Data on live fetuses found at caesarean sections did not show treatment related effect (see table 13). Examination of fetuses (tables 14-15) **did not show external, visceral or skeletal abnormality due to the treatment.**

Table 13 — Group Mean Caesarian Data — Project No. 314/28

Foetal Data

F Generation

Parameter	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 15 mg/kg	Group 4 - 60 mg/kg	ANOVA F-Probability
Number of foetuses	198	131	164	89	
Mean number of foetuses per dam	13.2 ± 3.2	10.9 ± 5.6	13.7 ± 1.4	11.1 ± 2.8	
Mean litter weight (g)	45.2 ± 11.3	36.5 ± 18.3	46.3 ± 5.3	36.0 ± 8.9	0.1612
Mean foetal weight (g) overall +	3.27 ± 0.25	3.45 ± 0.42	3.39 ± 0.24	3.25 ± 0.15	0.3000
Mean foetal weight (g) males +	3.35 ± 0.25	3.47 ± 0.42	3.48 ± 0.28	3.38 ± 0.20	0.5862
Mean foetal weight (g) females +	3.18 ± 0.27	3.32 ± 0.42	3.28 ± 0.22	3.13 ± 0.16	0.4250
Number of males	105	75	83	44	
Number of females	93	58	81	45	
Mean proportion of male foetuses	52.8 ± 14.4	61.1 ± 18.1	50.1 ± 17.6	47.2 ± 21.1	

+ calculated from litter means

Table 14 — Group Mean Caesarian Data — Project No. 314/28

Malformation, Variation Data

F Generation

Parameter	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 15 mg/kg	Group 4 - 60 mg/kg
Number of foetuses examined externally	198	131	164	89
Number of foetuses with external malformations	0	3	2	0
% of foetuses examined	0.0	2.3	1.2	0.0
Number of litters with externally malformed foetuses	0	2	2	0
Number of foetuses examined viscerally	101	67	83	47
Number of foetuses with visceral malformations	1	0	0	0
% of foetuses examined	1.0	0.0	0.0	0.0
Number of litters with viscerally malformed foetuses	1	0	0	0
Number of foetuses examined viscerally	101	67	83	47
Number of foetuses with visceral variations	0	0	0	0
% of foetuses examined	0.0	0.0	0.0	0.0
Number of litters with foetuses showing visceral variations	0	0	0	0
Number of foetuses examined skeletally	97	64	81	42
Number of foetuses with skeletal malformations	3	0	1	1
% of foetuses examined	3.1	0.0	1.2	2.4
Number of litters with skeletally malformed foetuses	2	0	1	1
Number of foetuses examined skeletally	97	64	81	42
Number of foetuses with skeletal variations	94	61	81	41
% of foetuses examined	96.9	95.3	100.0	97.6
Number of litters with foetuses showing skeletal variations	15	11	12	8
Total number of malformed foetuses	4	3	3	1
Total number of litters with malformed foetuses	3	2	2	1
Average per cent malformed foetuses	2.0	2.3	1.8	1.1

Table 15 — Foetal Defects (Key, nature and incidence) — Project No. 314/28

P Generation

Parameter		Group 1 - 0 mg/kg		Group 2 - 5 mg/kg		Group 3 - 15 mg/kg		Group 4 - 40 mg/kg	
Key	Type of defect	Total number of affected foetuses	Percentage of foetuses examined	Total number of affected foetuses	Percentage of foetuses examined	Total number of affected foetuses	Percentage of foetuses examined	Total number of affected foetuses	Percentage of foetuses examined
	External malformations								
1	Umbilical hernia	0	0.0	1	0.8	1	0.6	0	0.0
3	Tail developed rudimentarily	0	0.0	2	1.5	1	0.6	0	0.0
24	Oedema on the neck	0	0.0	1	0.8	0	0.0	0	0.0
	Visceral malformations								
37	Cerebral ventricuii enlarged	1	1.0	0	0.0	0	0.0	0	0.0
	Skeletal malformations								
50	Scoliosis	3	3.1	0	0.0	1	1.2	1	2.4

Table 15 (cont.) — Foetal Defects (Key, nature and incidence) — Project No. 314/28

P Generation

Parameter		Group 1 - 0 mg/kg		Group 2 - 5 mg/kg		Group 3 - 15 mg/kg		Group 4 - 40 mg/kg	
Key	Type of defect	Total number of affected foetuses	Percentage of foetuses examined	Total number of affected foetuses	Percentage of foetuses examined	Total number of affected foetuses	Percentage of foetuses examined	Total number of affected foetuses	Percentage of foetuses examined
	Skeletal variations								
61	Frontal incompletely ossified	2	2.1	0	0.0	0	0.0	0	0.0
62	Parietal incompletely ossified	28	28.9	8	12.5	22	27.2	12	28.6
63	Interparietal incompletely ossified	83	85.6	47	73.4	73	90.1	36	85.7
64	Occipital incompletely ossified	56	57.7	33	51.6	45	55.6	20	47.6
65	Jugal incompletely ossified	2	2.1	0	0.0	4	4.9	0	0.0
67	Mandible incompletely ossified	3	3.1	0	0.0	0	0.0	0	0.0
68	Squamosal incompletely ossified	24	24.7	7	10.9	14	17.3	6	14.3
# 71	Single extra rib								
75	Wavy rib(s)	5	5.2	4	6.3	3	3.7	1	2.4
# 76	Rib(s) of uneven thickness								
77	Rib(s) incompletely ossified	4	4.1	3	4.7	6	7.4	3	7.1
# 78	13th pair of ribs shortened								
# 79	Rib(s) shortened								
80	Only 12 ribs on the left side	0	0.0	0	0.0	0	0.0	2	4.8
82	Bipartite vertebra(e)	0	0.0	1	1.6	2	2.5	0	0.0
83	Vertebra(e) incompletely ossified	40	41.2	30	46.9	28	34.6	11	26.2
# 84	Vertebra(e) with one ossification site								
85	Basal incompletely ossified	20	20.6	6	9.4	13	16.0	4	9.5
86	Sternebra(e) not ossified	72	74.2	40	62.5	61	75.3	30	71.4
87	Sternebra(e) incompletely ossified	22	22.7	21	32.8	29	35.8	16	38.1
# 88	Sternebra(e) asymmetrically ossified								
# 93	Rib(s) without connection to the vertebral column								
95	Pelvic girdle partly incompletely ossified	5	5.2	4	6.3	4	4.9	1	2.4
96	Pelvic girdle partly not ossified	1	1.0	0	0.0	0	0.0	0	0.0
97	One or more of the metacarpals 1 to 5 not ossified or 1 to 4 incompletely ossified	40	41.2	23	35.9	33	40.7	10	23.8
98	One or more of the metatarsals 2 to 5 not ossified or 2 to 4 incompletely ossified	2	2.1	0	0.0	0	0.0	0	0.0
# 99	Zygoma incompletely ossified								

only used for pups which died during lactation

Pregnancy and Fertility Analysis by the reviewer:

It is not clear from Sponsor's Table 10 if the Sponsor counted number of corpora lutea from all animals deployed in the caesarean section in order to determine the effect of the treatment on ovulation process and early pregnancy. Therefore, the reviewer analyzed individual ovarian and uterus data from Appendix III as shown below.

Data for fetuses at caesarean section

Observation	Control	5 mg/kg	15 mg/kg	60 mg/kg
Number of females mated and pregnant (n)	15	12	12	9
Corpora Lutea, Total	263	196	195	118
Corpora Lutea/Litter	17.5	16.5	16.5	14.8
Implantation, Total	213	147	171	110
Implantation/Litter	14.2	12.3	14.3	12.2
% Preimplantation Loss	17.2%	26.2%	12.6%	17.6%
Total # intra-uterine death	15	16	7	21
Intra-uterine death/litter	1.0	1.3	0.6	2.3
% Post implantation Loss	7.6%	10.2%	3.7%	18.4%
Total # Live Fetus	198	131	164	89
# Live Fetus/Litter	13.2	10.9	13.7	11.1

Above data clearly suggest that **there was more post-implantation loss at 60 mg/kg among rats became pregnant.**

The reviewer also constructed a database for each female rat deployed in the caesarean-section study from Appendix 1 of report as shown below.

Animal #, Control	Animal #, 5 mg/kg	Animal #, 15 mg/kg	Animal #, 60 mg/kg
1, Pregnant	31, Pregnant	61, Pregnant	91, Pregnant
3, Pregnant	33, Pregnant	63, Pregnant	94, Pregnant
5, Pregnant	35, Pregnant	65, Pregnant	105, Pregnant
7, Pregnant	37, Pregnant	67, Pregnant	107, Pregnant
9, Pregnant	39, Pregnant	69, Pregnant	109, Pregnant
11, Pregnant	41, Pregnant	73, Pregnant	111, Pregnant
13, Pregnant	43, Pregnant	75, Pregnant	117, Pregnant
15, Pregnant	45, Pregnant	79, Pregnant	119, Pregnant

Animal #, Control	Animal #, 5 mg/kg	Animal #, 15 mg/kg	Animal #, 60 mg/kg
17, Pregnant	51, Pregnant	81, Pregnant	113, Pregnant, All pups died in uterus
19, Pregnant	55, Pregnant	83, Pregnant	93, Died before mating
21, Pregnant	57, Pregnant	87, Pregnant	95, Died before mating
23, Pregnant	59, Pregnant	89, Pregnant	97, No insemination after mating, Not Pregnant
25, Pregnant	40, Not Pregnant, Mated	71, Not Pregnant, Mated	99, Not Pregnant after mating
27, Pregnant	47, Died, Pregnant	77, Not Pregnant, Mated	101, Not Pregnant after mating
29, Pregnant	53, Not Pregnant after mating	85, Not inseminated after start of mating	103, Not Pregnant after mating
			115 Died before mating
Total= 15 Pregnants	Total= 13 Pregnant; 2 Not Pregnant after mating	Total=12 Pregnant; 3 Not Pregnant after Mating	Total=9 Pregnant; 3, Died before Mating; 4 Not Pregnant after Mating

Based on individual data from Appendix 1, following table on mating is presented below.

Female Rats, Caesarean Section

Observation	Group 1	Group 2	Group 3	Group 4
	Control	**5 mg/kg**	**15 mg/kg**	**60 mg/kg**
Total Deployed for Mating	15	15	15	17
Total Mated	15	15	15	14
Not Pregnant after Mating	0	2 (13%)	3 (20%)	4 (29%)
Died before Mating	0	0	0	3
Pregnant	15 (100%)	13 (86%)	12 (80%)	10 (71%)

The Sponsor did not present any historical control data in the study report. However, [ ]CD (SD) rats showed 95.5% pregnancy among mated rats. Based on the individual data, Milnacipran did not show lack of libido effect. However, **fertility was reduced dose dependently at 5, 15 and 60 mg/kg in female rats.** The no-effect dose for fertility was not determined. b(4)

Dams allowed to deliver:

Data for those dams allowed to deliver and nurse pups to weaning period are shown in **Sponsor's table 17 below. The treatment had** no effect on the gestation period. However, the number of pups found dead at delivery and post-natal survival was reduced at 15 and 60 mg/kg. The weight of pups at birth was also reduced in 15 and 60 mg/kg. However, the treatment had no effect on the pupillary and startle reflex of F_1 pups at the end of weaning. The treatment had no effect on the malformation of pups.

Table 17 — Group Mean Litter Data — Viability and Rearing Data — Project No. 314/28

F Generation

Parameter	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 15 mg/kg	Group 4 - 60 mg/kg
Duration of gestation in days	21.7 ± 0.5	21.8 ± 0.4	21.7 ± 0.5	21.5 ± 0.5
Mean number of implantations	14.9 ± 2.3	13.9 ± 1.7	14.7 ± 1.9	12.4 ± 2.0
Number of pups found alive	164	176	164	100
Mean number per female	12.6 ± 3.2	12.6 ± 1.3	12.6 ± 2.1	9.1 ± 5.2
Number of pups found dead	6	8*	14	30**
Mean number per female	0.5 ± 0.8	0.4 ± 1.1	1.1 ± 1.8	2.7 ± 3.7
Number of malformed pups	0	1	0	0
Percentage of total pups born	0.0	0.6	0.0	0.0
Number of litters	0	1	0	0
Mean live birth index in per cent	96.7	97.1	92.5	78.8

* one additional pup with malformed tail killed on day 1 p.p.
** one complete litter with eight pups found dead on day of birth; not evaluated for mean and standard deviation

Table 17 (cont.) — Group Mean Litter Data — Viability and Rearing Data — Project No. 314/28

F Generation

Parameter	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 15 mg/kg	Group 4 - 60 mg/kg
Number of pups alive day 1	164	176*	164	100
Mean number per female	12.6 ± 3.2	12.6 ± 1.3	12.6 ± 2.1	9.1 ± 5.2
Number of pups alive day 4	161	172	150	74
Mean number per female	12.4 ± 3.1	12.3 ± 1.2	10.3 ± 4.7	6.7 ± 5.6
Mean viability index (day 4 to 1) in per cent	98.5	98.4	86.0	63.0
Number of pups alive day 7	160	170	130	71
Mean number per female	12.3 ± 3.1	12.1 ± 1.3	10.0 ± 4.9	6.5 ± 5.7
Mean viability index (day 7 to 4) in per cent	99.4	98.8	90.5	83.3
Number of pups alive day 14	152	162	123	61
Mean number per female	11.7 ± 2.7	11.6 ± 1.2	9.5 ± 4.5	5.5 ± 5.5
Mean viability index (day 14 to 7) in per cent	95.7	95.5	95.3	78.3
Number of pups alive day 21	150	159	118	60
Mean number per female	11.5 ± 2.6	11.4 ± 1.3	9.1 ± 4.5	5.5 ± 5.5
Mean viability index (day 21 to 14) in per cent	98.8	98.1	95.2	97.9
Mean weaning index in per cent	94.0	92.5	82.0	66.9
Sex ratio males : females (day 1)	39.6 : 60.4	54.9 : 45.1	48.8 : 51.2	47.0 : 53.0
Sex ratio males : females (day 21)	41.3 : 58.7	53.5 : 46.5	50.0 : 50.0	40.0 : 60.0

* one male pup with malformed tail killed on day 1 p.p.

