ TRAECHTIGER MUTTERTIERE
MEAN BODYWEIGHT DIFFERENCE OF PREGNANT DAMS
(BODY WEIGHT INCREASE)



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*: MEHRFACHBELEGUNG - MULTIPLE POINTS
0:KONTR -CONTR 1: 0.1 MG/KG 2: 0.5 MG/KG 3: 1.5 MG/KG

*** $p < 0.001$

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Fig. C.3.c.1.(cont.)

SND 919 CL2Y RATTEN / RATS

SEGM. 2

KOERPERGEWICHT TRAECHTIGER MUTTERTIERE (G) ~~AUFZUCHT-GRUPPE~~
BODYWEIGHTS OF PREGNANT DAMS (G) SPONTANEOUS DELIVERY GROUP
MITTELWERTE / MEAN VALUES

		N	1	7	11	16	22
KONTR -CONTR CONTROL	MW	10	237.9	259.9	275.2	308.2	388.7
	SD		9.9	10.8	14.4	14.6	24.1
0.1 MG/KG	MW	12	236.0	263.8	281.7	316.2	387.8
	SD		15.4	16.3	18.8	21.8	30.8
0.5 MG/KG	MW	10	237.8	261.7	275.2	308.1	394.1
	SD		9.8	8.6	8.0	8.9	16.6
1.5 MG/KG	MW	10	234.6	261.8	255.0***	275.9***	308.8***
	SD		7.5	8.8	8.6	16.8	52.9

MITTLERE GEWICHTSDIFFERENZ TRAECHTIGER MUTTERTIERE
MEAN BODYWEIGHT DIFFERENCE OF PREGNANT DAMS
(BODY WEIGHT INCREASE)

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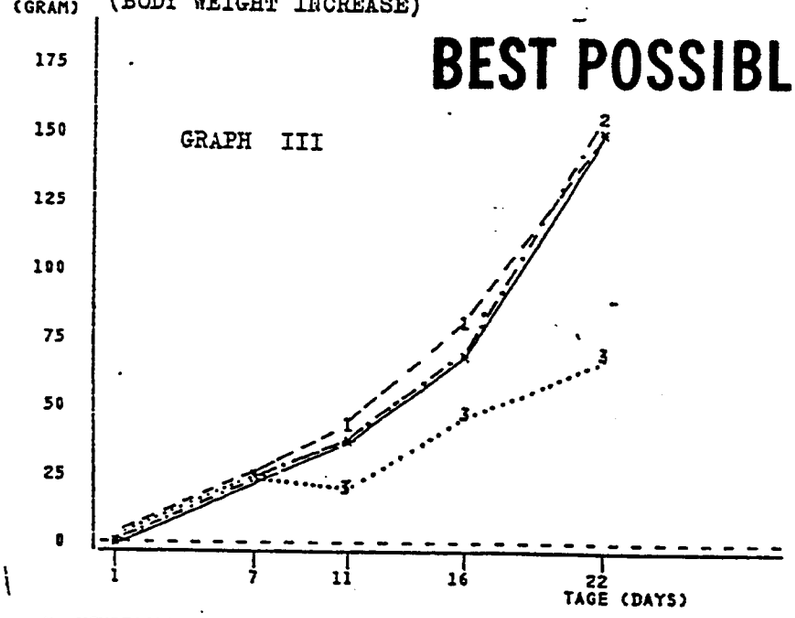

X: MEHRFACHBELEGUNG - MULTIPLE POINTS
0:KONTR -CONTR 1: 0.1 MG/KG 2: 0.5 MG/KG 3: 1.5 MG/KG

*** $p < 0.001$

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Tab. C.3.c.1

SND 919 CL2Y RATTEN / RATS

SEGM. 2

WURFDATEN / LITTER DATA SUMMENWERTE / SUM VALUES	KAISERSCHNITT-GRUPPE CAESAREAN SECTION GROUP			
	KONTROLLE CONTROL	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG
GESAMTZAHL DER TIERE TOTAL NUMBER OF ANIMALS	24	24	24	24
TODESFAELLE MATERNAL MORTALITY	0	0	0	0
TOTALRESORPTIONEN TOTAL RESORPTIONS	0	0	0	18
SPONTANWUERFE NATURAL DELIVERIES	0	0	0	0
TIERE MIT LEB. FETEN ANIM. WITH VIABLE FET.	19	21	18	4
GELBKOERPER CORPORA LUTEA	261	294	247	312
IMPLANTATIONEN IMPLANTATIONS	240	267	233	311
LEBENDE FETEN VIABLE FETUSES	232	250	222	51
MAENNLICH IN % MALE IN %	51	54	58	55
WEIBLICH IN % FEMALE IN %	49	46	42	45
TOTE FETEN DEAD FETUSES	0	0	0	0
RESORPTIONEN RESORPTIONS	8	17	11	260
FRUEH IN % EARLY IN %	50	76	73	100
SPAET IN % LATE IN %	50	24	27	0
MISSBILDUNGEN MALFORMATIONS	0	2	1	0
VARIATIONEN VARIATIONS	1	6	5	0

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Tab. C.3.c.2.

SND 919 CL2Y RATTEN / RATS

SEGM. 2

WURFDATEN / LITTER DATA
MITTELWERTE / MEAN VALUES

KAISERSCHNITT-GRUPPE
CAESAREAN SECTION GROUP

		KONTROLLE CONTROL	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG	
GEWICHT / FET (G)	MW	5.31	5.45	5.38	5.41	
WEIGHT / FETUS (G)	SD	0.23	0.30	0.22	0.32	
PLAZ.GEW. / FET (G)	MW	0.45	0.49	0.46	0.46	
WEIGHT PLAC./ FETUS (G)	SD	0.05	0.10	0.06	0.03	
CORPORA LUTEA / MUTTERT.	MW	13.7	14.0	13.7	14.2	
CORPORA LUTEA / DAM	SD	1.3	2.4	0.6	1.5	
IMPLANTATIONEN / MUTTERT.	MW	12.6	12.7	12.9	14.1	
IMPLANTATIONS / DAM	SD	3.0	4.1	2.0	1.5	
LEBENDE FETEN / MUTTERT.	MW	12.2	11.9	12.3	2.3	***
VIABLE FETUSES / DAM	SD	3.0	3.9	2.1	5.1	
TOTE FETEN / MUTTERT.	MW	0.0	0.0	0.0	0.0	
DEAD FETUSES / DAM	SD	0.0	0.0	0.0	0.0	
RESORPTIONEN / MUTTERT.	MW	0.4	0.8	0.6	11.8	***
RESORPTIONS / DAM	SD	0.6	1.2	1.1	5.4	
MISSBILDUNGEN / MUTTERT.	MW	0.0	0.1	0.1	0.0	
MALFORMATIONS / DAM	SD	0.0	0.3	0.2	0.0	
VARIATIONEN / MUTTERT.	MW	0.1	0.3	0.3	0.0	
VARIATIONS / DAM	SD	0.2	0.5	0.6	0.0	
LEB. FETEN IN % IMPL.	MW	98.8	97.6	98.5	6.0	***
VIABLE FET. IN % IMPL.	SD	2.2	3.8	3.5	26.9	
RESORPTIONEN IN % IMPL.	MW	1.2	2.4	1.5	94.0	***
RESORPTIONS IN % IMPL.	SD	2.2	3.8	3.5	26.9	
PRAEIMPLANTAT.-VERLUST	MW	2.5	5.1	1.5	0.1	
PRE IMPLANTAT. LOSS %	SD	8.2	11.0	5.3	0.3	
POSTIMPLANTAT.-VERLUST	MW	1.2	2.4	1.5	94.0	***
POST IMPLANTAT. LOSS %	SD	2.2	3.8	3.5	26.9	
MISSB. IN % LEB. FET.	MW	0.0	0.1	0.1	0.0	
MALFORM. IN % VIAB. FET.	SD	0.0	0.7	0.6	0.0	
VARIAT. IN % LEB. FET.	MW	0.1	2.1	1.7	0.0	
VARIAT. IN % VIAB. FET.	SD	1.4	5.5	6.5	0.0	

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*** $p < 0.001$

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Tab. C.3.c.3.

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BEFUNDE AN FETEN
FINDINGS IN FETUSES

KAISERSCHNITT-GRUPPE
CAESAREAN SECTION GROUP

	KONTR. CONTOL	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG
EXTERNAL				
ATRESIA ANI	0	1[a]	0	0
SIRENIFORM MALFORM.. OEDEMA, GASTROSCHISIS	0	1[b]	0	0
SKELETAL				
BENT AND SHORTENED LONG BONES, SYNOSTOSIS OF VERTEBRAE AND RIBS	0	1[b]	0	0
SPLIT VERTEBRAL BODY	0	0	1[c]	0
"HOUR-GLASS" SHAPED VERTEBRA	0	1[d]	0	0
VISCERAL				
DILATATION OF RENAL PELVIS	1[e]	5[f]	4[g]	0
DISPLACEMENT OF LEFT KIDNEY	0	0	1[h]	0
ADDITIONAL SKELETAL FINDINGS				
UNOSSIFIED 5TH STERNEBRA	3	3	4	0
REDUCED OSSIFIED 5TH STERNEBRA	2	1	6	0

a FETUS NO 101/14
b FETUS NO 116/2
c FETUS NO 213/5
d FETUS NO 107/7
e FETUS NO 017/3
f FETUS NO 101/4, 106/2, 114/15, 119/12, 124/16
g FETUS NO 210/10, 216/9, 216/11, 219/7
h FETUS NO 209/9

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Tab. C.3.c.4.

SND 919 CL2Y RATTEN / RATS

SEGM. 2

	WURFDATEN / LITTER DATA SUMMENWERTE / SUM VALUES		AUFZUCHT-GRUPPE SPONTANEOUS DELIVERY GROUP	
	KONTROLLE CONTROL	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG
GESAMTZAHL DER TIERE TOTAL NUMBER OF ANIMALS	12	12	12	12
TODESFAELLE MATERNAL MORTALITY	0	0	0	1
TOTALRESORPTIONEN TOTAL RESORPTIONS	0	0	0	7
TRAECHTIGE TIERE PREGNANT ANIMALS	10	12	10	10
LEBENDE JUNGTIERE VIABLE YOUNG	134	133	138	39
MAENNLICH IN % MALE IN %	46	49	41	49
WEIBLICH IN % FEMALE IN %	54	51	59	51
TOTE JUNGTIERE DEAD PUPS	0	1	0	0
MISSBILDUNGEN MALFORMATIONS	0	0	0	0
VARIATIONEN VARIATIONS	0	0	0	0
JUNGTIERE 7. TAG PUPS DAY 7	131	130	133	38
JUNGTIERE 14. TAG PUPS DAY 14	131	130	132	38
JUNGTIERE 21. TAG PUPS DAY 21	131	130	132	38
VERENDETE JUNGTIERE MORTALITY IN OFFSPRING	3	3	6	1

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Tab. C.3.c.5.