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b(4)

Fertility of F_1 generation male and female rats (untreated) were comparable among all groups as shown in table 20 below.

Table 20 Test Animals and Group Indices Project No. 314/28

F1 Generation

Parameter	Group 1 - untreated (P Gen.: 0 mg/kg)	Group 2 - untreated (P Gen.: 5 mg/kg)	Group 3 - untreated (P Gen.: 15 mg/kg)	Group 4 - untreated (P Gen.: 60 mg/kg)
Number of mated animals: Males	25	25	24 *	19 **
Number of mated animals: Females	25	25	25	25
Number of inseminated females	22	21 B	20 +	22 B
Number of pregnant females	20	18	17	19
Number of non pregnant animals	2	1	0	1
Number of pregnant animals which died	0	0	0	0
Number of non pregnant animals which died	0	0	0	0
Number of animals which aborted	0	0	0	0
Number of animals with 100 per cent intra-uterine death	0	0	0	0
Number of animals with live foetuses at necropsy	20	18	17	19
Mating performance in days	4.0	3.6	3.3	4.2
Insemination index in %	88.0	84.0	80.0	88.0
Fecundity index in %	90.9	94.7	100.0	95.0
Fertility index in %	80.0	78.3	77.3	82.6

* one of these animals paired with two female animals
** six of these animals paired with two female animals
\+ for three of these animals pregnancy status inadvertently not determined, therefore not evaluated
B for two of these animals pregnancy status inadvertently not determined, therefore not evaluated

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ON ORIGINAL

Mated F1 female rats were sacrificed on gestation day 20 to examine corpora lutea, implantations and post implantation loss. Table 24 below showed that the F_1 female rats obtained from 15 and 60 mg/kg treated Parent generation or F0 rats had a lower ovulation than control rats. F_1 female rats at 15 and 60 mg/kg also showed a slight reduction in implantation and live fetuses (see table 25).

Table 24 — Group Mean Caesarian Data — Ovarian and Uterine Data — F1 Generation — Project No. 314/28

Parameter		Group 1 - untreated (P Gen.: 0 mg/kg) n = 20	Group 2 - untreated (P Gen.: 5 mg/kg) n = 18	Group 3 - untreated (P Gen.: 15 mg/kg) n = 17	Group 4 - untreated (P Gen.: 60 mg/kg) n = 19
Corpora lutea	total number	344	307	276	282
	mean number	17.2 ± 2.7	17.1 ± 1.5	16.2 ± 2.1	14.8 ± 2.7 (1,2)
Implantations	total number	292	271	225	230
	mean number	14.6 ± 2.8	15.1 ± 2.7	13.2 ± 4.0	12.1 ± 3.9 (1,2)
Per cent pre-implantation loss		14.6	12.1	19.4	18.5

Remark: Number in parentheses indicate by group number which groups are significantly different from the marked group
(p < 0.05; Kruskal Wallis Analysis of Variance followed by Wilcoxon 2-sample test)

Table 25 — Group Mean Caesarian Data — Project No. 314/28

Implantation Data
(calculated from animals with live foetuses in utero at necropsy)

F1 Generation

Parameter		Group 1 - untreated (P Gen.: 0 mg/kg) n = 20	Group 2 - untreated (P Gen.: 5 mg/kg) n = 18	Group 3 - untreated (P Gen.: 15 mg/kg) n = 17	Group 4 - untreated (P Gen.: 60 mg/kg) n = 19
Implantations	total number	292	271	225	230
	mean number	14.6 ± 2.8	15.1 ± 2.7	13.2 ± 4.0	12.1 ± 3.9 (1,2)
Live foetuses	total number	265	260	213	214
	mean number	13.3 ± 3.5	14.4 ± 2.5	12.5 ± 3.8	11.3 ± 3.6 (2)
	% of implantations	90.9	96.2	94.9	93.6
Early resorptions	total number	26	11	12	16
	mean number	1.3 ± 2.3	0.6 ± 0.9	0.7 ± 0.8	0.8 ± 0.8
Late resorptions	total number	1	0	0	0
	mean number	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Dead foetuses	total number	0	0	0	0
	mean number	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total intra-uterine deaths	total number	27	11	12	16
	mean number	1.4 ± 2.3	0.6 ± 0.9	0.7 ± 0.8	0.8 ± 0.8
% Post-implantation loss		9.1	3.8	5.1	6.4

Remark: Number in parentheses indicate by group number which groups are significantly different from the marked group
(p < 0.05; Kruskal Wallis Analysis of Variance followed by Wilcoxon 2-sample test)

Caesarean data showed F_1 females had more female pups from 60 mg/kg treated Parent generation rats (see table 26).

Table 26 — Group Mean Caesarian Data — Foetal Data — F1 Generation — Project No. 314/28

Parameter	Group 1 - untreated (P Gen.: 0 mg/kg)	Group 2 - untreated (P Gen.: 5 mg/kg)	Group 3 - untreated (P Gen.: 15 mg/kg)	Group 4 - untreated (P Gen.: 60 mg/kg)	ANOVA F-Probability
Number of foetuses	265	260	213	214	
Mean number of foetuses per female	13.3 ± 3.5	14.4 ± 2.5	12.5 ± 3.8	11.3 ± 3.6 (2)*	
Mean litter weight (g)	42.6 ± 11.9	47.4 ± 9.2	41.7 ± 13.5	39.6 ± 12.1	0.2412
Mean foetal weight (g) overall +	3.22 ± 0.31	3.29 ± 0.31	3.32 ± 0.28	3.56 ± 0.31 (1,2,3)	0.0054
Mean foetal weight (g) males +	3.27 ± 0.36	3.38 ± 0.31	3.38 ± 0.33	3.66 ± 0.36 (1,2,3)	0.0066
Mean foetal weight (g) females +	3.16 ± 0.31	3.21 ± 0.30	3.23 ± 0.26	3.47 ± 0.31 (1,2,3)	0.0102
Number of males	140	132	100	99	
Number of females	125	120	105	115	
Mean proportion of male foetuses	51.9 ± 14.2	50.5 ± 10.8	53.8 ± 10.4	43.9 ± 16.6	

+ calculated from litter means

Remark: Number in parentheses indicate by group number which groups are significantly different from the marked group ($p < 0.05$; Analysis of Variance followed by Newman-Keuls test)

*Remark: Number in parentheses indicate by group number which groups are significantly different from the marked group ($p < 0.05$; Kruskal Wallis Analysis of Variance followed by Wilcoxon 2-sample test)

However, external malformation of F_2 pups at caesarean section was not evident (see table 27).

Table 27 — Group Mean Caesarian Data — Malformation, Variation Data — F1 Generation — Project No. 314/28

Parameter	Group 1 - untreated (P Gen.: 0 mg/kg)	Group 2 - untreated (P Gen.: 5 mg/kg)	Group 3 - untreated (P Gen.: 15 mg/kg)	Group 4 - untreated (P Gen.: 60 mg/kg)
Number of foetuses examined externally	265	260	213	214
Number of foetuses with external malformations	1	0	0	1
% of foetuses with external malformations	0.4	0.0	0.0	0.5
Number of litters with externally malformed foetuses	1	0	0	1
Total number of malformed foetuses	1	0	0	1
Total number of litters with malformed foetuses	1	0	0	1
Average per cent malformed foetuses	0.4	0.0	0.0	0.5

Summary of the study finding:

Male and female Sprague Dawley rats were treated at 5, 15 and 60 mg/kg for segment 1 fertility, early development and second generation reproductive performance assessment. No treatment related mortality was noted. Both male and female rats demonstrated about 10% or greater decreased body weight gain at 60 mg/kg. The high dose reached an

optimal level of dosing for the study. Therefore, the choice of doses for the study was acceptable.

Mating performance data showed that the treatment had no effect on the mating performance of rats up to 60 mg/kg.

The caesarean data in female rats showed there was more post-implantation loss at 60 mg/kg. About 15, 20 and 50% of pregnant rats had dead fetuses at caesarean section on day 20 comparable to 0% in the control. Moreover, analysis of individual data in female rats at caesarean section revealed that the fertility was reduced in female rats dose dependently at 5, 15 and 60 mg/kg. The fecundity index and fertility index were reduced at 5, 15 and 60 mg/kg as shown in the Sponsor's table # 1. However, examination of live fetuses did not show external, visceral or skeletal abnormality due to the treatment at caesarean section.

The treatment of pregnant rats had no effect on the gestation period up to 60 mg/kg. However, number of pups found dead at delivery and post-natal survival was reduced at 15 and 60 mg/kg. The surviving pups during weaning did not show an adverse effect on the reflex or any treatment-related malformation.

F_1 generation reproductive performance in male and female rats (untreated rats but parents were treated) showed normal mating pattern. However, ovulation in F_1 female obtained from 15 and 60 mg/kg treated P generation were reduced. It is possible that treatment with milnacipran had an endocrine effect on the maturing rats that was not observed in adult rats treated with milnacipran.

F_2 pups obtained from caesarean section of F_1 females had more female pups at 60 mg/kg than male pup. No external malformation was noted.

Conclusion:

Mating performance of rats was not affected up to 60 mg/kg (360 mg/m^2). However, fertility of rats was reduced at 5 mg/kg (30 mg/m^2), 15 mg/kg (90 mg/m^2) and 60 mg/kg (360 mg/m^2). No skeletal or visceral abnormality was noted in surviving pups. Milnacipran had no effect on the gestation period. Post-natal survival of pups was reduced at 15 (90 mg/m^2) and 60 (360 mg/m^2) treated female rats. In a multigeneration study, rats obtained from milnacipran treated parental generation, showed reduced ovulation at 15 mg/kg (90 mg/m^2) and 60 mg/kg (360 mg/m^2). Therefore, treatment had no effect on mating. However, reduced fertility and embryocidal effect of female rats was observed at 5 mg/kg (30 mg/m^2) and higher doses.

Pregnancy category C should be designated for milnacipran.

Embryofetal development

Study title: F2207: Oral (gavage) teratology study in the New Zealand white rabbits

Key study findings: The NOEL was 5 mg/kg. No maternal toxicity was observed up to 60 mg/kg. However, single extra rib was noted in rabbits at 15 and 60 mg/kg as a variation. No teratogenicity was observed in the study. The study is acceptable and reached MTD.

Study no.: T019
Volume # M4, and page #: 1
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: March 1985
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: F2207, lot # D9032, and % purity: 101%

Methods

Doses: 5, 15, 60 mg/kg
Species/strain: New Zealand white
Number/sex/group:

Group	Dose, mg/kg	# Inseminated rabbits
1	Control, vehicle	21
2	5	22
3	15	21
4	60	22

Route, formulation, volume, and infusion rate: The drug substance was dissolved in distilled water and administered once daily by oral gavage on gestation days 6 to 18. The volume of dose was adjusted daily.

Satellite groups used for toxicokinetics: None
Study design: Female rabbits were mated with male rabbits from same strain. Female rabbits were injected with HCG to ensure ovulation. The day of mating was considered to be day 0 of the pregnancy. Pregnant animals were sacrificed on gestation day 28 to examine fetuses following caesarean section.

Parameters and endpoints evaluated: During the treatment and thereafter all animals were observed daily for sign of toxicity and mortality. The body weight was recorded on pregnancy days 0, 6, 12, 18, 24 and 28. Surviving animals were sacrificed on gestation day 28 by IV injection of anesthetics (T-61). Gross examination of organs was performed at necropsy for the dams, ovaries and uteri were removed. Following parameters were recorded:

1. Number of corpora lutea
2. Number of live fetuses
3. Early resorption
4. Late resorption
5. Number of early or late dead fetuses
6. Fetal weight and sex

Fetuses were examined for external and visceral malformation. Skeletal abnormality was determined for half of eviscerated fetuses following dehydration of soft tissues in 95% ethanol. The skeletal remains were stained with Alizarin red for light microscopic examinations. The remaining half of litters were fixed in Bouin's fixative and observed for organ system variations and deformations according to the method of Wilson. Implantations in non-pregnant animals were determined from uteri treated with ammonium sulphide.

Results

Mortality (dams): Mortality data are shown in the table below.

Mortality:

Group, dose	Animal #	Day of death	Remark
1, control	6	17	Signs of pneumonia
1, control	8	23	Signs of pneumonia, aborted
2, 5 mg/kg	22	6	Sacrificed due to injury
2, 5 mg/kg	82	6	Intubation error
3, 15 mg/kg	51	24	Signs of pneumonia
3, 15 mg/kg	54	12	Edema of lung, gavage error
3, 15 mg/kg	55	19	Edema of lung, cut surface of liver
3, 15 mg/kg	58	24	Signs of pneumonia, spleen enlargement, reddening of gastric mucosa, cut surface on liver
4, 60 mg/kg	68	18	Signs of pneumonia
4, 60 mg/kg	71	16	Signs of pneumonia, swelling of gastric mucosa
4, 60 mg/kg	78	12	Intubation error

No clinical signs were reported at 5, 15 and 60 mg/kg in most of rabbits. Yet large number of animals in the control and treated groups showed reddened gastric mucosa. The sign of pneumonia was also noted in dead animals in control and treated groups. It is possible that deaths could be attributed to handling and intubation process rather than systemic toxicity to the treatment.

Clinical signs (dams): No clinical signs were reported at 5, 15 and 60 mg/kg in most of rabbits.

Body weight (dams): The average body weight (kg) of rabbits at the start, end of treatment and at necropsy is shown below.

Day	Control	5 mg/kg	15 mg/kg	60 mg/kg
6	3.7	3.6	3.7	3.6
18	4.0	3.9	4.0	3.9
28	4.2	4.2	4.2	4.1

Above data suggest that the treatment had no effect on the body weight gain of pregnant rabbits.

Food consumption (dams): Not provided

Toxicokinetics: Nil

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

A slight reddening of gastric mucosa was noted in 5, 7, 7, 11 rabbits at control, 5, 15, 60 mg/kg, respectively at necropsy. Distribution of events among control and treated groups do not suggest its relationship to the treatment.

Pregnancy data are shown below.

Parameter	Control	5 mg/kg	15 mg/kg	60 mg/kg
Pregnant	19	22	21	22
Corpora lutea/litter	12.0	11.1	11.3	10.7
Implantations/litter	6.9	6.7	8.4	7.8
% Pre-implantation loss	40.9	36.1	23.8	26.0
% Post-implantation loss	9.2	8.8	4.3	13.0
Fetuses/litter	6.3	6.2	8.1	7.4
Total intra-uterine death/litter	0.5	0.5	0.3	0.7
Total deaths	8	7	5	14
Mean Litter	238	234	295	262

weight (g)				
Mean Fetal weight	37.6	37.8	36.7	35.2

Animal #74 at 60 mg/kg showed total uterine deaths that contributed higher incidences of intrauterine deaths at 60 mg/kg (from 8 in the control to 14 at 60 mg/kg). Therefore, intrauterine deaths were not considered to be treatment related in the pregnancy data.

Offspring (malformations, variations, etc.):

Increased incidences of single extra rib were noted at 15 and 60 mg/kg based on individual animal data. Total incidences were 6, 3, 6, and 14 at control, 5, 15, and 60 mg/kg, respectively. No other skeletal variation was noted due to the treatment. Individual animal data for single extra rib are shown below.

Control	**Control**	**5 mg/kg**	**5 mg/kg**	**15 mg/kg**	**15 mg/kg**	**60 mg/kg**	**60 mg/kg**
Animal#	**#Pup**	**Animal#**	**#Pup**	**Animal#**	**#Pup**	**Animal#**	**#Pup**
4	1	38	1	43	2	63	1
11	1	40	1	50	2	64	2
12	1	81	1	52	1	66	2
15	1			46	1	72	2
16	1					73	1
20	1					75	1
						76	2
						77	3
Total	6		3		6		14

There were increased incidences of variation e.g. single extra rib observed in rabbits at 60 mg/kg dose. Occurrence of single extra rib in two pups in same animal was considered to be higher than the control and low dose groups where single extra rib was noted in one pup within the litter. Considering this, the incidence of single extra ribs/litter was also present at 15 mg/kg. No historical control data were provided by the Sponsor. The no effect dose was 5 mg/kg. There were no signs of maternal toxicity at the high dose of 60 mg/kg. Therefore, the MTD was not reached in this study. However, based on findings from a preliminary study (#T011), the high dose of 60 mg/kg is close to the MTD because 100 mg/kg showed deaths in rabbits. Therefore, a repeat of embryo-fetal development study in the rabbit would not be needed.

Conclusion of the rabbit teratogenicity study:

Pregnant rabbits were treated at 5, 15 and 60 mg/kg during gestation days 6-18 and sacrificed on gestation day 28 for examination of teratogenic potential. The high dose of 60 mg/kg was tolerated without any maternal toxicity. No teratogenicity was noted. However a variation e.g. increased incidence of single extra rib was noted at 15 and 60 mg/kg. Based on the data, the 5 mg/kg dose was NOEL and due to variations noted at maternally non-toxic dose, Pregnancy category C should be designated based on this

study. The high dose reached MTD when the data for a preliminary study was considered.

Study title: F2207, Oral teratology study in the mouse

Key study findings: Treatment at 5, 25 and 125 mg/kg did not show any skeletal and visceral malformations in pregnant mice. However, fetal weight was reduced at 25 and 125 mg/kg. No maternal toxicity was noted at any dose. Based on the maternal toxicity data, MTD was not clearly defined in this species. The NOEL dose was 5 mg/kg.

Study no.: T015
Volume #M4, and page #: 1
Conducting laboratory and location: [ ] b(4)
Date of study initiation: May 30, 1986
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: Milnacipran Hydrochloride, lot # D9032 and % purity: 101%

Methods

Doses: 5, 25, 125 mg/kg/day
Species/strain: Female mice, [ ] NMRI b(4)
Number/sex/group: 25
Route, formulation, volume, and infusion rate: Oral gavage, 10 mL/kg as aqueous solution
Satellite groups used for toxicokinetics: Nil
Study design:

Group number	Group description	Dose level mg/kg/day	Concentration mg/ml	Dose volume ml/kg
1	Control	0	0.0	10
2	Low	5	0.5	10
3	Intermediate	25	2.5	10
4	High	125	12.5	10

Animals were treated once a day from gestation days 6 to 15. Control animals received distilled water.

Parameters and endpoints evaluated:

Mice were observed twice daily for toxicity and mortality. The body weight was recorded on gestation days 0, 6, 10, 15, and 18. Animals were sacrificed on gestation day 18 by carbon dioxide inhalation. Ovaries and uteri were removed to record live fetuses, early and late fetal deaths, weight, and sex of fetuses. Fetuses were examined for external malformations. About half of fetuses were sacrificed, eviscerated, digested with

95% ethanol. Skeletons were stained with Alizarin red for the determination of skeletal changes. The remaining half were fixed in Bouin's fixative and examined for visceral changes according to the method of Wilson. Uteri from non-pregnant females were treated with ammonium sulphide for the determination of implantations. Variations (no functional significance), retardation (delayed development), and malformation (lethal) were recorded in fetuses.

Results

Mortality (dams): One control animal died on day 15 due to gavage error. No other mortalities were recorded.

Clinical signs (dams): No treatment related clinical signs were reported. One control animal showed total death of fetuses at necropsy.

Body weight (dams):

The average body weight of mice is shown in the Sponsor's table below.

Table 2 Group Mean Body Weight (g) Project No. 314/27

Days of gestation	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 25 mg/kg	Group 4 - 125 mg/kg
0	23.8 ± 1.7	24.1 ± 1.6	25.2 ± 2.1*	24.5 ± 2.2
6	27.5 ± 1.7	27.7 ± 1.4	28.9 ± 1.9*	28.2 ± 2.5
10	31.7 ± 2.1	31.8 ± 1.8	33.0 ± 1.9*	31.7 ± 2.6
15	41.6 ± 3.2	42.4 ± 3.2	43.6 ± 3.6*	41.5 ± 3.9
18	51.1 ± 4.7	52.7 ± 5.1	53.8 ± 4.8*	50.2 ± 5.3

*) $p < 0.05$ (Student's t-test)

Above data table did not suggest any treatment-related effect on the body weight.

Food consumption (dams): Not recorded

Toxicokinetics: Not determined

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Pregnancy data are shown from the Sponsor's table 1 below. There was no mortality to dams in any drug treated groups.

Table 1 Test Animals Project No. 314/27

Parameter	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 25 mg/kg	Group 4 - 125 mg/kg
Number of inseminated animals	25	25	25	25
Number of pregnant animals	23	20	23	21
Percentage	92.0	80.0	92.0	84.0
Number of non-pregnant animals	2	5	2	4
Number of pregnant animals which died	1	0	0	0
Number of non-pregnant animals which died	0	0	0	0
Number of animals with 100 % intra-uterine death	1	0	0	0
Number of animals with live foetuses at necropsy	21	20	23	21

The caesarean data did not show evidence of treatment related pre-implantation loss; however, the treatment started after the implantation. Total uterine deaths between control and treated mice were comparable as shown in Sponsor's table below.

Table 6 Group Mean Caesarian Data Project No. 314/27

- Implantation Data -

(calculated from animals with live foetuses in utero at necropsy and total intra-uterine deaths)

Parameter		Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 25 mg/kg	Group 4 - 125 mg/kg
implantations	total number mean number per dam	267 12.1 ± 3.6	268 13.4 ± 2.8	318 13.8 ± 2.9	260 12.4 ± 3.2
early intra-uterine deaths	total number mean number per dam	24 1.1 ± 1.5	16 0.8 ± 1.1	26 1.1 ± 1.1	12 0.6 ± 0.9
early-late intra-uterine deaths	total number mean number per dam	5 0.2 ± 0.4	4 0.2 ± 0.4	4 0.2 ± 0.4	9 0.4 ± 1.0
late intra-uterine deaths	total number mean number per dam	2 0.1 ± 0.4	1 0.1 ± 0.2	3 0.1 ± 0.3	0 0.0 ± 0.0
total intra-uterine deaths	total number mean number per dam	31 1.4 ± 1.5	21 1.1 ± 1.4	33 1.4 ± 1.2	21 1.0 ± 1.3
% post-implantation loss		15.8	8.0	10.2	7.4

Fetal data showed a slight decrease in the mean fetal weight at 125 mg/kg that was statistically significant. A decrease in the female fetal weight was also noted at 25 mg/kg. These data are shown in the Sponsor's table below.

Table 7 Group Mean Caesarian Data Project No. 314/27

- Foetal Data -

Parameter	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 25 mg/kg	Group 4 - 125 mg/kg
Number of foetuses	237	247	285	239
Mean number of foetuses per dam	11.3 ± 2.8	12.3 ± 3.0	12.4 ± 2.9	11.4 ± 2.9
Mean litter weight (g)	13.0 ± 3.2	14.5 ± 3.5	14.3 ± 3.2	12.7 ± 3.2
Mean foetal weight (g)*	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1++
Mean foetal weight (g) males**	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1+
Mean foetal weight (g) females**	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1+	1.1 ± 0.1+
Number of males	133	129	153	148
Number of females	104	118	132	91
Sex ratio in per cent males : females	56.1 : 43.9	52.2 : 47.8	53.7 : 46.3	61.9 : 38.1

*) calculated from individual weights
**) calculated from litter means
+) p < 0.05 (Student's t-test)
++) p < 0.001 (Student's t-test)

Data for the minimal fetal weight observed in some dams is shown below.

Observation	Control	5 mg/kg	25 mg/kg	125 mg/kg
Weight (g)	0.9	0.8	0.8	0.7
Dam #	7, 22, 25,	52	71, 78	84, 100

Offspring (malformations, variations, etc.):

Data on external, visceral, and skeletal examination did not show any treatment related change. Data are shown from the Sponsor's table below.

Table 8 Group Mean Caesarian Data Project No. 314/27

- Malformation, Variation Data -

Parameter	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 25 mg/kg	Group 4 - 125 mg/kg
Number of foetuses examined externally	237	247	285	239
Number of foetuses with external malformations	0	0	1	1
% of foetuses examined	0.0	0.0	0.4	0.4
Number of litters containing foetuses with external malformations	0	0	1	1
Number of foetuses examined viscerally	121	126	145	123
Number of foetuses with visceral malformations	3	1	2	1
% of foetuses examined	2.5	0.8	1.4	0.8
Number of litters containing foetuses with visceral malformations	2	1	2	1
Number of foetuses examined viscerally	121	126	145	123
Number of foetuses with visceral variations	2	2	2	3
% of foetuses examined	1.7	1.6	1.4	2.4
Number of litters containing foetuses with visceral variations	2	2	2	2
Number of foetuses examined skeletally	116	123	140	116
Number of foetuses with skeletal malformations	0	0	0	0
% of foetuses examined	0.0	0.0	0.0	0.0
Number of litters containing foetuses with skeletal malformations	0	0	0	0
Number of foetuses examined skeletally	116	123	140	116
Number of foetuses with skeletal variations	70	83	96	76
% of foetuses examined	60.3	67.5	68.6	65.5
Number of litters containing foetuses with skeletal variations	18	20	22	21
Total number of malformed foetuses	3	1	3	2
Total number of litters with malformed foetuses	2	1	3	2
Average per cent malformed foetuses	1.3	0.4	1.1	0.8

Summary of mouse segment 2 reproductive safety study:

Pregnant mice were treated with milnacipran between gestation days 6 to 15 at 5, 25, and 125 mg/kg. Animals were sacrificed on gestation day 18 to determine the effect of the treatment on organogenicity and fetal development. The drug-treatment did not show any maternal toxicity, embryocidal effect, variations, or malformations up to 125 mg/kg. However, fetal weight was reduced compared to the control group following treatment with 25 (female) and 125 mg/kg milnacipran. Based on the data, The NOEL was 5 mg/kg.