SND 919 CL2Y RATTEN / RATS

SEGM. 2

WURFDATEN / LITTER DATA
MITTELWERTE / MEAN VALUES

AUFZUCHT-GRUPPE
SPONTANEOUS DELIVERY GROUP

		KONTROLLE CONTROL	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG
TRAGEZEIT DER MUTTERTIERE	MW	22.0	22.1	22.0	22.0
GESTATION PERIOD OF DAMS	SD	0.0	0.3	0.0	0.0
	N	10	12	10	10
GEWICHT / JUNGTIER 1.TAG	MW	5.88	6.24	5.95	6.04
WEIGHT / PUP AT DAY 1	SD	0.15	0.34	0.26	0.47
GEWICHT / JUNGTIER 21.TAG	MW	29.58	34.76	31.14	32.82
WEIGHT / PUP AT DAY 21	SD	2.59	6.98	4.83	4.01
GEWICHTSZUNAHME 1.-21.TAG	MW	23.69	28.51	25.19	26.78
BODYW. INCR. DAY 1-21	SD	2.56	6.80	4.79	3.88
LEB.JUNGT./MUTTERT.1.TAG	MW	13.4	11.1	13.8	3.9 ***
VIABLE PUPS/ DAM DAY 1	SD	1.4	3.3	2.0	6.3
TOTE JUNGT./MUTTERT.1.TAG	MW	0.0	0.1	0.0	0.0
DEAD PUPS / DAM DAY 1	SD	0.0	0.3	0.0	0.0
LEB.JUNGT./MUTTERT.7.TAG	MW	13.1	10.8	13.3	3.8 ***
VIABLE PUPS / DAM DAY 7	SD	1.2	3.5	2.3	6.1
LEB.JUNGT./MUTTERT.21.TAG	MW	13.1	10.8	13.2	3.8 ***
VIABLE PUPS /DAM DAY 21	SD	1.2	3.5	2.2	6.1
JUNGTIERVERLUST % 1.-21.T.	MW	0.6	0.5	2.2	0.1
PUPS LOSS % DAY 1-21	SD	1.6	3.0	2.7	0.3
MISSBILDUNGEN / MUTTERT.	MW	0.0	0.0	0.0	0.0
MALFORMATIONS / DAM	SD	0.0	0.0	0.0	0.0
VARIATIONEN / MUTTERTIER	MW	0.0	0.0	0.0	0.0
VARIATIONS / DAM	SD	0.0	0.0	0.0	0.0
MISSBILDUNGEN % LEB.JUNGT.	MW	0.0	0.0	0.0	0.0
MALFORMATIONS IN % VIAB.P.	SD	0.0	0.0	0.0	0.0
VARIATIONEN % LEB.JUNGT.	MW	0.0	0.0	0.0	0.0
VARIATIONS IN % VIAB.PUPS	SD	0.0	0.0	0.0	0.0

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*** $p < 0.001$

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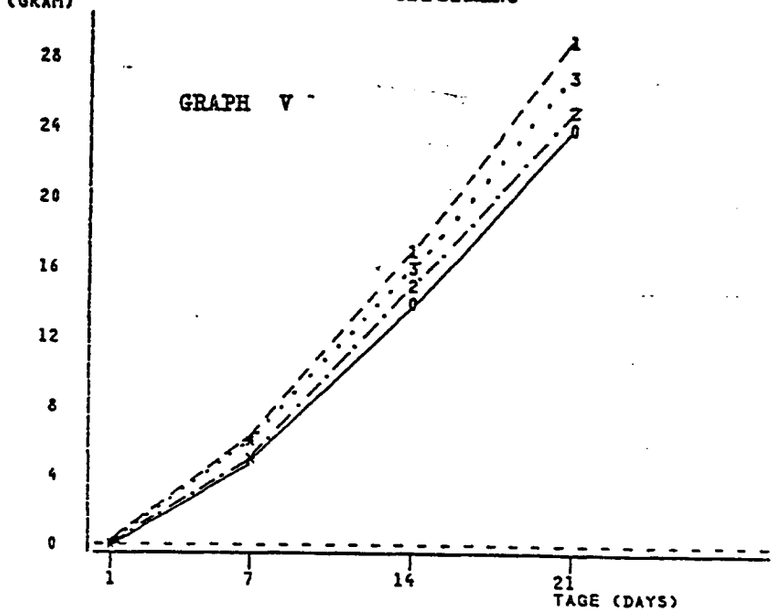
Fig. C.3.c.2.

SND 919 CL2Y RATTEN / RATS

SEGM. 2

MITTLERE GEWICHTSZUNAHME LEBENDER JUNGTIERE (G) AUFZUCHT-GRUPPE
MEAN BODYWEIGHT INCREASE OF VIABLE SPONTANEOUS DELIVERY GROUP
OFFSPRING



%: MEHRFACHBELEGUNG - MULTIPLE POINTS
0:KONTR -CONTR 1: 0.1 MG/KG 2: 0.5 MG/KG 3: 1.5 MG/KG

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Tab. C.3.c.6

SND 919 CL 2 Y

RATTEN/RATS

SEGM. 2

BEOBACHTUNGEN DER F_1-GENERATION DER GRUPPE DER SPONTANGEBURTEN

OBSERVATIONS OF F_1-OFFSPRING IN SPONTANEOUS DELIVERY GROUP

90

ERGEBNISSE IN PROZENT / RESULTS IN PER CENT

DOSIS DOSE	AUSGEWERTETE WÜRFE EVALUATED LITTERS	AUFSTELLEN DER OHREN ERECTION OF PINNAE		FELLWACHSTUM FUR GROWTH		LAUFEN MIT ANGEHOBENEM BAUCH RUNNING WITH RAISED VENTER		DURCHBRECHEN DER OBEREN SCHNEIDEZÄHNE ERUPTION OF MAXILLARY INCISORS		AUGENÖFFNUNG EYE OPENING	
		TAG 4	DAY 5	TAG 6	DAY 7	TAG 12	DAY 13	TAG 12	DAY 13	TAG 15	DAY 16
KONTROLLE/ CONTROL	10	-	96.2	-	100.0	98.5	100.0	95.4	100.0	10.7	67.2
0.1 MG/KG	12	7.6	99.2	6.2	100.0	99.2	100.0	96.2	100.0	22.3	76.9
0.5 MG/KG	10	-	97.0	-	100.0	99.2	100.0	96.2	100.0	12.1	55.3
1.5 MG/KG	3	2.6	100.0	-	100.0	100.0	100.0	97.4	100.0	18.4	81.6

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Tab. C.3.c.7

SND 919 CL 2 Y

RATTEN/RATS

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ERGEBNISSE VON FUNKTIONS- UND VERHALTENSTESTE DER F_1-GENERATION

RESULTS OF FUNCTION AND BEHAVIOUR TESTS IN F_1-OFFSPRING

DOSIS DOSE	ANZAHL WÜRFE No. OF LITTERS	ANZAHL TIERE No. OF ANIMALS	AUF RICHTE-REFLEX RIGHTING-REFLEX %	SEHTEST PUPILLARY REACTION %	HÖRTEST HEARING-TEST %		
					80 Hz	4000 Hz	16000 Hz
KONTROLLE CONTROL	10	131	100.0	100.0	100.0	100.0	100.0
0.1 MG/KG	12	130	100.0	100.0	100.0	100.0	100.0
0.5 MG/KG	10	132	100.0	100.0	100.0	100.0	100.0
1.5 MG/KG	3	38	100.0	100.0	100.0	100.0	100.0

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Boehringer Ingelheim KG
Report/Study No.: R 49

C.3.c.8

Table 8: Geometric mean values and ranges of plasma concentrations [ng/ml] and AUC [1-24 h] of SND 919 CL 2 Y in rats on day 12 of gestation after multiple administration by gavage

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dose	hours				AUC
[mg/kg]	1	2	4	24	[ng/ml·h]
0.1	7.32	5.98	2.92	0.00	44.78
0.5	33.25	26.75	13.27	0.63	213.20
1.5	77.74	68.95	40.10	1.84	620.01

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C.3.d. Segment II in Rabbits: Reproduction and Teratogenicity

	Main Study	PK Study
Document #(s):	BI Document U87-0805 Upjohn TR 7219-94-073	BI Document U94-0020 Upjohn TR 7256-94-026
Sponsor Volume:	1.44	1.59

Summary:

Pramipexole was administered by gavage at doses of 0, 0.1, 1.0 and 10.0 mg/kg/day to pregnant Himalayan rabbits /dose) from day 6 to 18 of gestation. Fetuses were delivered by Caesarean section on day 29, and maintained in an incubator for 24 hrs. After sacrifice, the offspring were examined for skeletal and visceral abnormalities. A supplemental toxicokinetic study was conducted in 4 rabbits/dose on day 12 of gestation.

Overt drug-related changes were restlessness and agitation with the high dose, and dose-dependent decreases in body weight gain and fecal output. One animal died after the third drug treatment due to cardiovascular collapse. Drug exposure in the LD group was at least equivalent to, and generally exceeded, the exposure of humans receiving the projected PPX maintenance dose of 1.5 mg, t.i.d. Pathological findings in other dams at sacrifice were considered spontaneous findings for this strain. No embryotoxic or fetotoxic effects of pramipexole were identified. Increases in the rate of resorption, which occurred at a very high rate in rats, did not occur in rabbits. No dose-related trends in the incidence of malformations were observed in newborns. Thus, pramipexole appears to be devoid of reproductive toxicities in rabbits at doses up to 10 mg/kg, and at exposures that are well-above expected human exposures.

Methods:

Dosages: 0.1, 1.0, 10.0 mg/kg/day (Drug Lot: Batch I - main study, Batch IV - PK study; prepared in distilled water) from day 6 to day 18 (inclusive) of gestation in the main study, and day 6 to 12 in the TK study.

Low dose is 10 times the expected human dose (at the time of study initiation). The high dose was selected based on a pilot study, in which a reduction in body weight was produced by this dose.

Route of Administration: oral (gavage)

Species/Number: Main Study - 72 mated females
TK Study - 16 mated females

Mean initial weight/age: 2317 g / 5-6 months

Parameters monitored/Intervals:

Clinical - daily

Body weight	-	days 1, 6-18, and 29 of gestation, and days 1, 6, 13, and 20 of lactation
Food consumption	-	not measured
Plasma Concs	-	day 12 (0, 2, 4, 8 and 24 hrs postdose)

Termination:

Dams were sacrificed on day 29 of gestation. Routine pathological examinations of the dams were done, and the number of corpora lutea, fetal viability/loss, and implantations and resorptions were determined. Viable newborns were maintained in an incubator for 24 hrs, at which point they were weighed, examined for malformations and skeletal variations, and necropsied.

Statistics:

Statistical comparisons were made by one-way ANOVA followed by Newman-Keuls test for multiple comparisons, or Fisher's exact test.

Results:

Effects on Dams (Main Study)

Mortality: Three deaths occurred during the study, but only one was likely drug-related; an HD dam died on day 3 apparently because of circulatory collapse.

Clinical Signs:

Restlessness and agitation in HD animals between days 10-18.
Reduced fecal output on several days in 8 LD, 16 MD, and 17 HD.

Body Weight Gain:

Dose-dependent, significant decreases in all 3 groups on day 7, in MD and HD groups from days 8-12, and in HD group on days 13-14. The effect waned with increasing duration of treatment (Fig. C.3.d.1).

Gestation:

One control and one LD dam were not pregnant at the end of the study.
Dams with no living offspring were excluded from statistical analyses.
No miscarriages occurred during the study.

Necropsy:

None of the identified pathologies appeared to be drug-related.

TR No.: 7219-94-073

Fig. C.3.d.1.

SND 919 CL2Y. KANINCHEN / RABBITS

SEGM. 2

KOERPERGEWICHT TRAECHTIGER MUTTERTIERE (G)
BODYWEIGHTS OF PREGNANT DAMS (G)
MITTELWERTE / MEAN VALUES

		N	0	6	12	18	29
KONTROLLE CONTROL	MW	17	2284.1	2351.8	2350.6	2370.6	2558.8
	SD		148.7	160.3	180.2	180.5	170.1
0.1 MG/KG	MW	15	2304.7	2349.3	2318.0	2316.0	2515.3
	SD		158.1	155.3	161.6	181.1	198.2
1 MG/KG	MW	18	2310.6	2345.0	2271.1	2336.1	2549.4
	SD		135.3	165.7	154.8	201.2	164.5
10 MG/KG	MW	17	2336.5	2397.6	2287.6	2354.7	2542.4
	SD		138.1	174.0	172.5	152.2	149.8

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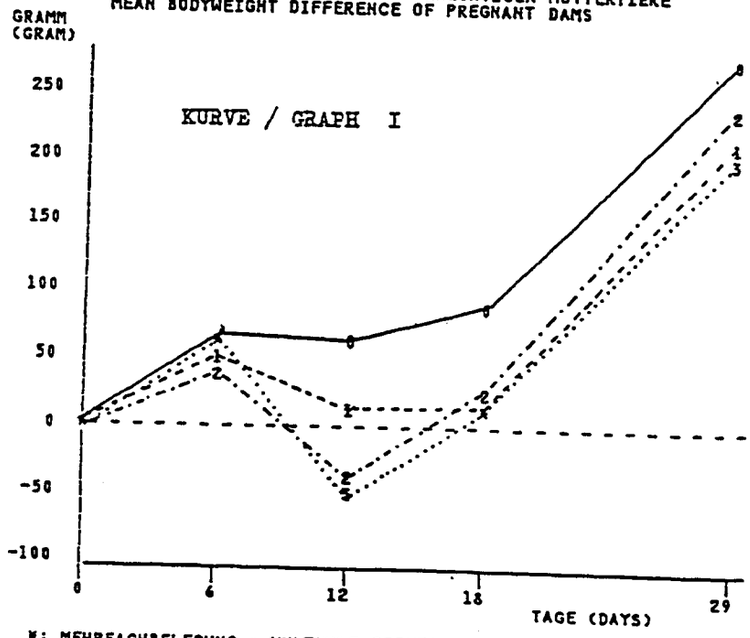
MITTLERE GEWICHTSDIFFERENZ TRAECHTIGER MUTTERTIERE
MEAN BODYWEIGHT DIFFERENCE OF PREGNANT DAMS



M: MEHRFACHBELEGUNG - MULTIPLE POINTS
0:KONTR -CONTR 1: 0.1 MG/KG 2: 1 MG/KG 3: 10 MG/KG

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Autopsy findings in the dams

At the autopsy of the dams after the ovario-hysterectomy (or after death) the following findings were obtained:

Animal no. 006: Approx. bean-sized subcapsular pale area on the liver.
(Histological finding: subcapsular focal, small- to large droplet fatty infiltration).

Animal no. 008: Right uterine horn segmented and empty.

Animal no. 102: Fracture in the region of the 4th lumbar vertebra.

Animal no. 105: Right uterine horn segmented and empty.

Animal no. 111: Agenesia of the accessory lobe of the lung.

Animal no. 118: Foamy contents in the bronchi, multiple haemorrhages in all lobes of the lung.

Animal no. 208: Agenesia of the left uterine horn.

Animal no. 304: Severe lung congestion with oedema.
Extensive haemorrhages in the tissue of the mammary gland (Histopathological finding: recent haemorrhages in the subcutis and in the intra- and inter-lobular mammary tissue).

Animal no. 310: Pyometra of right uterine horn.

Further random findings were parovarian cysts and necroses of the fatty tissue in the retroperitoneal adeps renis with the following incidence:

	Contr.	0.1 mg/kg	1 mg/kg	10 mg/kg
parovarian cysts	5	5	2	1
necroses of fatty tissue	1	3	0	3

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Effects on Litters

No significant differences were evident among groups in mean litter values of number of corpora lutea, implantations, viable/dead fetuses, and resorptions. Mean fetal birth weights and placental weights also did not differ among groups. There were no drug-related trends in the distribution of "runts" (<65% mean fetal weight; 4 Con, 8 LD, 2 HD) and fetal deaths between 0-24 hrs after birth (5 Con, 6 LD 4 MD) (Tab. C.3.d.1-2).

Tab. C.3.d.1. BEST POSSIBLE COPY

SEGM. 2

WURFDATEN / LITTER DATA
SUMMENWERTE / SUM VALUES

	KONTROLLE CONTROL	0.1 MG/KG	1 MG/KG	10 MG/KG
GESAMTZAHL DER TIERE TOTAL NUMBER OF ANIMALS	18	18	18	18
TODESFAELLE MATERNAL MORTALITIES	0	2	0	1
TOTALRESORPTIONEN TOTAL RESORPTIONS	0	0	0	0
SPONTANWUERFE NATURAL DELIVERIES	0	0	0	0
TIERE MIT LEB. FETEN ANIM. WITH VIABLE FET.	17	15	18	17
GELBKOERPER CORPORA LUTEA	179	157	183	192
IMPLANTATIONEN IMPLANTATIONS	133	114	124	127
LEBENDE FETEN VIABLE FETUSES	113	99	115	104
MAENNLICH IN % MALE IN %	44	49	47	60
WEIBLICH IN % FEMALE IN %	56	51	53	40
TOTE FETEN DEAD FETUSES	0	0	0	0
RESORPTIONEN RESORPTIONS	20	15	9	23
FRUEH IN % EARLY IN %	60	60	89	70
SPAET IN % LATE IN %	40	40	11	30
MISSBILDUNGEN MALFORMATIONS	1	1	1	2
VARIATIONEN VARIATIONS	6	2	6	5

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Tab. C.3.d.2.

SND 919 CL2Y KANINCHEN / RABBITS

SEGM. 2

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WURFDATEN / LITTER DATA
MITTELWERTE / MEAN VALUES

		KONTROLLE CONTROL	0.1 MG/KG	1 MG/KG	10 MG/KG
GEWICHT / FET (G)	MW	38.93	35.80	38.65	38.07
WEIGHT / FETUS (G)	SD	5.13	5.08	4.41	4.75
PLAZ.GEW. / FET (G)	MW	4.92	4.55	4.62	4.63
WEIGHT PLAC./ FETUS (G)	SD	0.86	0.72	0.66	0.90
CORPORA LUTEA / MUTTERT.	MW	10.5	10.5	10.2	11.3
CORPORA LUTEA / DAM	SD	2.4	2.1	2.0	1.9
IMPLANTATIONEN / MUTTERT.	MW	7.8	7.6	6.9	7.5
IMPLANTATIONS / DAM	SD	2.2	1.4	1.5	1.8
LEBENDE FETEN / MUTTERT.	MW	6.6	6.6	6.4	6.1
VIABLE FETUSES / DAM	SD	2.0	1.7	1.8	2.1
TOTE FETEN / MUTTERT.	MW	0.0	0.0	0.0	0.0
DEAD FETUSES / DAM	SD	0.0	0.0	0.0	0.0
RESORPTIONEN / MUTTERT.	MW	1.2	1.0	0.5	1.4
RESORPTIONS / DAM	SD	1.3	0.9	0.8	1.7
MISSBILDUNGEN / MUTTERT.	MW	0.1	0.1	0.1	0.1
MALFORMATIONS / DAM	SD	0.2	0.3	0.2	0.3
VARIATIONEN / MUTTERT.	MW	0.4	0.1	0.3	0.3
VARIATIONS / DAM	SD	0.6	0.4	0.8	0.7
LEB. FETEN IN % IMPL.	MW	91.0	90.8	96.8	89.8
VIABLE FET. IN % IMPL.	SD	6.5	6.6	6.5	9.9
RESORPTIONEN IN % IMPL.	MW	9.0	9.2	3.2	10.2
RESORPTIONS IN % IMPL.	SD	6.5	6.6	6.5	9.9
PRAEIMPLANTAT.-VERLUST	MW	23.4	25.2	31.1	32.5
PRE IMPLANTAT. LOSS %	SD	4.1	2.5	1.4	2.7
POSTIMPLANTAT.-VERLUST	MW	9.0	9.2	3.2	10.2
POST IMPLANTAT. LOSS %	SD	6.5	6.6	6.5	9.9
MISSB. IN % LEB. FET.	MW	0.1	0.1	0.1	0.2
MALFORM. IN % VIAB. FET.	SD	1.3	1.9	0.8	1.9
VARIAT. IN % LEB. FET.	MW	1.8	0.3	1.1	0.9
VARIAT. IN % VIAB. FET.	SD	5.6	1.8	6.6	4.6

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CALCULATIONS OF PERCENTAGES ARE ARCSINE-TRANSFORMATIONS

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Fetal Necropsies

A relatively even distribution was evident in the incidence of malformations and variations among fetuses from different treatment groups (Tab. C.3.d.3). The distribution of anomalies compared favorably to historical data. Deficient ossification of the 5th sternal anlage was noted with a slightly higher frequency in fetuses of the MD group (40% vs. 27.9-29.2% in the other 3 treatment groups).