The treatment of dams did not reach MTD in the absence of any maternal toxicity. Normally an effect on fetus is examined at maternally non-toxic doses. Using that end point, fetal toxicity was present. However, maternal toxicity is a confirmation of adequacy of dosing. In this study they could have increased the dose more but we have an effect on the fetus already. Considering this, the reviewer is not recommending repeating the study at MTD.

Prenatal and postnatal development

Study title: F2207-Effects of the test article on peri- and post-natal development of the rat when administered orally (by gavage) during late gestation and lactation.

Key study findings: Data suggest that the treatment with milnacipran at 60 mg/kg had an adverse effect on survival and weight of F_1 pups. A NOEL was not established in the study. The study was not designed to allow for the determination of a NOAEL.

Study no.: T071

Volume #M4, and page #: 1
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: Dec 5, 1990
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: F2207, lot # [redacted] **and % purity:** 99.33% b(4)

Methods

Doses: 60 mg/kg
Species/strain: Sprague Dawley mated female rats [redacted] OFA.SD strain b(4)
Number/sex/group: See study design
Route, formulation, volume, and infusion rate: Animals were treated orally by gavage at 5 mL/kg once a day. The test substance was dissolved in purified water before the study. Water was used in the vehicle control.
Satellite groups used for toxicokinetics: Nil
Study design:

Experimental design is shown in the table below.

Group	Period of treatment	Number	Dose, mg/kg/oral
1	G 15 - L21	15	0
2	G 15 - L-21	15	60
3	G 15 - L21	15	60
4	L 1 - L21	15	60
5	G 15 - L21	15	60
6	G 15 - L21	15	0

G=gestation, L=lactation

Rats weighed between 215-270 g at gestation day 0.

Milnacipran treatment was given from gestation day 15 to lactation day 21 (L21), except rats in group 4 were treated during lactation day 1 to lactation day 21. Offspring from groups 5 and 6 dams were cross-fostered on day 1 of lactation to determine the effect of the drug on pups when exposed to milnacipran in utero. Groups 1 and 6 served as vehicle control groups.

The dose was selected on the basis of a previous study (#T030, fertility study in rats, reviewed above) in which fetal effects were observed when dams were treated before mating, during mating, gestation, and lactation period. Rats treated at 15 and 60 mg/kg showed a reduction in the post-natal viability. However, the Sponsor did not use a lower dose to characterize the no effect dose in this study.

Parameters and endpoints evaluated:

Mortality, clinical signs, body weight, duration of gestation, litter size, live and dead fetuses, and nursing behavior were noted during the study. After delivery, pups were examined for external appearance, bodyweight, and sex. Physical development was assessed by the intra-litter onset for pinna unfolding, incisor eruption, and eye opening.

The following behavioral and functional studies were conducted: behavioral and functional tests e.g., righting reflex on day 8, gripping reflex on day 17, papillary and auditory reflex on 21, spontaneous activity on day 14 during the weaning period.

At the end of study all animals were sacrificed.

Results

F_0 in-life: No mortality or clinical signs was reported due to the treatment in the Parent generation during gestation.

Average body weight data are shown in the table below.

Group	Treatment, Days	Dose, mg/kg	Body weight Day G0 (g)	Body weight Day G21 (g)	BW gain (g)
1	G15-L21	Vehicle	238	410	172
2	G15-L21	60	236	381	145
3	G15-L21	60	235	391	156
4	L1-L21	60	238	389	151
5	G15-L21	60	236	373	137
6	G15-L21	Vehicle	240	384	144

Rats in the drug treated groups did not show substantial change in the body weight gain during gestation period. The Sponsor stated that some of the dams already delivered when the weight was recorded that introduced variability in the body weight on day G21. Therefore, average body weight gain (g) in dams during entire gestation and immediately after delivery on day L1 is shown in the table below.

Group	Treatment	Dose, mg/kg	Day G0	Day L1	BW gain
1	G15-L21	vehicle	238	319	81
2	G15-L21	60	236	305	69
3	G15-L21	60	235	296	61
4	L1-L21	60	238	319	81
5	G15-L21	60	236	295	59
6	G15-L21	Vehicle	240	319	79

Rats in groups 1, 4, and 6 were not treated with milnacipran during gestation and showed comparable weight gain during gestation. However, treated rats in groups 2, 3, and 5 had a reduction in body weight gain during gestation period.

Above data suggest that a reduction in the weight gain due to the treatment with 60 mg/kg.

Mean body weight (g) during lactation is shown in the table below.

Group	Treatment	Dose, mg/kg	L1	L21	BW gain, g
1	G15-L21	Vehicle	319	342	23
2	G15-L21	60	305	333	28
3	G15-L21	60	296	331	37
4	L1-L21	60	319	330	11
5	G15-L21	60	295	324	29
6	G15-L21	Vehicle	319	347	28

Mean body weight of P generation rats during nursing period was comparable to control except rats in group 4 that showed a lower body weight gain when treated during lactation period only.

Milnacipran had no effect on the duration of gestation and live birth.

F_0 necropsy: No data were provided.

F_1 physical development:

Viability of pups up to postnatal day 4 was reduced in treated groups compared to control. Viability of pups in group 6 control that were fostered to drug treated group 5 rats was also reduced. Data suggest that in utero exposure of pups to milnacipran during gestation day 15 to delivery had an effect on the postpartum survival. Viability data are shown in the table below.

Group	Treatment period	% viability on post partum day 4	BW, male pup (at birth)	BW, female pup (at birth)
1	G15-L21 (control)	99	7.21	6.86
2	G15-L21	78	6.29	5.84*
3	G15-L21	61	5.83*	5.53*
4	L1-L21	89	7.23	6.52
5	G15-L21	80	7.31	6.85
6	G15-L21 (control)	66	6.39*	6.04*

* Statistically significant

The mean birth weight was slightly but significantly reduced for pups delivered by dams treated with milnacipran during gestation day 15 to lactation day 21 and for pups cross-fostered (group 6). **Data suggest that the treatment with milnacipran had an adverse**

effect on survival and weight of F_1 pups that was not due to alternation in maternal behavior.

Weight of F_1 pups at the end of weaning on day 21 showed a reduction in group 2-5 pups. Data are shown in the table below.

Group	Treatment period	F_1, male (g)	F_1, female (g)
1	Control	52.47	50.61
2	G15 - L21	45.75 (↓12.8%)	44.63 (↓11.8%)
3	G15 - L21	45.78 (↓12.8%)	44.76 (↓11.6%)
4	L1 - L21	43.08 (↓17.9%)	40.80 (↓19.4%)
5	G15 - L21	48.83 (↓6.9%)	47.28 (↓6.6%)
6	Control	51.06	53.63

F_1 behavioral evaluation:

Spontaneous activity on postnatal day 14 did not show any drug related effect. Eye opening, incisor eruption, gripping reflex, auditory reflex, pupil reflex and righting reflex was comparable between treated and control pups. Pinna unfolding was slightly slow in group 3 pups. The data are shown from Sponsor's table below.

EFFECTS OF THE TEST ARTICLE ON PERI- AND POST-NATAL DEVELOPMENT IN THE RAT — MF 314521

MEAN AGE IN DAYS OF PUPS REACHING CRITERION — SUMMARY

PAGE 1

DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
EYE OPENING	MEAN	14.65	14.03	14.12	14.40	14.67	14.24
	S.D.	0.57	1.17	0.53	0.76	0.66	0.78
	N	13	9	8	11	10	9
pups reaching criterion	%	100	100	100	100	100	100
INCISOR ERUPTION	MEAN	9.27	9.67	9.63	9.19	9.27	8.99
	S.D.	0.60	1.81	1.06	0.96	1.07	0.95
	N	13	9	8	11	10	9
pups reaching criterion	%	100	100	100	100	100	100
PINNA UNFOLDING	MEAN	2.69	2.83	2.97	2.66	2.18	2.95
	S.D.	0.54	1.12	0.91	0.97	0.88	0.90
	N	13	9	6	12	12	9
pups reaching criterion	%	100	91	76	150	97	78
GRIPPING REFLEX	MEAN	17.00	17.00	17.00	17.00	17.00	17.00
	S.D.	0.00	0.00	0.00	0.00	0.00	0.00
	N	13	9	8	11	10	9
pups reaching criterion	%	100	100	100	100	100	100
AUDITORY REFLEX	MEAN	21.00	21.00	21.00	21.00	21.00	21.00
	S.D.	0.00	0.00	0.00	0.00	0.00	0.00
	N	13	9	8	11	10	9
pups reaching criterion	%	100	100	100	100	100	100
PUPIL REFLEX	MEAN	21.00	21.00	21.00	21.00	21.00	21.00
	S.D.	0.00	0.00	0.00	0.00	0.00	0.00
	N	13	9	8	11	10	9
pups reaching criterion	%	100	100	100	100	100	100
SURFACE RIGHTING REFLEX	MEAN	8.00	8.00	8.00	8.00	8.00	8.00
	S.D.	0.00	0.00	0.00	0.00	0.00	0.00
	N	13	9	8	11	10	9
pups reaching criterion	%	100	100	100	100	100	100

N = Number of litters
% pups reaching criterion = total number of pups with measure present/total number tested on first day of test.

SPONTANEOUS ACTIVITY - MEAN DATA

	TOTAL TIME (secs) ENGAGED IN :			TOTAL	TOTAL TIME (secs) SPENT IN :		
	Ambulatory activity	Small movements	Inactivity	DISTANCE TRAVELLED (cm)	Central region	Corner regions	Lateral regions
GROUP 1							
MEAN	0.5	33.0	146.0	287.3	29.9	97.1	52.4
S.D.	0.9	27.4	28.1	85.6	46.5	61.4	45.7
N	13	13	13	13	13	13	13
GROUP 2							
MEAN	14.6	56.6	105.0	516.9	6.3	126.9	45.0
S.D.	21.1	40.9	61.9	387.0	8.3	51.3	43.5
N	9	9	9	9	9	9	9
GROUP 3							
MEAN	7.2	32.5	139.7	327.7	2.4	148.6	28.4
S.D.	13.4	46.4	59.5	306.6	4.3	59.2	53.3
N	8	8	8	8	8	8	8
GROUP 4							
MEAN	2.3	30.7	146.5	278.9	12.4	100.3	64.6
S.D.	5.0	38.7	43.5	176.3	23.7	72.7	65.0
N	11	11	11	11	11	11	11
GROUP 5							
MEAN	0.9	25.8	152.1	260.0	57.0	95.0	27.4
S.D.	1.4	28.0	29.9	97.7	78.2	80.4	34.2
N	10	10	10	10	10	10	10
GROUP 6							
MEAN	2.4	43.9	133.1	325.1	37.8	76.1	65.6
S.D.	3.8	46.5	50.2	175.4	59.2	57.3	43.2
N	9	9	9	9	9	9	9

F_1 reproduction: Not evaluated

Conclusion of the study:

A pre and post natal reproductive safety study was conduced at 60 mg/kg in pregnant rats treated at gestation day 15 to lactation day 21. Data suggest that the treatment with milnacipran had an adverse effect on survival and weight of F_1 pups. However, surviving pups (F_1) did not show abnormality in the physical development and behavioral assays. Since the study was conducted at a fixed dose, the NOEL is not known.

Study title: Peri and postnatal study in rats treated orally with TN-912

Key study findings: It was concluded that prenatal and post-natal study showed a reduction of survival of F1 pups at 5 mg/kg and higher doses. The treatment had no effect on gestation and delivery. The study was conducted at MTD. The NOEL was not established. The QA statement was not provided. However, the study is acceptable.

Study no.: T081
Volume # M4, and page #: 1
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: Not indicated in the report
GLP compliance: Yes
QA reports: yes ( ) no (x)
Drug: TN-912, lot #[redacted]-006, and % purity: above 99.9% (certificate of analysis was provided on June 9, 2008)

Methods

Doses: 5, 20 and 80 mg/kg

Species/strain: Mated female Wistar rats [redacted] Wistar SPF strain, body weight between 157-233 g at gestation day 0 b(4)

Number/sex/group: Study design and groups are shown below.

Test Groups	Dose Levels* (mg/kg)	Concentration (%)	No. of Animals w/ Successful Copulation	Animal No.
Control	0	0	24	1101 - 1124
Low	5	0.1	24	2101 - 2124
Intermediate	20	0.4	24	3101 - 3124
High	80	1.6	24	4101 - 4124

*: as bulk, purity conversion not done

The dose level was determined from results of the fertility study.

Route, formulation, volume, and infusion rate: The drug substance was dissolved in water for injection and the vehicle was used in the control animals. The drug solution was administered orally once daily by gavage at 0.5 mL/100 g from gestation day 17 to post-partum day 21.

Satellite groups used for toxicokinetics: Nil

Study design: Animals were observed three times daily during the treatment and once a day in other period of the study for abnormal behavior, toxicity, and mortality. The body weight of dams were recorded on days 0, 4, 7, 11 and 14 of gestation and daily from gestation days 17 until delivery. The body weight of dams was recorded on lactation days 0, 2, 4, 7, 11, 14, 17, and 21. The food consumption was recorded on gestation days 1, 4, 8, 11, 14, 17 and 20. The food consumption was also recorded on

post partum days 2, 4, 7, 11, 14, 17 and 21. Dams were sacrificed under ether anesthesia on lactation day 22 to examine ovaries for corpora lutea and uteri for implantations and resorptions. Any gross changes were noted and abnormal tissues were fixed in 10% formalin.

Parameters and endpoints evaluated:

The length of gestation and percent delivery index (# pregnant delivered live pups / # of pregnant rats) were recorded. The number of live and still births was determined. Any variation of organs in still-born pups was determined according to Wilson's techniques. External malformations, gender and body weights of surviving pups were recorded. The body weight of weaning pups was recorded twice a week up to day 21 and once a week up to day 70.

At least 4 male and 4 female pups/litter were randomly culled for weaning by their dams on day 4. The remaining pups were sacrificed under ether anesthesia and fixed in 10% formalin. Furthermore, 2 pups/sex/litter were selected on day 22 for further development and the remaining pups were sacrificed under ether anesthesia.

The following physical and developmental parameters were recorded for F_1 pups during the development and sacrificed on day 70 by ether anesthesia except those animals used for reproductive performance: pinna detachment (days 4 and 7), abdominal hair (days 7 and 11), eruption of lower incisor (days 11 and 14), opening of eyelids (days 14 and 17), testicular descent (days 21 and 28), opening of vagina (days 35 and 42). Viability of F_1 pups were recorded up to day 70. Viability at the time of birth (birth index), on day 4 (viability index) and on day 21 (weaning index) were recorded.

The following behavioral and functional assessments of F_1 pups were recorded: righting reflex, pupillary reflex, pinna reflex, corneal reflex, and auditory reflex were recorded on day 21. Mobility and functional coordination was tested using an open-field test on lactation week 5. Learning ability using water-filled multiple T-maze test was carried out at 7-8 weeks of age. Pups were trained to swim to reach the destination. The time taken to reach the goal and number of errors were recorded.

Reproductive performance:

One male and one female F_1 pups per litter at 10-12 weeks of age were mated. Body weights of F_1 females with confirmed copulation were recorded on days 0, 4, 8, 12, 16 and 20 of gestation. Animals were sacrificed on gestation days 20 for examination of ovaries and uteri using procedures similar to that described above.

Results

F_0 in-life: No treatment related clinical signs were observed except one animal at 20 mg/kg showed a palpable mass at the left inguinal region. Several dams were sacrificed

due to dead fetuses. However, there was no death of dams due to the treatment during gestation period.

During the lactation period, 3, 2, 2, and 14 dams were sacrificed at several time points due to the death of pups at control, 5, 20, and 80 mg/kg, respectively. Therefore, the treatment at 80 mg/kg showed postnatal deaths to rats.

The average body weight (g) of dams during gestation period is shown in the table below.

Dose	Day G0	Day G17	Day G20	BW gain, G17-20
Group 1, control	190	281.8	316.4	34.8
Group 2, 5 mg/kg	191.6	276.4	309.6	33.2
Group 3, 20 mg/kg	188.9	283.6	311.7	28.1*
Group 4, 80 mg/kg	189.7	280.3	304.8*	24.4*

*Statistically significant

Data suggest that there was a statistically significant reduction (20% or more) in the average body weight gain during gestation days 17 and 20 at 20 and 80 mg/kg that was due to the treatment.

The average body weight (g) during the lactation period day 1 and day 21 is shown in the table below.

Dose, mg/kg	L1	L21	BW gain, L1-21
0	252.7	278.4	22.5
5	244.3	272.3	25.6
20	251.1	269.9*	18.9
80	244.0	263.3*	15.6

Above data show that the body weight of dams was reduced at 20 and 80 mg/kg was reduced more than 15% during the lactation period.

The food consumption was slightly reduced before delivery at 80 mg/kg. The average food consumption (g/rat/day) is shown below from the Sponsor's table.

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Table 5 Peri- and post-natal study in rats treated orally with TN-912

Food consumption of F0 dams during the gestation period

Dose mg/kg		Administration 1	4	8	11	14	17	20a)
0	No.	23	23	23	23	23	23	23
	Mean	16.4	21.1	21.9	23.9	23.1	24.3	18.7
	S.D.	3.4	2.8	2.7	2.1	2.4	1.9	2.6
5	No.	22	22	22	22	22	22	22
	Mean	16.7	20.5	21.0	23.4	22.5	24.0	17.8
	S.D.	2.7	2.2	1.7	2.2	2.7	2.5	2.6
20	No.	23	23	23	23	23	23	23
	Mean	17.6	21.0	21.5	23.8	23.5	24.3	18.2
	S.D.	2.3	2.0	2.4	2.6	2.9	3.0	1.6
80	No.	24	24	24	23 b)	24	24	24
	Mean	17.5	22.0	21.7	23.6	22.6	24.1	16.5**
	S.D.	3.5	2.7	2.1	3.2	2.1	2.4	2.5

No.: No. of dams
a) : Day of gestation
Unit : g/rat/day
b) : Data of one dam was excluded from statistical analysis, because a large amount of spilt food was noticed.
** : p<0.01 (Significant difference from control)

The mean food consumption was 44.5, 42.4, 42.1, and 37.4 at control, 5, 20, and 80 mg/kg at the end of lactation day 21. The reduced food consumption contributed to the reduced weight gain of dams at 80 mg/kg.

The delivery data are shown in the table below (from the Sponsor's table 7).

Table 7 Peri- and post-natal study in rats treated orally with TN-912

Delivery data on F0 dams

Dose mg/kg		No. of pregnant females	No. of a) females	Delivery index % b)	Gestation period	No. of implantations	No. of stillborn (%)c)	Live born: No. of live born Total	Male	Female	Sex ratio (Male/Female)	Ext. d) malf. (%)e)	Live birth index % f)
0	Total	23	23	100.0		332	9(2.9)	301	163	138	1.18	0(0.0)	90.7
	Mean				22.0	14.4		13.1	7.1	6.0			
	S.D.				0.0	2.0		2.1	1.8	1.9			
5	Total	22	22	100.0		322	22(7.5)*	270	151	119	1.27	0(0.0)	83.9
	Mean				22.0	14.6		12.3	6.9	5.4			
	S.D.				0.2	1.5		2.3	2.2	2.1			
20	Total	23	23	100.0		339	27(8.7)	283	148	135	1.10	0(0.0)	83.5*
	Mean				22.0	14.7		12.3	6.4	5.9			
	S.D.				0.2	1.6		2.9	2.0	2.5			
80	Total	24	24	100.0		362	44(13.6)**	283	151	132	1.14	0(0.0)	78.2**
	Mean				22.0	15.1		11.8*	6.3	5.5			
	S.D.				0.4	1.9		2.2	1.8	1.9			

a): No. of females delivered with live pup
b): (No. of females delivered with live pup / No. of pregnant females) × 100
c): (No. of stillborn pups / No. of stillborn and live born pups) × 100
d): No. of live born pups with external malformations
e): (No. of live born pups with external malformations / No. of live born pups) × 100
f): (No. of live born pups / No. of implantations) × 100
* : p<0.05 ; ** : p<0.01 (Significant difference from control)

There was no change in the gestation period and delivery. However, there was a reduction in the live birth and increase in still births at 5, 20, and 80 mg/kg. Based on the pregnancy data, there was no NOEL in rats when treated with milnacipran at late pregnancy. Data from Sponsor's table 13 suggest that still born pups did not have a trend of visceral changes that could lead to fetal deaths.

F_0 necropsy: There were no treatment related changes at necropsy in dams except hardened node at left inguinal region in one rat at 20 mg/kg.

F_1 physical development:

The average body weight of F_1 pups from birth to post natal day 70 is shown from the **Sponsor's table.**

Table 9 Peri- and post-natal study in rats treated orally with TN-912

Body weights of F1 males

Dose mg/kg		0	4	7	11	14	17	21	28	35	42	49	56	63	70a)
0	No.	23	21 b)	20 c)	20	20	20	20	20	20	20	20	20	20	20
	Mean	5.3	8.2	13.7	21.6	28.2	34.8	43.7	76.7	125.0	177.3	231.0	276.2	311.3	340.4
	S.D.	0.4	1.4	2.0	2.9	3.4	4.0	5.4	8.1	11.0	13.8	16.1	17.1	16.8	20.5
5	No.	22	21 d)	20 e)	20	20	20	20	20	20	20	20	20	20	20
	Mean	5.2	7.8	12.6	20.1	26.6	32.1*	41.0	70.9*	116.9*	166.6*	218.2**	267.6**	291.2**	318.2**
	S.D.	0.4	1.4	1.7	2.3	2.7	3.1	4.2	6.6	8.2	11.5	13.5	16.4	16.4	18.0
20	No.	23	21 f)	21	21	21	21	21	21	21	21	21	21	21	21
	Mean	4.0**	7.3*	11.5**	18.6**	24.7**	30.5**	38.5**	67.5**	110.9**	160.3**	209.9**	250.8**	283.3**	311.3**
	S.D.	0.4	0.7	1.4	2.4	2.9	3.4	4.2	6.7	12.0	13.4	15.8	16.7	18.1	20.2
80	No.	24	7 g)	7	7	7	7	7	7	7	7	7	7	7	7
	Mean	4.6**	6.5**	9.5**	16.1**	19.3**	23.6**	29.7**	53.5**	92.9**	137.6**	181.3**	220.9**	253.9**	275.9**
	S.D.	0.4	1.3	2.3	3.4	3.9	3.8	4.9	9.7	14.5	16.9	21.1	23.8	25.6	29.7

No.: No. of dams
a) : Day after birth
b) : Two litters died on day 4 after birth.
c) : One litter died on day 7 after birth.
d) : One litter died on day 4 after birth.
e) : One litter died on day 5 after birth.
f) : One litter died on day 3 and one litter died on day 4 after birth.
g) : Eight litters died on day 1, four litters died on day 2, two litters died on day 3 and three litters died on day 4 after birth.
* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Unit : g

Table 10 Peri- and post-natal study in rats treated orally with TN-912

Body weights of F1 females

Dose mg/kg		0	4	7	11	14	17	21	28	35	42	49	56	63	70a)
0	No.	23	21 b)	20 c)	20	20	20	20	20	20	20	20	20	20	20
	Mean	4.9	7.6	12.5	20.3	26.8	33.0	41.6	68.6	108.0	134.7	159.7	178.8	197.4	210.2
	S.D.	0.4	1.1	1.8	2.4	2.9	3.2	4.4	7.2	9.1	11.2	11.8	12.9	14.0	15.6
5	No.	22	21 d)	20 e)	20	20	20	20	20	20	20	20	20	20	20
	Mean	4.9	7.4	11.6	18.6	24.9*	30.7*	39.1	67.0	101.7	130.7	154.4	174.1	191.7	204.8
	S.D.	0.5	1.4	2.0	2.7	3.1	3.6	4.7	7.1	9.2	9.8	11.9	11.3	13.0	13.6
20	No.	22 f)	22	21 e)	21	21	21	21	21	21	21	21	21	21	21
	Mean	4.7	6.8*	10.8**	17.5**	23.4**	29.0**	36.7**	62.5*	96.8**	126.2**	148.5**	159.3*	188.4**	200.4*
	S.D.	0.4	1.2	1.6	2.4	3.1	3.3	4.3	7.1	9.2	8.6	10.3	11.2	11.5	13.3
80	No.	24	7 g)	7	7	7	7	7	7	7	7	7	7	7	7
	Mean	4.8**	6.2**	9.2**	15.0**	19.9**	24.9**	31.1**	56.1**	90.3**	118.2**	139.0**	158.6**	176.1**	190.1**
	S.D.	0.4	1.2	1.9	2.8	3.0	3.2	3.9	5.0	7.9	8.6	10.8	10.3	12.8	14.8

No.: No. of dams
a) : Day after birth
b) : Two litters died on day 4 after birth.
c) : One litter died on day 7 after birth.
d) : One litter died on day 4 after birth.
e) : One litter died on day 5 after birth.
f) : One litter of F1 female was not born.
g) : Eight litters died on day 1, four litters died on day 2, two litters died on day 3 and three litters died on day 4 after birth.
* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Unit : g

The average body weight of male F_1 pups showed slower weight gain during post-natal development at 5, 20, and 80 mg/kg. The body weight gain of female F_1 pups showed slower growth during the post-natal development at 20 and 80 mg/kg.

A significant reduction in the post-natal death was reported within post-natal days 4 and 21 in F_1 pups at 80 mg/kg as shown in the Sponsor's table below.

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Table 11 Peri- and post-natal study in rats treated orally with TN-912

Viability index of pups

Dose mg/kg		No. of dams	Before culling: Day 0 Live number c)	Before culling: Day 4 Live number c)	Before culling: Survival index on day 4 after birth % a)	No. of dams	After culling: Day 4 Live number c)	After culling: Day 21 Live number c)	After culling: Weaning index on day 21 after birth % b)
0	Total	23	301	253	84.1	21	168	158	94.0
	Mean		13.1	11.0			8.0	7.5	
	S.D.		2.1	4.1			0.0	1.8	
5	Total	22	270	220	81.5	21	159	154	96.9
	Mean		12.3	10.0			7.6	7.3	
	S.D.		2.3	3.6			1.1	1.8	
20	Total	23	283	238	84.1*	22	168	163	97.0
	Mean		12.3	10.3			7.6	7.4	
	S.D.		2.9	3.5			1.2	1.8	
80	Total	24	283	60	21.2**	7	49	46	93.9
	Mean		11.8*	2.5**			7.0	6.6	
	S.D.		2.2	4.3			1.9	1.8	

a): (No. of live pups on day 4 / No. of live born pups on day 0) × 100
b): (No. of live pups on day 21 / No. of live pups on day 4) × 100
c): No. of live pups
* : p<0.05 ; ** : p<0.01 (Significant difference from control)

The death in weaning rats at 80 mg/kg was not attributed to abnormal visceral changes.