Tab. C.3.d.3.

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SEGM. 2

MISSBILDUNGEN UND VARIATIONEN
MALFORMATIONS AND VARIATIONS

SUMMENWERTE / COLLECTIVE VALUES

BEFUNDE FINDINGS	KONTR. CONTR.	0.1 MG/KG	1 MG/KG	10 MG/KG
MALFORMATIONS				
MULTIPLE MISSBILDUNGEN MULTIPLE MALFORMATIONS	0	1	0	0
STERNEBRA-SYNOSTOSEN SYNOSTOSIS OF STERNABRAE	1	0	0	1
AGENESIE DER GALLENBLASE ABSENCE OF GALLBLADDER	0	0	1	0
AGENESIE EINES WIRBELKÖRPERS MISSING OF ONE VERTEBRA	0	0	0	1
VARIATIONS				
FLEXUR DER VORDERPFOTE(N) FLEXURAE OF FORE PAW(S)	5	1	4	4
13. RIPPE 13TH RIB	1	0	0	0
AGENESIE 12. RIPPE MISSING OF 12TH RIB	0	1	0	0
HYPOPLASIE 12. RIPPE SHORTENED 12TH RIB	0	0	0	1
AGENESIE LUNGENANHANGSLAPPEN MISSING OF ACCESSORIUS LOBE OF THE LUNG	1	1	3	0
RUNTS	4	8	0	2

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Plasma Concentrations (Satellite Study)

PPX was detectable in all plasma samples. The highest concentrations were noted 2 hr after dosing. Increases in AUC were generally dose proportional. PK parameters ($C_{2\,hr}$, AUC_{1-24}) associated with the lowest test dose were equivalent to or slightly greater than those of humans receiving the projected PPX maintenance dose of 1.5 mg, t.i.d. ($Cmax_{ss}$ = ng/ml, AUC_{0-8} =)

Table 7: Geometric mean value and ranges of plasma concentrations [ng/ml] after oral administration of SND 919 CL 2 Y in pregnant rabbits

hours	dose [mg/kg] 0.1*)	1.0	10.0
0	0.49	12.23	104.38
2	8.66	92.16	886.99
4	6.36	89.93	788.86
8	4.03	52.26	506.26
24	0.44	10.40	102.38

Table 8: AUC (0 - 24 hours) values [ng/ml·h] with geometric means and ranges

animal	Dose [mg/kg] 0.1	1	10
1	(72.81)*)	1120.41	10370.14
2	69.89	1094.90	8047.06
3	75.00	1228.85	11726.36
4	104.70	905.50	10935.35
geometric - mean - range	81.87	1080.90	10170.79

C.3.e. Segment III in Rats: Perinatal and Postnatal Toxicity

Document #(s): BI Document U89-0091
Upjohn TR 7219-94-076

Sponsor Volume: 1.44

Summary:

Pramipexole was administered by gavage at doses of 0, 0.1, 0.5 and 1.5 mg/kg/day to female Wistar rats (24/dose) from day 16 of gestation to day 21 of the rearing phase (lactation). Overt drug-related changes were agitation and increased activity in the MD and HD dams during lactation. Food intake was also decreased at the high dose. No *in utero* toxicities were apparent. The major toxicity observed was an impairment of pup development reflected by a decreased body weight gain during the lactation/rearing phase, and a slight (insignificant) delay in eye-opening in pups of the MD and HD dams. The sponsor suggests that the basis of this effect is CNS activation in the dams leading to an inability of the pups to suckle; no evidence to support this hypothesis was presented. An alternative explanation is the inhibitory effect of PPX on prolactin release impairs milk-production in the dams. Despite the reduced body weight development, fertility of the F1 offspring was not impaired. No remarkable drug-related changes in litter parameters or at necropsy of the pups were evident. Since the development and maintenance of pregnancies of HD rats were comparable to the other groups, in contrast to the Segment I and II studies where PPX dramatically impaired pregnancy, it may be concluded that the deleterious effects of PPX on rat reproduction occur at the early, prolactin-dependent implantation stage of pregnancy (circa days 6-8).

Methods:

Dosages: 0.1, 0.5, 1.5 mg/kg/day (Drug Lot: Batch III; prepared in distilled water) from day 16 of gestation to day 21 of rearing.

Low dose is 5 times the expected human dose (at the time of study initiation). The high dose was selected based on a previous Segment II study, in which signs of maternal and fetal toxicity were apparent.

Route of Administration: oral (gavage)

Species/Number: 96 inseminated females (24/group)

Approximate initial weight/age: 218 g / 10 weeks

Parameters monitored/Intervals:

Clinical	-	daily
Body weight	-	days 1, 7, 11, 16, and 22 of gestation, and daily during lactation

Food consumption	-	weekly
Litter parameters	-	Size, live/still births, and gross abnormalities were recorded. Non-pregnant females were excluded from mean calculations. Litters were reduced to 8 pups (4/sex) at day 4 post-partum. Functional, maturational and behavioral tests were conducted on the pups. At day 21, dams were necropsied, and half the offspring were examined for visceral abnormalities. The remaining weanlings were tested for swimming ability, Preyer reflex, pupillary reflex, water T-maze, and fertility (1 male and 1 female per litter).

Statistics

Statistical comparisons were made by Bartlett's test, one-way ANOVA, Newman-Keuls test for multiple comparisons, and Chi-square and Fisher's exact test.

Results:

Effects on Dams

Mortality: One control dam died from maladministration.

Clinical Signs:

Restlessness and agitation at MD and HD during lactation.

Body Weight Gain:

No significant effects during gestation, but significantly increased in the LD group on day 3 and 4 of lactation (Fig. C.3.e.1).

Food Intake:

Non-significant decrease in HD group (%) during week 3 of gestation and the 3 week lactation period.

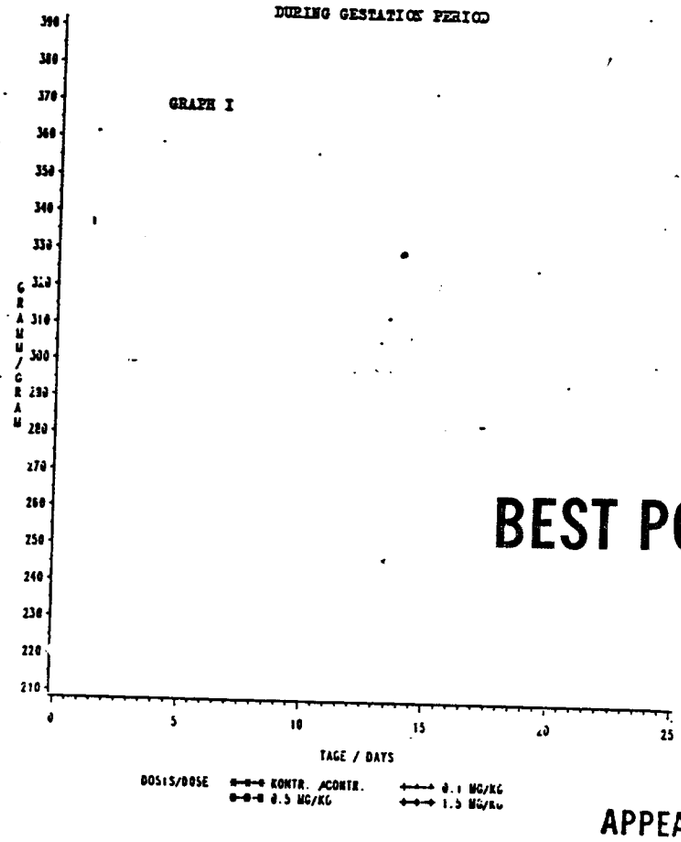
Gestation:

Nine of 96 females were not pregnant (1 CON, 3 LD, 2 MD, 3 HD). All except 1 HD dam delivered on day 23.

Necropsy:

No significant drug-related pathologies were found. One control dam had pulmonary edema, and MD dam had blood and caliculi in the urinary tract.

Fig. C.3e.1.



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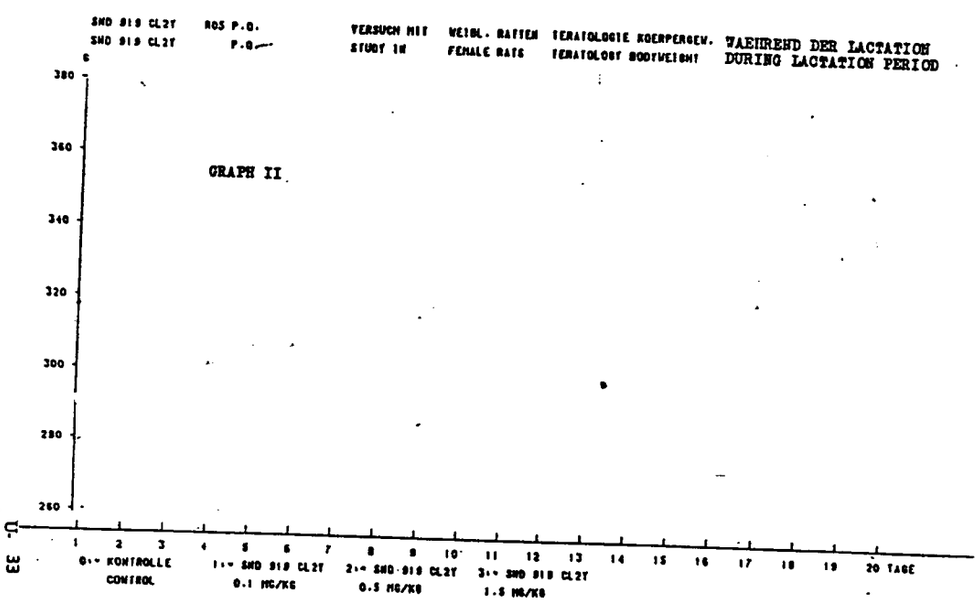

Effects on fetuses and pups

The most notable effects of PPX in the offspring were significant reductions in body weight at days 4 and 21, and body weight gain between days 1-4 and 4-21 in pups from MD and HD dams (Fig. C.3.e.2). Mortality in pups during rearing was due to cannibalism (1 CON, 1 LD, and 1 HD litter) (Tables C.3.e.1-2). With respect to maturational parameters, a slight (insignificant) delay in eye-opening was noted. There were no impairments in swimming, visual, auditory or memory tests (Tab. C.3.e.3). At autopsy, 2 LD and 1 MD weanling had hydronephrosis.

Fertility in Offspring

All females were inseminated within a 10 day mating period except for 1 control, 1 LD, and 1 MD offspring. Three of 80 inseminated females did not become pregnant (1 control, 1 LD, and 1 MD). Mean body weight of the HD group was significantly reduced during gestation, although body weight gain was increased (Tab. C.3.e.4). There were no differences among groups in corpora lutea number, implantations, viable embryos, or resorptions (Tab. C.3.e.5). At autopsy, one dam was diagnosed with hepatocellular necrosis.

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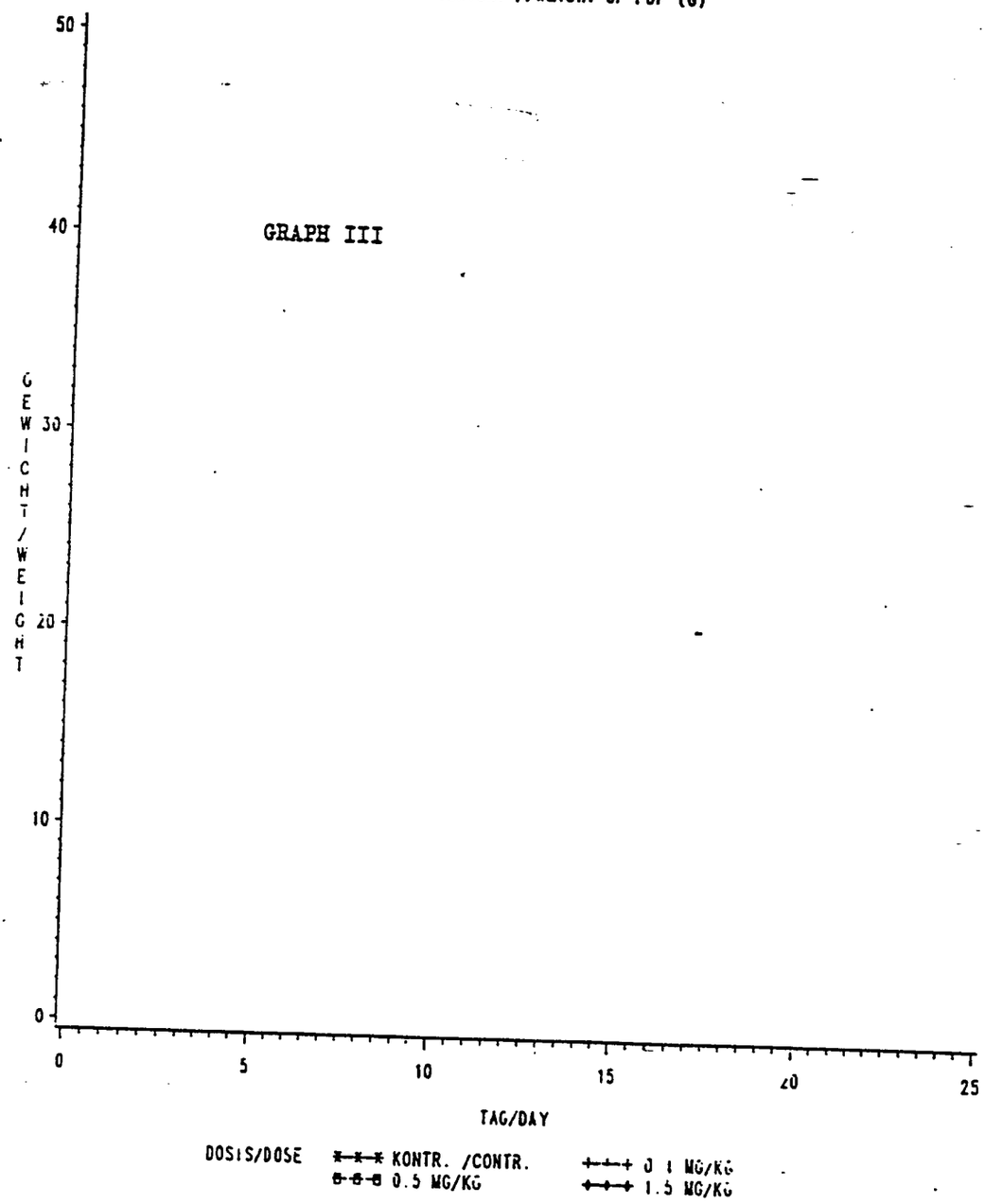
Fig. C.3.e.2.

— BOEHRINGER INGELHEIM KG —

SND 919CL2Y VERS.NR./STUDY.NO. R05 SEGM. 3

JUNGTIERGEWICHT /. WEIGHT OF PUP (G) RATTEN/RATS



TR No.: 7219-94-076

Tab. C.3.e.1

- BOEHRINGER INGELHEIM KG -

SND 919CL2Y

VERS.NR./STUDY.NO.: R05 SEGM. 3

RATTEN/RATS

WURFDATEN / LITTER DATA
SUMMENWERTE / SUM VALUES

	KONTR. /CONTR.	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG
GESAMTZAHL TIERE TOTAL NUMBER ANIMALS	24	24	24	24
TODESFAELLE MATERNAL MORTALITY	0	0	1	0
FEHLWUERFE ABORTIONS	0	0	0	0
TRAECHTIGE TIERE PREGNANT ANIMALS	23	21	21	21
TOTE JUNGTIERE STILLBIRTHS	1	1	0	1
JUNGTIERE 1. TAG VIABLE PUPS DAY 1	279	276	278	272
MAENNLICH IN % MALE IN %	53.05	46.74	52.52	54.04
WEIBLICH IN % FEMALE IN %	46.95	53.26	47.48	45.96
JUNGTIERE 4. TAG VIABLE PUPS DAY 4	261	273	276	255
JUNGTIERE 4*. TAG VIABLE PUPS DAY 4*	174	165	168	160
JUNGTIERE 7. TAG VIABLE PUPS DAY 7	173	157	168	158
JUNGTIERE 14. TAG VIABLE PUPS DAY 14	173	157	167	158
JUNGTIERE 21. TAG VIABLE PUPS DAY 21	173	157	167	158
VERENDETE JUNGTIERE MORT. IN PUPS 1 - 4	18	3	2	17
VERENDETE JUNGTIERE MORT. IN PUPS 4* -21	1	8	1	2
MISSBILDUNGEN MALFORMATIONS	0	2	1	0
VARIATIONEN VARIATIONS	0	0	0	0

* NACH WURFREDUZIERUNG/AFTER LITTER REDUCTION

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TR No.: 7219-94-076

Tab. C.3.e.2.

- BOEHRINGER INGELHEIM KG -

SKD 919CL2Y

VERS.NR./STUDY.NO.: R05 SEGM. 3

RATTEN/RATS

WURFDATEN / LITTER-DATA
MITTELWERTE / MEAN-VALUES

		KONTR. /CONTR.	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG
TRAGEZEIT DER MUTTERTIERE	MW	22.00	22.00	22.00	22.05
GESTATION PERIOD OF DAMS	SD	0.00	0.00	0.00	0.22
ANZ. TIERE / NUMB. OF ANIM.	N	23.00	21.00	21.00	21.00
GEWICHT/JUNGTIER (G) TAG 1	MW	6.12	6.12	6.06	5.94
WEIGHT/PUP AT (G) DAY 1	SD	0.29	0.24	0.23	0.29
GEWICHT/JUNGTIER (G) TAG 4	MW	7.95	7.82	** 7.41	*** 7.00
WEIGHT/PUP (G) DAY 4	SD	0.46	0.52	0.49	0.52
GEWICHT/JUNGTIER (G) TAG 4*	MW	8.00	7.94	** 7.53	*** 7.11
WEIGHT/PUP (G) DAY 4*	SD	0.48	0.46	0.44	0.52
GEWICHT/JUNGTIER (G) TAG 21	MW	41.19	41.99	***36.03	***26.03
WEIGHT/PUP (G) DAY 21	SD	3.90	2.37	2.66	6.52
GEWICHTSZUN. (G) TAG 1-4	MW	1.76	1.63	*** 1.32	*** 1.02
BODYW. INCR. (G) DAY 1-4	SD	0.46	0.46	0.40	0.45
GEWICHTSZUN. (G) TAG 4*-21	MW	33.19	34.07	***28.48	***18.88
BODYW. INCR. (G) DAY 4*-21	SD	4.04	2.78	2.70	6.17
LEB.JUNGT./MUTTERT. TAG 1	MW	12.13	13.14	13.24	12.95
VIABLE PUPS/ DAM DAY 1	SD	2.40	2.89	1.81	2.20
TOTE JUNGT./MUTTERT. TAG 1	MW	0.04	0.05	0.00	0.05
DEAD PUPS / DAM DAY 1	SD	0.21	0.22	0.00	0.22
LEB.JUNGT./MUTTERT. TAG 4	MW	11.86	13.00	13.14	12.75
VIABLE PUPS/ DAM DAY 4	SD	2.36	2.90	1.85	2.10
LEB.JUNGT./MUTTERT. TAG 4*	MW	7.91	7.86	8.00	8.00
VIABLE PUPS/ DAM DAY 4*	SD	0.43	0.48	0.00	0.00
LEB.JUNGT./MUTTERT. TAG 21	MW	7.86	7.85	7.95	7.90
VIABLE PUPS/ DAM DAY 21	SD	0.47	0.49	0.22	0.31
JUNGTIERVER. % TAG 1-4	MW	6.31	1.09	0.81	8.52
PUPS LOSS % DAY 1-4	SD	20.70	2.77	2.69	22.16
JUNGTIERVERL. % TAG 4*-21	MW	0.57	4.76	0.60	1.25
PUPS LOSS % DAY 4*-21	SD	2.67	21.82	2.73	3.85
MISSBILDUNGEN / MUTTERTIER	MW	0.00	0.10	0.05	0.00
MALFORMATIONS / DAM	SD	0.00	0.44	0.22	0.00
RIATIONEN / MUTTERTIER	MW	0.00	0.00	0.00	0.00
RIATIONS / DAM	SD	0.00	0.00	0.00	0.00
MISSB. % LEB. JUNGT./TAG 1	MW	0.00	0.68	0.34	0.00
MALF. % VIAB. PUPS / DAY 1	SD	0.00	3.12	1.56	0.00
VARIAT. % LEB. JUNGT./TAG 1	MW	0.00	0.00	0.00	0.00
VARIAT. % VIAB. PUPS/ DAY 1	SD	0.00	0.00	0.00	0.00

** $p < 0.01$
*** $p < 0.001$

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CALCULATIONS OF PERCENTAGES ARE ARCSINE-TRANSFORMATIONS
* NACH WURFREDUZIERUNG/AFTER LITTER REDUCTION

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SND 919 CL 2 Y

Tab. C.3.e.3.