Among the surviving F_1 pups, opening of eye lids, appearance of abdominal hair and incisor eruption were retarded at 80 mg/kg as shown in the Sponsor's table below.

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Table 12 Peri- and post-natal study in rats treated orally with TN-912

External differentiation of pups

Dose	No. of dams	Pinna detachment		Appearance of abdominal hair		Eruption of lower incisor		Opening of eyelid		Descent of testis		Opening of vagina	
mg/kg		4	7	7	11	11	14	14	17	21	28	35	42 a)
0	21	253/253	160/160	0/160	160/160	102/160	159/159	6/159	158/158	22/ 77	40/ 40	39/ 40	40/ 40
		100.0	100.0	0.0	100.0	63.8	100.0	3.8	100.0	28.6	100.0	97.5	100.0
5	21	220/220	155/155	0/155	154/155b)	77/155	155/155	4/155	155/155	14/ 82	40/ 40	40/ 40	40/ 40
		100.0	100.0	0.0	99.4	49.7**	100.0	2.6	100.0	17.1	100.0	100.0	100.0
20	22	237/238	163/163	0/163	163/163	91/163	163/163	7/163	163/163	10/ 81	42/ 42	40/ 42	42/ 42
		99.6	100.0	0.0	100.0	55.8	100.0	4.3	100.0	12.3**	100.0	95.2	100.0
80	7	60/ 60	49/ 49	0/ 49	45/ 48c)	12/ 48	48/ 48	8/ 48	48/ 48	6/ 21	12/ 12	11/ 13	13/ 13
		100.0	100.0	0.0	93.8**	25.0**	100.0	16.7**	100.0	28.6	100.0	84.6	100.0

Upper: No. of pups differentiated / No. of pups observed
Lower: %
a): Day after birth
b): All pups differentiated on day 12 after birth.
c): All pups differentiated on day 13 after birth.
* : p<0.05 ; ** : p<0.01 (Significant difference from control)

F_1 behavioral evaluation:

Functional examinations of F_1 pups on post-natal day 21 did not show any change in reflexes when compared to the pup from vehicle treated dams. A slight reduction in latency (time taken to start movement) was noted in open field test at 5 weeks of age at 80 mg/kg in male F_1 pups. However, its relationship to the treatment is not known because a similar change was not noted in female F_1 pups.

No treatment related change in the water-filled maze test was noted in male and female F_1 pups. F_1 pups did not show any macroscopic changes at necropsy.

F_1 reproduction: Fertility index (mating and pregnancy) of F_1 generation males and females did not show any treatment-related change as shown in the Sponsor's table below.

Table 24 Peri- and post-natal study in rats treated orally with TN-912

Mating and fertility of F1 animals

Dose	Male				Female			
mg/kg	No. of males	Days until copulation Mean±S.D.	Copulation index (%) a)	Insemination index (%) b)	No. of females	Days until copulation Mean±S.D.	Copulation index (%) c)	Fertility index (%) d)
0	20	3.4±2.5	19/20(95.0)	18/19(94.7)	20	3.4±2.5	19/20(95.0)	18/19(94.7)
5	20	2.8±1.2	20/20(100.0)	20/20(100.0)	20	2.8±1.2	20/20(100.0)	20/20(100.0)
20	21	3.1±2.9	21/21(100.0)	19/21(90.5)	21	3.1±2.9	21/21(100.0)	19/21(90.5)
80	7	3.0±1.2	7/ 7(100.0)	7/ 7(100.0)	7	3.0±1.2	7/ 7(100.0)	7/ 7(100.0)

a): (No. of males with confirmed copulation / No. of males mated) X 100
b): (No. of inseminated males / No. of males with confirmed copulation) X 100
c): (No. of females with confirmed copulation / No. of females mated) X 100
d): (No. of pregnant animals / No. of females with confirmed copulation) X 100

The body weight gain at 80 mg/kg in F_1 animals during the gestation period was reduced. The body weight data from Sponsor's table 25 are shown below.

Table 25 Peri- and post-natal study in rats treated orally with TN-912

Body weights of F1 dams during the gestation period

Dose mg/kg		0	4	8	12	16	20a)	Body weight gains 0 - 20
0	No.	18	18	18	18	18	18	18
	Mean	228.5	242.7	256.4	275.8	298.7	348.6	120.1
	S.D.	16.2	17.1	17.3	19.6	19.5	20.6	9.3
5	No.	20	20	20	20	20	20	20
	Mean	223.5	237.1	249.9	267.8	290.7	337.2	113.7
	S.D.	16.3	16.5	17.4	18.6	19.8	23.0	10.5
20	No.	19	19	19	19	19	19	19
	Mean	219.8	234.2	247.7	266.4	286.4	333.8	114.0
	S.D.	20.7	20.4	21.2	22.3	22.3	25.8	12.6
80	No.	7	7	7	7	7	7	7
	Mean	199.6**	211.3**	222.3**	238.0**	259.7**	305.7**	106.1**
	S.D.	18.8	18.0	20.4	18.7	23.0	25.1	10.2

No.: No. of F1 dams
a) : Day of gestation
** : p<0.01 (Significant difference from control)

Unit : g

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The caesarean section data for F_1 females from Sponsor's table 26 are shown below.

Table 26 Peri- and post-natal study in rats treated orally with TN-912

Cesarean section data on F1 dams

Dose mg/kg	No. of F1 dams		No. of corpora lutea	No. of implantations	Implantation index x a)	No. of resorbed or dead fetuses: Total (X)b)	Early c)	Late d)	Live fetuses: No. of live fetuses: Total	Male	Female	Sex ratio (M/F)e)	Fetal body weight(g): Male	Female	Placental weight(g)	No. of f) live fetuses with ext. mal. (X)h)
0	18	Total	281	274	97.5	22(8.0)	22	0	252	123	129	0.95				0(0.0)
		Mean	15.6	15.2					14.0	6.8	7.2		3.33	3.03	0.42	
		S.D.	1.6	1.5					1.3	2.2	2.1		0.18	0.20	0.03	
5	20	Total	288	278	96.5	19(6.8)	19	0	259	140	119	1.18				0(0.0)
		Mean	14.4*	13.9*					13.0*	7.0	6.0		3.31	3.06	0.44	
		S.D.	1.8	1.7					1.6	2.1	2.1		0.16	0.18	0.04	
20	19	Total	272	266	97.8	24(9.0)	13	11	242	127	115	1.10				1(0.4)i)
		Mean	14.3*	14.0*					12.7*	6.7	6.1		3.29	3.04	0.43	
		S.D.	1.2	1.3					2.3	2.1	2.4		0.26	0.28	0.04	
80	7	Total	90	89	98.9	6(6.7)	5	1	83	36	47	0.77				0(0.0)
		Mean	12.9**	12.7**					11.9**	5.1	6.7		3.40	3.17	0.43	
		S.D.	1.1	1.0					1.1	2.0	2.5		0.23	0.14	0.03	

a): (No. of implantations / No. of corpora lutea) × 100
b): (No. of resorbed or dead fetuses / No. of implantations) × 100
c): Resorbed embryo and placental remnant
d): Early macerated fetus, late macerated fetus and dead fetus
e): No. of males / No. of females
f): No. of live fetuses with external malformations
h): (No. of live fetuses with external malformations / No. of live fetuses) × 100
i): Vestigial tail
* : p<0.05 ; ** : p<0.01 (Significant difference from control)

There was a reduction in ovulation, implantation and live fetuses at 80 mg/kg.

F_2 findings: No external malformation due to the treatment was noted in F_2 pups.

Summary of the study:

A pre and post-natal developmental toxicity study (segment 3) was conducted in Wistar rats at 5, 20 and 80 mg/kg/oral from gestation day 17 to post-natal day 21. The body weight gain was reduced at 20 and 80 mg/kg by 15% or more. No treatment related mortality was observed in dams. The treatment had no effect on the gestation and delivery of pups. However, a decrease in live birth was noted at 5, 20, and 80 mg/kg. The post-natal survival and physical development of F_1 pups was further impaired at 80 mg/kg. However, behavior and functional assays did not show a treatment related effect on the surviving F_1 pups.

Reproductive performance of F_1 rats did not show any effect on the mating performance. However, fertility was affected at 80 mg/kg in F_1 generation.

It was concluded that prenatal and post-natal study showed a reduction of survival of F_1 pups at 5 mg/kg and higher doses. A NOEL was not established. The study was conducted at MTD.

Study title: Peri- and postnatal study in rats treated orally with TN-912 (additional study)

Key study findings: NOEL for the study was 2.5 mg/kg

Study no.: T082
Volume #M4, and page #: 1
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: Not mentioned
GLP compliance: Yes
QA reports: yes ( ) no (x)
Drug: TN-912, lot #:[redacted]**-006, and % purity:** Above 99%

Methods

Doses: 1.25, 2.5 and 5 mg/kg/oral

Species/strain: Pregnant [redacted] Wistar SPF rats weighed 162-214 g at the beginning of gestation

Number/sex/group: b(4)

Test Groups	Dose Levels* (mg/kg)	Concentration (%)	No. of Animals w/ Successful Copulation	Animal No.
Control	0	0	24	1101 - 1124
Low	1.25	0.25	24	2101 - 2124
Intermediate	2.5	0.05	24	3101 - 3124
High	5	0.1	24	4101 - 4124

*: as bulk, purity conversion not done

The dose was selected on the basis of previous study.

Route, formulation, volume, and infusion rate: The test substance was dissolved in water for injection. Animals were treated with the vehicle or test solution orally by gavage at 0.5 mL/kg between gestation days 17 and lactation day 21.

Satellite groups used for toxicokinetics: Nil

Study design: Animals were observed daily three times during the treatment period for any toxicity and mortality. Animals were observed once daily during other period. The body weight was recorded on gestation days 0, 4, 7, 11, 14, daily between gestation days 17 up to the delivery. The body weight was recorded on lactation days 0, 2, 4, 7, 11, 14, 17, and 21. The food consumption was recorded on gestation days 1, 4, 8, 11, 14, 17, and 20. The food consumption was also recorded on lactation days 2, 4, 7, 11, 14, 17, and 21.

Pregnant animals were allowed to deliver and the length of gestation was recorded. Number of pregnant animals and number of animals delivered live pups were counted for the determination of delivery index. F_1 pups were culled to 4 pups/sex/litter on weaning day 4. F_1 animals were observed daily for viability up to day 70 after birth. The remaining pups were sacrificed by ether anesthesia.

Development of F_1 pups were examined as follows: pinna detachment on days 4 and 7, abdominal hair on days 7 and 11, eruption of lower incisor on days 11 and 14, opening of eye lids on days 14 and 17, testicular descent on days 21 and 28 and opening of vigina on days 35 and 42.

One male and one female F_1 rats/test group were allowed to mate at 10-12 weeks of age. Animals were sacrificed on gestation day 20 under ether anesthesia to determine pregnancy, corpora lutea, resorptions, live and dead fetuses. Live fetuses (F_2 pups) were examined for gender and external malformation. Half of the fetuses were fixed in **Bouin's fixative to examine visceral malformations** and other half was dehydrated with in 90% ethanol for skeletal examination.

Parameters and endpoints evaluated:

Treated dams were allowed to lactate for 21 days after delivery. The general conditions of F_1 pups were recorded. Dams were sacrificed on lactation day 22 under ether anesthesia to examine the ovary and uterus for corpora lutea and resorptions. Any tissues with macroscopic lesions were fixed in 10% formalin.

Number of still born F_1 pups were counted and fixed in **Bouin's fixative for visceral examination of any variations or malformations according to Wilson's techniques. The** body weight of F_1 dams were recorded twice a week up to weaning day 21 and once a week up to day 70. Any external malformation of live pups was recorded also.

During the development process of F_1 pups , birth index (# live birth/ # implantations x100), viability index (# alive on day 4 / # live pups at birth x 100) and weaning index (# live pups on day 21 / # culled on day 4 x 100) were recorded. F_1 pups died during the **lactation period were fixed in Bouin's** fixative for visceral examinations.

Results

F_0 in-life: No clinical signs were noted in any surviving dam during gestation and lactation period.

One dam at 2.5 mg/kg and one dam at 5 mg/kg died due to gavage error on gestation days 20 and 13, respectively.

One dam at 5 mg/kg died due to gavage error on the day of delivery. Two dams were necropsied on lactation days 4 and 11 at 5 mg/kg due to deaths of litters.

The average body weight (g) of dams during the gestation period is shown in the table below.

Dose, mg/kg	Day G0	Day G20	BW gain G17-G20
0	191.9	306.2	32.3
1.25	192.3	311.4	34.3
2.5	191.6	313.2	33.5
5	193.0	308.1	30.5

There was no effect on the body weight of dams during the gestation period except a slight reduction in the body weight gain at 5 mg/kg.