RATTEN/RATS

BEOBACHTUNGEN DER F_1-GENERATION DER GRUPPE DER SPONTANGEBURTEN

OBSERVATIONS OF F_1-OFFSPRING IN SPONTANEOUS DELIVERY GROUP

SEGM. 3

ERGEBNISSE IN PROZENT / RESULTS IN PER CENT

VERS.NR./STUDY NO. R 05

DOSIS / DOSE	AUSGEWERTETE WURFE / EVALUATED LITTERS	AUFSTELLEN DER OHREN / ERECTION OF PINNAE		FELLWACHSTUM / FUR GROWTH		LAUFEN MIT ANGEHOBENEM BAUCH / RUNNING WITH RAISED VENTER		DURCHBRECHEN DER OBEREN SCHNEIDEZÄHNE / ERUPTION OF MAXILLARY INCISORS		AUGENÖFFNUNG / EYE OPENING		
		TAG 4	DAY 5	TAG 6	DAY 7	TAG 12	DAY 13	TAG 12	DAY 13	TAG 15	DAY 16	DAY 17
KONTROLLE/ CONTROL	22	8.0	98.9	-	100.0	100.0	100.0	100.0	100.0	20.8	75.1	98.8
0.1 MG/KG	20	8.9	100.0	-	100.0	100.0	100.0	100.0	100.0	17.8	79.0	100.0
0.5 MG/KG	21	22.6	100.0	-	100.0	100.0	100.0	100.0	100.0	6.0	57.5	99.4
1.5 MG/KG	20	17.0	98.7	-	100.0	83.5	99.4	100.0	100.0	8.9	41.1	89.2

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Tab. C.3.e.4.

BOEHRINGER INGELHEIM KG REPRODUKTIONSTOXIKOLOGIE

SUBST./COMP. : SHD 919CL2Y
VERS.NR./STUDY NO. : R 05

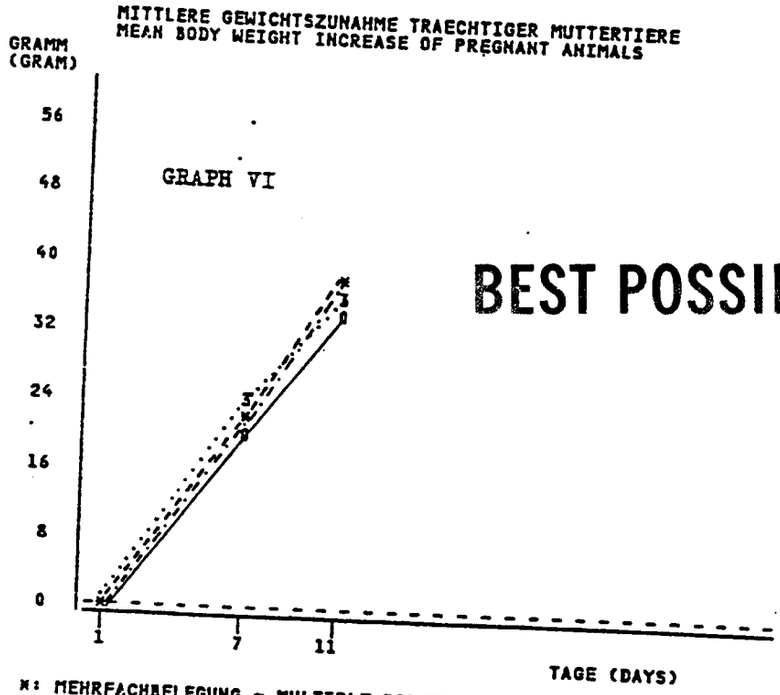
TIERART/SPEC. : RATTEN / RATS
SEGM. 3

KOERPERGEWICHT TRAECHTIGER MUTTERTIERE (G) (TAG 1, 7, 11)
BODY WEIGHT OF PREGNANT ANIMALS (G)(DAYS 1, 7, 11)
MITTELWERTE / MEAN VALUES F_1-GENERATION

		N	1	7	11
KONTROLLE CONTROL	MW	20	246.1	266.1	279.4
	SD		18.0	18.9	17.7
0.1 MG/KG	MW	18	239.1	260.5	277.1
	SD		18.6	18.9	19.0
0.5 MG/KG	MW	19	233.6	256.6	271.8
	SD		18.2	19.3	20.5
1.5 MG/KG	MW	20	213.3***	237.6***	250.1***
	SD		19.2	20.1	20.6

*** p < 0.001

MITTLERE GEWICHTSZUNAHME TRAECHTIGER MUTTERTIERE
MEAN BODY WEIGHT INCREASE OF PREGNANT ANIMALS



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X: MEHRFACHBELEGUNG - MULTIPLE POINTS
0:KONTR -CONTR 1: 0.1 MG/KG 2: 0.5 MG/KG 3: 1.5 MG/KG

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Tab. C.3.e.5.

WURFDATEN / LITTER DATA
SUMMENWERTE / SUM VALUES F_1-GENERATION
(GETOETET 14.-16. TAG / SACRIFICED DAY 14-16)

	KONTROLLE CONTROL	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG
GESAMTZAHL DER TIERE TOTAL NUMBER OF ANIMALS	21	19	20	20
TODESFAELLE MATERNAL MORTALITY	0	0	0	0
TOTALRESORPTIONEN TOTAL RESORPTIONS	0	0	0	0
SPONTANWUERFE NATURAL DELIVERIES	0	0	0	0
TIERE MIT LEB. FETEN ANIM. WITH VIABLE FET.	20	18	19	20
GELBKOERPER CORPORA LUTEA	313	291	300	313
IMPLANTATIONEN IMPLANTATIONS	304	277	290	309
LEBENDE FETEN VIABLE FETUSES	278	249	264	289
RESORPTIONEN RESORPTIONS	26	28	26	20

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WURFDATEN / LITTER DATA
MITTELWERTE / MEAN VALUES F_1-GENERATION
(GETOETET 14.-16. TAG / SACRIFICED DAY 14-16)

		KONTROLLE CONTROL	0.1 MG/KG	0.5 MG/KG	1.5 MG/KG
CORPORA LUTEA / MUTTERT. CORPORA LUTEA / DAM	MW	15.6	16.2	15.8	15.6
	SD	2.0	2.3	1.4	1.5
IMPLANTATIONEN / MUTTERT. IMPLANTATIONS / DAM	MW	15.2	15.4	15.3	15.4
	SD	2.8	3.3	2.4	1.5
LEBENDE KEIME / MUTTERT. LIFE EMBRYOS / DAM	MW	13.9	13.4	13.9	14.4
	SD	3.8	4.2	2.3	1.5
RESORPTIONEN / MUTTERT. RESORPTIONS / DAM	MW	1.3	2.0	1.4	1.0
	SD	2.5	2.7	1.7	1.1
LEBENDE KEIME IN % IMPL. LIFE EMBRYOS IN % IMPL.	MW	96.4	90.8	95.4	96.4
	SD	6.9	7.7	4.6	3.0
RESORPTIONEN IN % IMPL. RESORPTIONS IN % IMPL.	MW	3.6	7.6	4.6	3.6
	SD	6.9	6.5	4.6	3.0
PRAEIMPLANTAT.-VERLUST PRE IMPLANTAT. LOSS %	MW	0.6	1.2	0.6	0.2
	SD	3.6	5.6	3.7	1.2

PROZENTWERTBERECHNUNG UEBER ARCSIN-TRANSFORMATIONEN
CALCULATIONS OF PERCENTAGES ARE ARCSINE-TRANSFORMATIONS

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C.4. Genotoxicity

a. Testing for Point Mutagenic Activity of PPX (dihydrochloride) with *Salmonella typhimurium*
b. Testing for Point Mutagenic Activity of PPX (free base) with *Salmonella typhimurium*
c. Cell Transformation Assay in Syrian Hamster Embryo Cells
d. Induction of Chromosome Aberrations in Chinese Hamster Ovary Cells
e. V79 Gene Mutation Assay for HGPRT Mutants
f. *In vivo* Mouse Micronucleus Test

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

except for studies *d* (Cytotest) and *e* (NOTOX) (see text for addresses).

All studies complied with GLP.

C.4.a. Testing for Point Mutagenic Activity of PPX dihydrochloride with *Salmonella typhimurium*

Document #(s): BI Document U85-0554
Upjohn TR 7219-94-079
Sponsor Volume: 1.44

Summary:

Pramipexole was not mutagenic under the conditions employed in this study. The highest dose tested was appropriate. The strains employed for conferring of his-independence are not sensitive to A-T substitutions. A second study (C.4.b.) was conducted to meet this criterion.

Methods:

Drug concentrations: 10, 100, 500, 1000, 5000 µg/plate (Batch C of PPX dihydrochloride prepared in distilled water)

Positive controls/vehicles:
1-methyl-3-nitro-1-nitrosoguanidine/DMSO (dil. w. 50% DMSO)
2-nitrofluorene/DMSO
4-nitroquinoline-1-oxide/20%DMSO (dil. w. PBS)
9-aminoacridine/water
2-aminoanthracene/DMSO
benzo(a)pyrene/DMSO
emodin/DMSO

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Bacterial Tester Strains:

Strain	Genetic characteristics
TA1535	hisG46, ΔuvrB, rfa
TA1537	hisC3076, ΔuvrB, rfa
TA100	hisG46, ΔuvrB, rfa, R fact.
TA98	hisD3052, ΔuvrB, rfa, R fact.

Base-pair (G-C) substitutions or frameshift mutations in the **his** operon confer **his**-independence; **uvrB** gene deletion confers defective excision repair system; **rfa** mutation increases permeability to chemical mutagens; **R factor** increases sensitivity to mutagens.

Metabolizing System: S9 fraction prepared from Arochlor-induced rat (500 mg/kg given five days before tissue harvest)

Test Conditions: Test system components (agar, bacteria, test substance, +/- S9) mixed and poured onto plates (triplicate samples). Plates were incubated for two days at 37° and the number of revertants determined.

Results:

Spontaneous reversion frequencies on control plates were within historical control. Pramipexole did not increase mutation frequency above control values in any tester strain at any dose in the presence or absence of S9. PPX was not toxic to indicator cells. The positive controls produced the expected increases in mutation frequency (Tables C.4.a.1-4).

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Tab. C.4.a.1

Table 1: Induction of His^+ mutations in S. typhimurium by SNO 919 CL 2 in the absence of a metabolizing system (Experimental part No. MUT 0074/01)

Substance[a]	Concentration (µg/plate)	S9[b]	Number of His^+ revertants per plate[c,d]							
			TA1535		TA1537		TA100		TA98	
			n	$\bar{n}$	n	$\bar{n}$	n	$\bar{n}$	n	$\bar{n}$
Control		-	6 15 11	11	5 16 8	10	108 151 127	129	23 20 30	24
SNO 919 CL 2	10	-	8 11 13	11	6 7 5	6	148 154 139	147	22 27 28	26
	100	-	8 11 11	10	4 9 6	6	118 119 157	131	22 18 24	21
	500	-	6 6 4	5	6 7 9	7	116 153 122	130	23 26 20	23
	1000	-	7 8 5	7	4 7 9	7	120 152 144	139	28 17 23	23
	5000	-	5 8 4	6	9 6 4	6	160 140 121	140	30 18 17	22
MNNG	2.5	-	>2000 >2000 >2000		16 20 29	22	>2000 >2000 >2000		19 22 23	21
2-NF	10	-	13 6 8	9	116 131 132	126	>2000 >2000 >2000		>2000 >2000 >2000	

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Tab. C.4.a.2.

Table 2: Induction of His^+ mutations in S. typhimurium by SNO 919 CL 2 in the absence of a metabolizing system - repeat study (Experimental part No. MUT 0074/05)

Substance[a]	Concentration (µg/plate)	S9[b]	Number of His^+ revertants per plate[c,d]							
			TA1535		TA1537		TA100		TA98	
			n	$\bar{n}$	n	$\bar{n}$	n	$\bar{n}$	n	$\bar{n}$
Control		-	27 18 28	24	6 9 7	7	154 C 170	162	27 23 31	27
SNO 919 CL 2	10	-	28 25 24	26	5 8 6	6	171 170 133	158	29 30 19	26
	100	-	30 38 34	34	10 6 8	8	131 166 127	141	19 31 19	23
	500	-	14 14 14	14	4 8 8	7	142 143 167	151	26 18 26	23
	1000	-	18 20 24	21	8 6 11	8	161 160 162	161	27 25 20	24
	5000	-	20 10 24	18	7 7 8	7	139 151 142	144	17 27 17	20
4-NQO	1	-	98 88 58	81	29 29 25	28	>2000 >2000 >2000		549 515 C	532
9-AA	100	-	36 14 26	25	489 280 305	358	156 126 135	139	8 9 11	9

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Tab. C.4.a.3

Table 3: Induction of His$^+$ mutations in S. typhimurium by SKD 919 CL 2 in the presence of a metabolizing system (Experimental parts No. MUT 0074/02 and /03)

Substance[a]	Concentration (µg/plate)	S9[b]	Number of His$^+$ revertants per plate[c,d]							
			TA1535		TA1537		TA100		TA98	
			m	$\bar{m}$	m	$\bar{m}$	m	$\bar{m}$	m	$\bar{m}$
Control		+	9 13 12	11	9 7 7	8	167 190 174	177	31 49 44	41
SKD 919 CL 2	10	+	16 8 11	12	14 11 12	12	149 170 156	158	40 42 50	44
	100	+	4 9 17	10	6 8 7	7	177 182 168	176	53 42 36	44
	500	+	12 8 7	9	6 9 11	9	173 172 167	171	50 52 59	54
	1000	+	5 7 14	9	6 5 12	8	173 163 163	166	55 46 60	54
	5000	+	6 17 16	13	8 11 6	8	171 198 176	182	85 88 61	78
2-AA	0.5	+					279 535 468	427		
	2.5	+	375 406 384	388	351 372 368	364			1118 1268 808	1065
	0.5	-					157 151 168	159		
	2.5	-	13 5 9	9	4 20 9	11			41 31 28	33

Tab. C.4.a.4.

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Table 4: Induction of His$^+$ mutations in S. typhimurium by SKD 919 CL 2 in the presence of a metabolizing system - repeat study (Experimental part No. MUT 0074/04)

Substance[a]	Concentration (µg/plate)	S9[b]	Number of His$^+$ revertants per plate[c,d]							
			TA1535		TA1537		TA100		TA98	
			m	$\bar{m}$	m	$\bar{m}$	m	$\bar{m}$	m	$\bar{m}$
Control		+	4 14 10	9	3 13 7	8	159 151 153	154	37 35 35	36
SKD 919 CL 2	10	+	14 10 14	13	7 7 9	8	149 148 152	150	50 42 33	42
	100	+	15 7 2	8	6 8 11	8	153 159 149	154	37 41 38	39
	500	+	18 10 16	15	8 11 11	10	132 142 145	140	35 36 41	37
	1000	+	10 20 16	12	6 15 8	9	159 C 173	166	45 44 48	46
	5000	+	13 16 19	16	7 C 9	8	175 162 156	164	58 35 42	45
B(a)P	5	+	14 9 18	14	164 156 120	147	953 C 896	925	592 498 549	546
Emodin	10	+	11 10 8	10	498 509 475	494	274 314 349	312	36 36 40	37

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C.4.b. Testing for Point Mutagenic Activity of PPX (free base) with *Salmonella typhimurium*

Document #(s): BI Document U90-0639
Upjohn TR 7219-94-084

Sponsor Volume: 1.44

Summary:

This experiment confirms the previous study that PPX is not mutagenic in *S. typhimurium* strains sensitive to mutations that affect G-C sites. The absence of mutagenicity in the *E. Coli* strains suggests that PPX does not induce mutations by affecting A-T base pairs. The highest dose tested was appropriate, and positive controls produced the expected results.

Methods:

Drug concentrations: 10, 100, 500, 1000, 5000 µg/plate (Batch III of PPX free base prepared in DMSO)

Positive Controls/vehicles:
1-methyl-3-nitro-1-nitrosoguanidine/DMSO
1-ethyl-3-nitro-1-nitrosoguanidine/DMSO
2-nitrofluorene/DMSO
2-aminoanthracene/DMSO

Bacterial Tester Strains:

Strain	Genetic characteristics
TA1535	hisG46, ΔuvrB, rfa
TA1537	hisC3076, ΔuvrB, rfa
TA1538	hisD3052, ΔuvrB, rfa
TA100	hisG46, ΔuvrB, rfa, R fact.
TA98	hisD3052, ΔuvrB, rfa, R fact.

Base-pair (G-C) substitutions or frameshift mutations in the **his** operon confer **his**-independence; **uvrB** gene deletion confers defective excision repair system; **rfa** mutation increases permeability to chemical mutagens; **R factor** increases sensitivity to mutagens.

In addition, the WP2uvrA strain of E. Coli was used. Point mutations in the trp operon of this strain confer trp-independence. It is sensitive to the base-pair substituting (A-T) activity of alkylating agents.

Metabolizing System: S9 fraction prepared from Arochlor-induced rat (500 mg/kg given five days before tissue harvest)

Test Conditions: Test system components (agar, bacteria, test substance, +/- S9) mixed and poured onto plates (triplicate samples). Plates were incubated for two days at 37° and the number of revertants determined.

Results:

Spontaneous reversion frequencies on control plates were within historical control. PPX did not increase mutation frequency above control values in any tester strain at any dose in the presence or absence of S9. PPX was not toxic to indicator cells. The positive controls produced the expected increases in mutation frequency (Tables C.4.b.1-4).

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Tab. C.4.b.1

Table 1. Induction of mutations in the absence of a metabolizing system

Experiment 1 (MUT 0193/01 and /05)

Substance[a]	Conc. (µg/pl.)	S9[b]	Number of revertants per plate[c] TA1535	TA1537	TA1538	TA100	TA98	WP2uvrA
Solvent control (DMSO, 100 µl/pl.)		-	3	29	13	103	19	14
			15	18	13	91	19	27
			5	25	14	104	20	25
			8	24	13	99	19	22
SND 919 BSY	10	-	6	34	11	107	25	20
			5	23	15	93	23	15
			5	23	20	95	29	13
			5	27	15	98	26	16
	100	-	2	24	14	107	15	13
			7	33	11	108	20	13
			3	22	13	66	22	15
			4	26	13	94	19	14
	500	-	3	23	17	83	26	16
			6	23	19	95	15	15
			2	23	13	81	19	16
			4	23	16	86	20	16
	1000	-	4	36	13	86	9	18
			3	36	14	99	28	12
			4	31	19	78	17	15
			4	34	15	88	18	15
	5000	-	5	20	11	81	18	17
			4	40	14	81	19	15
			4	26	9	62	13	14
			4	29	11	75	17	15
MNNG (1)	2.5	-	>2000	165	>2000	>2000	>2000	1006
2NF (2,3,4,5)	10		>2000	160	>2000	>2000	>2000	798
ENNG (6)	2.5		>2000	168	>2000	>2000	>2000	728
				164				845
Titer of the bacterial suspension (x10⁹/ml)			3.2 (338, 298, 327)[d]	2.2 (249, 196, 215)[d]	2.5 (235, 233, 274)[d]	0.6 (60, 49, 62)[d]	1.9 (205, 198, 162)[d]	1.9 (196, 196, 182)[d]

Tab. C.4.b.2.