The body weight (g) during lactation period (Lo-L21) is shown in the table below.

Dose, mg/kg	L0	L21	BW gain L1-21
0	241.3	266.1	23.7
1.25	240.4	268.3	27.7
2.5	245.5	268.1	22.6
5	244.0	262.3	18.1*

The body weight gain during lactation was significantly reduced from 23.7 g in the control to 18.1 g at 5 mg/kg dose during lactation period. However, its biological significance is unknown because the body weights on days L0 and L21 were not affected by the treatment. The food consumption during the gestation period was not affected by the treatment. However, the food consumption during the lactation period was increased at 2.5 and 5 mg/kg after lactation day 11 that was statistically significant.

The delivery information for dams is shown in the Sponsor's table below.

Table 7 Peri- and post-natal study in rats treated orally with TN-912 (additional study)
Delivery data on F0 dams

Dose		No. of pregnant	No. of	Delivery index	Gestation	No. of implan-	No. of still-	Live born					Live birth index
								No. of live born				Ext. d) malf.	
mg/kg		females	a) females	x b)	period	tations	born (%)c)	Total	Male	Female	Sex ratio (Male/Female)	(%)e)	x f)
0	Total	23	23	100.0		339	13(4.2)	296	150	148	1.03	1(0.3)h)	87.3
	Mean				22.2	14.7		12.9	6.5	6.3			
	S.D.				0.3	3.1		2.9	2.3	2.6			
1.25	Total	23	23	100.0		380	8(2.4)	325	173	152	1.14	0(0.0)	92.9*
	Mean				22.0	16.2		14.1	7.5	6.6			
	S.D.				0.2	1.3		1.2	2.3	2.1			
2.5	Total	22	22	100.0		329	16(5.1)	295	145	149	0.98	0(0.0)	89.7
	Mean				22.0	15.0		13.4	6.6	6.8			
	S.D.				0.1	1.3		1.8	1.8	2.0			
5	Total	23g)	23	100.0		344	8(2.5)	307	161	146	1.10	1(0.3)h)	89.2
	Mean				22.0	15.0		13.3	7.0	6.3			
	S.D.				0.1	1.3		1.6	1.7	1.6			

a): No. of females delivered with live pup.
b): (No. of females delivered with live pup / No. of pregnant females) X 100.
c): (No. of stillborn pups / No. of stillborn and live born pups) X 100.
d): No. of live born pups with external malformations.
e): (No. of live born pups with external malformations / No. of live born pups) X 100.
f): (No. of live born pups / No. of implantations) X 100.
g): One dam was excluded from statistical analysis, because it was killed by intubation error on day 13 of lactation.
h): Vestigial tail.
* : p<0.05 (Significant difference from control).

The number of live birth was not affected by the treatment.

F_0 necropsy: No treatment related macroscopic change was reported in Sponsor's table 8.

F_1 physical development: The viability data for F_1 pups are shown in the Sponsor's table below.

Table 11 Peri- and post-natal study in rats treated orally with TN-912 (additional study)
Viability index of pups

Dose mg/kg		No. of dams	Before culling: Day 0 Live number c)	Before culling: Day 4 Live number c)	Before culling: Survival index on day 4 after birth % a)	No. of dams	After culling: Day 4 Live number c)	After culling: Day 21 Live number c)	After culling: Weaning index on day 21 after birth % b)
0	Total	23	295e)	249	84.4	21f)	159	153	96.2
	Mean		12.8	10.8			7.6	7.3	
	S.D.		2.9	4.6			1.1	1.4	
1.25	Total	23	325	273	84.0	22g)	170	160	94.1
	Mean		14.1	11.9			7.7	7.3	
	S.D.		1.2	3.7			0.9	1.8	
2.5	Total	22	295	279	94.6	22	176	175	99.4
	Mean		13.4	12.7			8.0	8.0	
	S.D.		1.8	2.1			0.0	0.2	
5	Total	23d)	306e)	278	90.8	22h)	176	168	95.5
	Mean		13.3	12.1			8.0	7.6	
	S.D.		1.6	3.2			0.0	1.7	

a): (No. of live pups on day 4 / No. of live born pups on day 0) × 100.
b): (No. of live pups on day 21 / No. of live pups on day 4) × 100.
c): No. of live pups.
d): One dam was excluded from statistical analysis, because it was killed by intubation error on day 13 of lactation.
e): One pup was excluded from statistical analysis, because it had external malformation (vestigial tail).
f): Two dams were necropsied on day 1 and 4 of lactation, because all pups died.
g): One dam was necropsied on day 3 of lactation, because all pups died.
h): One dam was necropsied on day 4 of lactation, because all pups died.

Survival index on day 4 and weaning index on day 21 of lactation for F_1 pups was not affected by the treatment.

The body weight gain of F_1 male and female during weaning and development was not affected by the treatment.

Opening of vagina on day 35 in F_1 offspring was reduced in 63% pups at 5 mg/kg compared to 89% in the control pups. No historical control data were provided for the time taken for opening of vagina in the Wistar rats. However, the range is between 28 and 39 days for Sprague Dawley rats obtained from [ ] b(4)
No visceral abnormality in still born pups and pups that died during post-natal period was noted. Surviving F_1 generation rats also did not show visceral abnormality at necropsy.

F_1 behavioral evaluation: Not evaluated

F_1 reproduction: Reproductive performance and fertility of F_1 rats were comparable to the control as shown in the Sponsor's table below.

Table 19 Peri- and post-natal study in rats treated orally with TN-912 (additional study)
Cesarean section data on F1 dams

Dose mg/kg	No. of F1 dams		No. of corpora lutea	No. of implantations	Implantation index × a)	No. of resorbed or dead fetuses				Live fetuses						Placental weight(g)	No. of f) live fetuses with ext. mal. (%)h)
						Total (%)b)		Early c)	Late d)	No. of live fetuses Total	Male	Female	Sex ratio (M/F)e)	Fetal body weight(g) Male	Female		
0	18	Total	262	247	94.3	18(7.3)		16	2	229	122	107	1.14				0(0.0)
		Mean	14.6	13.7						12.7	6.8	5.9		3.32	3.06	0.43	
		S.D.	1.8	3.4						3.6	2.6	2.0		0.23	0.12	0.08	
1.25	i) 18	Total	260	242	93.1	18(7.4)		13	5	224	116	108	1.07				0(0.0)
		Mean	14.4	13.4						12.4	6.4	6.0		3.33	3.09	0.42	
		S.D.	1.7	2.7						2.5	2.5	2.0		0.23	0.19	0.03	
2.5	i) 19	Total	269	263	97.8	13(4.9)		11	2	250	131	119	1.10				0(0.0)
		Mean	14.2	13.8						13.2	6.9	6.3		3.33	3.11	0.43	
		S.D.	2.0	1.9						1.9	1.7	2.1		0.19	0.16	0.04	
5	i) 19	Total	255	254	99.6*	14(5.5)		13	1	240	122	118	1.03				0(0.0)
		Mean	13.4	13.4						12.6	6.4	6.2		3.29	3.08	0.43	
		S.D.	1.7	1.7						1.8	1.8	2.3		0.21	0.23	0.03	

a): (No. of implantations / No. of corpora lutea) × 100.
b): (No. of resorbed or dead fetuses / No. of implantations) × 100.
c): Resorbed embryo and placental remnant.
d): Early macerated fetus, late macerated fetus and dead fetus.
e): No. of males / No. of females.
f): No. of live fetuses with external malformations.
h): (No. of live fetuses with external malformations / No. of live fetuses) × 100.
i): One dam was excluded from statistical analysis, because it was not confirmed on the day of copulation.
* : p<0.05 (Significant difference from control).

F_2 findings: Not determined

Summary of the study:

Above data suggest that the no effect dose (NOEL) for dams and offspring was 2.5 mg/kg. The compliance statement was not provided in the report. However, the study is acceptable.

Summary of the reproductive safety studies:

Fertility and reproductive safety of milnacipran was investigated in rats for segment 1 study, mice and rabbits for segment 2 studies and in rats for segment 3 studies.

The fertility study was conducted at 5, 15, and 60 mg/kg in Sprague Dawley rats. Based on the data, the high dose reached maximum tolerated dose and the study is acceptable. The mating performance of male and female rats was not affected by the treatment up to 60 mg/kg. However, caesarean data showed decreased fertility at 5 (30 mg/m^2), 15 (90 mg/m^2) and 60 (360 mg/m^2) mg/kg. The Sponsor also conducted another fertility study at 5, 20, and 80 mg/kg in Wistar rats. Female rats were treated up to gestation days 7. Mating performance was delayed at 20 and 80 mg/kg. Fertility was reduced at 80 mg/kg. A NOEL was 5 mg/kg for effects on fertility. However, the biological responses in two studies was similar and the effect on Sprague Dawley rats at 5 mg/kg was considered to

be the most sensitive. Therefore, it was decided that milnacipran reduced fertility at 5 mg/kg. The package insert should indicate that the treatment with milnacipran affected the fertility of rats at 5 mg/kg. The no-effect dose was not established.

Although the segment 2 studies were conducted in mice and rabbits, the design of segment 1 study in rats included the teratogenicity assessment. Therefore, data for malformation and variations due to the treatment with milnacipran were assessed in rats, mice and rabbits.

Rats were treated at 5 (30 mg/m^2), 15 (90 mg/m^2) and 60 (360 mg/m^2) mg/kg during implantation and organogenicity. Rats did not show external, visceral or skeletal abnormalities due to the treatment (no teratogenicity was noted). However, the percent of intrauterine deaths were 0, 15, 20, and 50% pregnant rats at control, 5, 15 and 60 mg/kg, respectively. The no-effect dose was not determined.

The mouse teratogenicity study (segment 2) was conducted at 5 (15 mg/m^2), 25 (75 mg/m^2) and 125 (375 mg/m^2) mg/kg during gestation days 6 and 15. No visceral or skeletal malformations were reported due to milnacipran. Fetal weights were reduced at 25 and 125 mg/kg. Therefore, no teratogenicity was noted. The no effect dose (NOEL) was 5 mg/kg.

The rabbit teratogenicity study (segment 2) was conducted at 5 (60 mg/m^2), 15 (180 mg/m^2) and 60 (720 mg/m^2) mg/kg during gestation days 6 and 18. Although no teratogenicity was observed, single extra rib was observed as a variation at 15 and 60 mg/kg. The no effect dose (NOEL) was 5 mg/kg.

Three prenatal and post natal studies (segment 3) were conducted in rats. The segment 1 study was also designed to examine the effect of treatment on delivery and post-natal development.

The post-natal data from rats treated at 5, 15 and 60 mg/kg showed no effect on the gestation. However, number of still births and post-natal deaths were increased at 15 and 60 mg/kg. Although reflex development in F_1 rats was not affected by the treatment, fertility of second generation (F_1) was reduced at 15 and 60 mg/kg. **The treatment might have an impact on the development of neuro-endrocrine systems in rats when exposed to milnacipran in utero.** The no-effect dose (NOEL) for post-natal development was 5 mg/kg based on study #T 030.

Another segment 3 study (#T081) in rats confirmed a similar deleterious effect on post-natal survival even at 5 mg/kg and higher doses in pregnant rats. However, behavioral and physical development parameters of the F_1 generation (offspring of treated mothers) were not affected. Fertility index was not affected at 5, 20, and 80 mg/kg in F_1 generation rats in study # T081 (untreated rats weaned by treated mothers) unlike that observed in study #T030.

Since the no-effect dose was not determined in the study # T081, a third segment 3 study (T082) was conducted at 1.25, 2.5 and 5 mg/kg. Viability of F_1 pups was not affected at 1.25, 2.5, and 5 mg/kg. However, time taken for the opening of vagina was increased at 5 mg/kg. Reproductive function and fertility of F_1 rats was not affected. Data showed that the no-effect dose for peri and post natal treatment in pregnant rats was 2.5 mg/kg. However, based on combined effects of pre and post natal effect of milnacipran in pregnant rats in several studies, the reviewer considered that 5 mg/kg (30 mg/m^2) increased post-natal deaths in rats. The no effect dose for segment 3 study was 2.5 mg/kg.

Conclusions:

Treatment with milnacipran reduced fertility in rats at 5 mg/kg (30 mg/m^2) due to increased dead fetuses. The no-effect dose was not established.

Mice and rats treated with milnacipran during organogenicity did not show teratogenicity at 125 mg/kg (375 mg/m^2) and 5 mg/kg (30 mg/m^2), respectively. However, intrauterine deaths were increased at 5 mg/kg (30 mg/m^2) in rats. Milnacipran showed extra single rib in pregnant rabbits at 15 mg/kg (180 mg/m^2). The no effect dose was 5 mg/kg in pregnant rabbits.

Milnacipran had no effect on gestation period and delivery of rats. However, increased post-natal deaths were noted in rats at 5 mg/kg (30 mg/m^2) and higher doses. The effect of milnacipran on fertility and pregnancy was noted at maternally non-toxic dose. Therefore, findings in pregnant animals are considered to be treatment related and Pregnancy Category C should be designated for milnacipran. A summary of reproductive safety is shown in the table below.