Table 2. Induction of mutations in the absence of a metabolizing system

Experiment 2 (MUT 0193/02 and /05)

Substance[a]	Conc. (µg/pl.)	S9[b]	Number of revertants per plate[c] TA1535	TA1537	TA1538	TA100	TA98	WP2uvrA
Solvent control (DMSO, 100 µl/pl.)		-	9	9	15	107	31	23
			19	7	19	129	20	23
			24	9	14	95	30	19
			17	8	16	110	27	22
SND 919 BSY	10	-	8	26	C	126	28	8
			3	22	11	133	31	18
			4	3	29	104	15	17
			5	17	20	121	25	14
	100	-	7	11	15	115	27	16
			14	3	12	116	30	13
			4	10	13	117	27	15
			8	8	13	116	28	15
	500	-	8	7	20	89	14	14
			5	4	19	104	23	10
			6	23	15	137	14	12
			6	11	18	110	17	12
	1000	-	3	18	18	96	35	14
			7	9	16	82	28	15
			9	5	18	105	16	14
			6	11	17	94	26	14
	5000	-	3	3	11	86	11	17
			5	8	20	101	14	11
			7	7	6	107	10	9
			5	6	12	98	12	12
MNNG (1)	2	-	1279	145	>2000	>2000	>2000	928
2NF (2,3,4,5)	10		1597	143	>2000	>2000	>2000	994
ENNG (6)	2.5		1482	183	>2000	>2000	>2000	968
			1453	157				963
Titer of the bacterial suspension (x10⁹/ml)			2.3 (244, 242, 200)[d]	1.8 (194, 181, 171)[d]	3.0 (294, 302, 297)[d]	0.8 (92, 62, 72)[d]	1.8 (185, 170, 174)[d]	1.9 (199, 178, 206)[d]

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Tab. C.4.b.3.

Table 3. Induction of mutations in the presence of a metabolizing system

Experiment 1 (MUT 0193/03 and /05)

Substance[a]	Conc. (µg/pl.)	S9[b]	Number of revertants per plate[c] TA1535	TA1537	TA1538	TA100	TA98	WP2uvrA
Solvent control (DMSO, 100 µl/pl.)		+	6	6	17	86	34	26
			6	14	16	106	29	11
			4	7	24	105	37	17
			5	9	19	99	33	18
SND 919 BSY	10	+	7	14	30	102	26	16
			5	12	37	100	33	14
			7	14	25	108	18	23
			6	13	31	103	26	18
	100	+	11	7	15	105	30	c
			2	11	26	100	28	15
			5	13	30	94	29	13
			6	10	24	100	29	14
	500	+	7	8	31	124	31	16
			6	12	14	107	42	19
			4	9	34	95	25	14
			6	10	26	109	33	16
	1000	+	2	17	17	107	25	11
			11	9	36	141	23	19
			6	9	26	129	34	9
			6	12	26	126	27	13
	5000	+	7	7	23	121	34	17
			10	4	26	117	34	17
			7	4	18	118	25	14
			8	5	22	119	31	16
2AA (7.4.5) (1.2) (5)	0.5	+	168	239	554	338	275	304
	2.5		207	245	472	358	255	301
	5		218	250	482	396	264	490
			198	245	503	364	265	365
Titer of the bacterial suspension (x10⁸/ml)			2.3 (194, 244, 247)[d]	2.0 (175, 235, 193)[d]	2.9 (292, 318, 244)[d]	0.9 (86, 88, 82)[d]	2.0 (195, 178, 220)[d]	1.9 (196, 196, 182)[d]

Tab. C.4.b.4.

Table 4. Induction of mutations in the presence of a metabolizing system

Experiment 2 (MUT 0193/04 and /05)

Substance[a]	Conc. (µg/pl.)	S9[b]	Number of revertants per plate[c] TA1535	TA1537	TA1538	TA100	TA98	WP2uvrA
Solvent control (DMSO, 100 µl/pl.)		+	14	5	18	122	41	9
			6	3	27	131	38	19
			9	11	22	126	50	25
			10	6	22	126	43	18
SND 919 BSY	10	+	13	8	24	107	50	16
			11	5	24	123	47	20
			6	4	27	116	50	15
			10	6	25	115	49	17
	100	+	12	9	19	121	46	22
			13	8	26	102	42	23
			7	11	23	119	28	16
			11	9	23	114	39	20
	500	+	5	11	30	110	34	24
			4	6	25	102	31	11
			9	14	23	134	39	18
			6	10	26	115	35	18
	1000	+	5	16	30	107	39	18
			6	7	23	122	45	9
			8	7	24	105	40	18
			6	10	26	111	41	15
	5000	+	7	7	28	108	33	16
			7	4	20	100	27	13
			7	6	22	128	31	12
			7	6	23	112	30	14
2AA (7.4.5) (1.2) (5)	0.5	+	334	177	354	313	319	351
	2.5		367	172	375	346	360	561
	5		341	194	381	332	336	369
			347	181	370	330	338	427
Titer of the bacterial suspension (x10⁸/ml)			2.3 (232, 214, 244)[d]	1.3 (126, 110, 146)[d]	2.0 (200, 198, 194)[d]	0.5 (50, 45, 41)[d]	1.7 (167, 182, 165)[d]	1.9 (199, 178, 206)[d]

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C.4.c. Cell Transformation Assay in Syrian Hamster Embryo Cells

Document #(s): BI Document U87-1074
Upjohn TR 7219-94-082

Conducted by:

Sponsor Volume: 1.44

Summary:

In this transformation experiment, no reproducible transforming potential of PPX was detected in Syrian Hamster Embryo cells. The dosage range used in this study (μg/ml) was limited by cytotoxicity criteria established by the sponsor (minimum desired survival rate of %). This cytotoxicity level is below that established in the newer guidelines (no more than 20% survival), as is the exposure range (desired upper treatment levels for mammalian cells is 5 mg/ml or 10 mM = 2.9 mg/ml for PPX).

Since this assay is not in the core battery for genotoxicity testing, a repetition of the test at higher doses is not required. In view of the relatively low transformation rate with the positive controls, the utility of this test in predicting genotoxic potential is questionable.

Methods:

Drug Concentrations and Exposures: PPX dihydrochloride (Batch I) prepared in nutrient medium at the following concentrations:

Without S9 activation:

4 hr:	10, 25, 40, 100 μg/ml
48 hr:	10, 25, 40, 100 μg/ml

With S9 activation:

4 hr:	10, 40, 200, 400 μg/ml

Positive Controls/vehicles:

Without S9 activation:

1-methyl-3-nitro-1-nitrosoguanidine/DMSO (0.5 μg/ml medium)

With S9 activation:

Benzo(a)pyrene/DMSO (5 μg/ml medium)

(final concentration of DMSO in medium was 1%)

Test System:

Cultures prepared from 12-14 day Syrian Hamster embryos (decapitated, eviscerated), and propagated in 175 cm^2 (feeder cells) or 25 cm^2 (target cells) flasks. Cultures were exposed to the test substance for the indicated times (4 or 48 hr). Cultures were maintained for up to 10 days after the initial seeding, then fixed and stained. Colonies within each flask were examined for morphological transformations:

- criss-cross growth in marginal zones of colonies
- three-dimensional piling up of cells in center of the colony
- reduced cytoplasm to nuclear ratio

One or two thousand colonies per test condition were scored (100 colonies in 10 or 20 flasks). A response was considered positive if two or more transformed colonies occur in flasks treated with test article. Historical control frequency of spontaneous alterations is 0.05 - 0.1%

Metabolizing System:

S9 fraction prepared from Arochlor-induced rat (500 mg/kg given five days before tissue harvest)

Results:

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Determination of cytotoxicity:

Experimental doses for the main study were selected based on the following survival/toxicity data:

Final concentration µg/ml	% relative survival without S9 mix 48 h	with S9 mix 4 h
0 = medium	100.0	100.0
5.0	89.4	96.0
10.0	87.8	95.6
50.0	54.3	84.9
100.0	43.0	71.6
250.0	0.0	59.6
500.0	0.0	38.7
750.0	0.0	2.2
1000.0	0.0	0.0
2900.0	0.0	0.0

According to these data the concentrations applied in the transformation study were chosen.

PPX was toxic at concentrations above 100 µg/ml in the absence of S9, and above 250 µg/ml in the presence of S9. Doses for the transformation experiment were 100 and 400 µg/ml in the absence and presence of S9, respectively.

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Transformation Experiment I (Tables C.4.c.1-2):

RESULTS WITHOUT S9 MIX

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4 h Treatment Interval

Test groups	number of colonies scored	transf. colonies	% transf. colonies	PE = % survival absolute	relative
untreated cells	1,000	0	0.0	45.7	100.0
MNNG 0.5 µg/ml	1,000	13	1.3	24.9	54.5
test article					
10.0 µg/ml	1,000	1	0.1	45.4	99.3
25.0 µg/ml	1,000	0	0.0	37.8	82.7
40.0 µg/ml	1,000	1	0.1	31.4	68.7
100.0 µg/ml	1,000	0	0.0	22.6	49.5

48 h Treatment Interval

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Test groups	number of colonies scored	transf. colonies	% transf. colonies	PE = % survival absolute	relative
untreated cells	1,000	1	0.1	44.0	100.0
MNNG 0.5 µg/ml	1,000	11	1.1	28.0	63.6
test article					
10.0 µg/ml	1,000	0	0.0	43.4	98.6
25.0 µg/ml	1,000	0	0.0	36.6	83.2
40.0 µg/ml	1,000	1	0.1	26.1	59.3
100.0 µg/ml	1,000	2	0.2	20.7	47.0

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RESULTS WITH S9 MIX

4 h Treatment Interval

Test groups	number of colonies scored	transf. colonies	% transf. colonies	PE = % survival absolute	relative
untreated cells	1,000	0	0.0	44.9	100.0
B(a)P 5 µg/ml	1,000	12	1.2	30.5	67.9
test article					
4.0 µg/ml	1,000	0	0.0	45.5	101.3
40.0 µg/ml	1,000	0	0.0	36.9	82.2
200.0 µg/ml	1,000	0	0.0	30.0	66.8
400.0 µg/ml	1,000	0	0.0	21.8	48.6

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Similar results were obtained in a repeat experiment in which 2000 colonies per condition were scored, except that no more than 1 transformed colony appeared in PPX-treated cultures at a given concentration.

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