Segment, Study #	Species	Observation	NOEL
1, T030	Rat (Sprague Dawley)	Fertility was reduced at 5 mg/kg	Not determined
1, T092	Rat (Wistar)	Delayed mating at 20 and 80 mg/kg, reduced fertility at 80 mg/kg	NOEL 5 mg/kg
2, T015	Mouse	No teratogenicity but fetal weight was reduced at 25 mg/kg	5 mg/kg
2, T030	Rat	No teratogenicity, increased intra-uterine deaths at 5 mg/kg	Not determined
2, T019	Rabbit	Single extra rib at 15 mg/kg as a variation	5 mg/kg
3, T071, T081, T082	Rat	No effect on gestation and delivery, increased post-natal deaths at 5 mg/kg	2.5 mg/kg

Certificate of analysis:

Study #	Batch #	Purity	Certificate provided or

T030		101%	not Yes
T092		Above 99.9%	Yes
T019		101%	Yes
T015		101%	Yes
T071		99.33%	Yes
T081		Above 99.9%	Yes
T082		Above 99.9%	Yes

b(4)

Labeling recommendations:

Fertility:

Sponsor's Proposed label for Fertility:

b(4)

Sponsor's Proposed label for Pregnancy:

b(4)

Reviewer's Proposed label for Pregnancy:

Pregnancy

b(4)

Nonteratogenic effect:

Same as recommended by the Sponsor

Labor and delivery:

Same as recommended by the Sponsor

Nursing mothers:

Same as recommended by the Sponsor

2.6.6.7 Local tolerance: No local tolerance studies were reviewed.

2.6.6.8 Special toxicology studies: No special toxicity study was reviewed.

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the Sponsor]

Table 7–4. Repeat-Dose Toxicity - A 52 Week Oral Toxicity Study of TN-912 in Rats

Test Article: Milnacipran HCl (TN-912)

Species/Strain: Ra — Wistar SPF
Initial Age: 4 weeks
Date of First Dose: M: 27 February 1991
F: 28 February 1991

Duration of Dosing: 52 weeks
Duration of Postdose: none
Method of Administration: oral gavage
Vehicle/Formulation: water for injection

Study No.: E 1 (3087)
Location in CTD: 4.2.3.2

b(4)

GLP Compliance: yes

Special Features: None

No Observed Adverse Effect Level: M: 3 mg/kg; F: 10 mg/kg

Daily Dose (mg/kg)	0 (Control)		1		3		10		30	
Gender: No. of Animals	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
Noteworthy Findings										
Died or Sacrificed Moribund[a]	1	0	0	0	1	0	0	0	0	0
BW (%)[b]	479.6 g	269.1 g	+1.2	-1.1	+1	-3	-1.9	-5	-5.2	-9.5 *
BWG (%)[b]	322.3 g	148.6 g	+5	-4	+4	-7	-5	-12	-15	-21 *
Food Consumption (g/animal/d) (%)[b]	14.0 g	14.0 g	0	0	+2.6	-3.6	+1.6	-5.7	-3.2	-8.6**
Clinical Findings	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-
Serum Chemistry										
BUN (mg/dL)	19.6	20.2	19.1	21.1	19.7	21.5	20.0	22.5*	18.5	22.7**
Urinalysis										
Volume (mL/24 h)	11.6	7.3	11.6	6.9	12.7	6.5	14.6**	7.0	13.9*	6.2
Sp.G.	1.066	1.060	1.068	1.070	1.069	1.074**	1.059	1.064	1.057	1.060

(continued)

Table 7–4. Repeat-Dose Toxicity - A 52 Week Oral Toxicity Study of TN-912 in Rats

Test Article: Milnacipran HCl (TN-912)

Species/Strain: Ra — Wistar SPF

Duration of Dosing: 52 weeks

Study No.: C 1 (3087)

b(4)

Daily Dose (mg/kg)	0 (Control)		1		3		10		30	
Gender: No. of Animals	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15	M:15	F:15
Organ Weights[c]										
Salivary Glands (mg%)	65	77.5	-	-	-	+9.7*	+13.8**	+10.3*	+15.4**	+18.1**
Epididymis (mg%)	113		-		-		-13.3**		-9.1*	
Seminal Vesicle (g%)	0.26		-		-		-19.2**		-23.1**	
Spleen (g%)	0.18	0.19	-	-	-	-	-	-15.8**	-16.7**	-10.5*
Prostate[d] (g%)	0.21		-		-		-		-23.8	
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Histopathology										
No. Examined	14	15	15	0	14	0	15	0	15	15
Liver- Vacuolation of Hepatocytes	1	0	0	-	0	-	2	-	10	0

a Death not test article-related.

b At the end of the dosing period. For controls, group means are shown; for treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

c Relative weights. For treated groups, percent differences from control are shown. Statistical significance is based on actual data (not on the percent differences). Recovery was seen during the 1-month recovery period.

d Prostates of high-dose males were microscopically similar in appearance to those of young adult males; may suggest growth suppression or aging-related changes.

Scheffe's test/Kruskal-Wallis nonparametric ANOVA/dimbrotion-free multiple comparison as appropriate: *p<.05; **p<.01.

- = no noteworthy findings; BUN = blood urea nitrogen; ANOVA = analysis of variance . BW = body weight; BWG = body weight change.

Table 7–7. Repeat-Dose Toxicity - F2207: 26-Week Oral (Gastric Intubation) Toxicity Study in the Cynomolgus Monkey

Test Article: Milnacipran HCl (F2207)

Species/Strain: Monkey/Cynomolgus
Initial Age: not given. M: 2.9–4.7 kg; F: 2.6–4.6 kg
Date of First Dose: 19 March 1985

Duration of Dosing: 26 weeks
Duration of Postdose: None
Method of Administration: Oral gavage
Vehicle/Formulation: Distilled water

Study No.: 314/22 (T020)
Location in CTD: 4.2.3.2

GLP Compliance: Yes

Special Features: Starting at Day 40, the high-dose level of 60 mg/kg/d was reduced to 40 mg/kg/d because of severe incidents of vomiting.
No Observed Adverse Effect Level: 15 mg/kg

Daily Dose (mg/kg)	0 (Control)		5		15		60/40	
Gender: No. of Animals	M:5[a]	F:5[a]	M:5[a]	F:4	M:5[a]	F:4	M:5[a]	F:5[a]
Noteworthy Findings								
Died or Sacrificed Moribund[b]	1	3	1	0	2	0	2	1
BW (%)[c]	4.1 kg	3.2 kg	-17	-	-	+3	-5	-
BWG (%)[c]	600 g	0 g	-67	-	-33	+17	-50	-17
Total Food Consumption (%)[c]	670	617	-2.4	-7.0	-1.3	-1.8	-1.3	-0.5
Clinical Observations								
Vomiting	-	-	1	2	2	2	4	5
Diarrhea	2	3	2	4	1	3	3	3
Ophthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Organ Weights								
Liver/BW (%)[d]	1.74	2.41	+13.8	-10.8	+6.3	-9.5	+23.6	+2.1

a Owing to deaths during the first weeks of the study (non-treatment-related), one additional animal was dosed.
b Deaths were not treatment related except for 1M and 1F at the high dose.
c At the end of the dosing period. For controls, group means are shown; for treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
d Relative weight differed from group mean control values in the direction indicated. Statistical significance is based on actual data (not on the percent differences).
- = no noteworthy findings; BW = body weight; BWG = body weight gain. ANOVA: *p<.05; **p<.01.

Table 7–8. Repeat-Dose Toxicity - Fifty-Two Week Oral Toxicity Study of TN-912 in Cynomolgus Monkeys

Test Article: Milnacipran HCl (TN-912)

Species/Strain: Monkey/Cynomolgus
Initial Age: 3–7 years
Date of First Dose: 21 May 1991

Duration of Dosing: 52 weeks
Duration of Postdose: None
Method of Administration: Oral gavage
Vehicle/Formulation: Distilled water for injection

Study No. [redacted] (T038)
Location in CTD: 4.2.3.2

GLP Compliance: Yes

b(4)

Special Features: None
No Observed Adverse Effect Level: 25 mg/kg

Daily Dose (mg/kg)	0 (Control)		2.5		7.9		25.0	
Gender: No. of Animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Toxicokinetics: AUC_{0-24h} (ng*h/mL)[a]								
Day 1	NE	NE	NE	NE	NE	NE	25912	25951
Day 14	NE	NE	NE	NE	NE	NE	21853[b]	32375
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
BW (%)[c]	4.585 kg	3.028 kg	+6	-1	+4	-	-5	+4
Clinical Observations								
Mydriasis	-	-	-	-	-	-	2	3
Emesis	-	-	-	-	-	-	1	1
Salivation	-	-	-	-	-	-	1	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-

a Toxicokinetic Bridging Study MLNTX06000; n=3/sex.
b Includes animals with emesis.
c At the end of the dosing period. For controls, group means are shown; for treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
Dunnett's test: *p<.05; **p<.01.
NE = not evaluated; - = no noteworthy findings; BW = body weight.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Milnacipran is a monoamine re-uptake inhibitor at neuronal endings. This NDA for milnacipran is submitted for the treatment of fibromyalgia at a maximum dose of 200 mg per day (3.3 mg/kg for a 60 kg subject or 122 mg/m^2). The Sponsor submitted non-clinical studies for pharmacology, safety pharmacology, toxicity studies, pharmacokinetic and metabolism, genetic toxicity, reproductive toxicity and carcinogenicity studies. In this review, repeat dose toxicity, genetic toxicity, reproductive toxicity and rat carcinogenicity studies are reviewed. Other studies would be reviewed by Dr. Bolan under a separate review.

Three repeat dose toxicity studies were reviewed for the NDA in cynomolgus monkeys and rats. A 6- month toxicity study was conducted in cynomolgus monkeys at 5, 15 and 60/40 mg/kg/oral doses. The high dose was reduced due to mortality in monkeys due to infections. A NOEL was not established in the study. The NOAEL was 40 mg/kg. Dose limiting adverse event was vomiting at all doses. There was no treatment related histopathological change observed in the study. The Sponsor obtained blood samples to determine the plasma levels as proof of absorption following oral dosing. However, PK parameters were not determined.

The repeat dose toxicity study was also conducted in cynomolgus monkeys up to 52 weeks of treatment at 2.5, 7.9, and 25 mg/kg. Mydriasis and vomiting was noted as dose limiting toxicity, NOEL was 7.9 mg/kg and NOAEL was 25 mg/kg (8.3 mg/kg human equivalent at equal surface area).

The third chronic study was conducted in Wistar rats at 1, 3, 10 and 30 mg/kg/oral for 52 weeks. No treatment related mortality was observed. The study is acceptable and conducted at MTD based on the loss of body weight gain. Male rats showed vacuolation in hepatocytes at 30 mg/kg. However, no transaminase elevation was noted in the plasma chemistry data. No other treatment related histopathological changes were noted. The NOEL was 10 mg/kg in male and 3 mg/kg in female rats. NOAEL was 30 mg/kg (4.2 mg/kg human equivalent for equal body surface area). Liver is the organ of toxicity in the rodent model.

The Sponsor submitted several mutagenicity studies to fulfill regulatory requirements to conduct recommended battery of tests. Review of these studies showed milnacipran is not mutagenic. However, the certificate of analysis for the Ames Assay was not submitted in the report. Therefore, the reviewer recommends that Ames Assay be repeated post approval (if approved) as a Phase IV requirement using a clinical batch for milnacipran.

Two species carcinogenicity studies were conducted with milnacipran under IND 63,736. One of the studies was conducted in CD rats for 104 weeks at 5, 15 and 50 mg/kg. The

study report was presented to CAC-EC on June 15, 2004. The CEC-EC recommended that the Sponsor conduct histopathology of thyroid gland from all animals and reevaluate the statistical analysis. The rat carcinogenicity review has been updated with respect to histopathology data and statistical review. Based on the review of experimental data and historical control data, the reviewer concluded that milnacipran was not carcinogenic in rats up to 50 mg/kg (300 mg/m^2). The maximum tolerated dose was achieved. The Sponsor provided proof of absorption from the dietary administration of the drug. However, in the absence of exposure data, the dose ratio between rat and human was expressed as actual doses not as the exposure ratio. Non-neoplastic findings of carcinogenicity data also suggested high incidences of vacuolation in hepatocytes at 15 and 30 mg/kg in male rats. As well as Keratitis in male and female rats at 50 mg/kg.

The updated histopathology data for the rat carcinogenicity study was presented to CAC-EC. The CAC-EC recommended that the tumor finding for thyroid C-cell in male rats needs to be addressed in the package insert.

The Sponsor also presented data for 104-week carcinogenicity study in CD-1 mice at 10, 30 and 100 mg/kg for 104 weeks. The review of data was presented to CAC-EC on June 15, 2004. The committee recommended that the Sponsor provide the exposure data in mice at doses tested to clarify whether the exposure was 25 times higher than the maximum recommended human dose. Alternately, the Sponsor could repeat the study in Tgras H2 transgenic mice. Accordingly, the Sponsor conducted the transgenic mouse assay to fulfill the regulatory requirement of second species for the carcinogenic risk assessment. The review for mouse carcinogenicity data in transgenic mice was completed by Dr. Elizabeth Bolan under a separate review. The review of CD-1 mouse carcinogenicity data is not included in this review because the study did not test up to the maximum tolerated dose and the Sponsor chose to repeat the study in transgenic mice as the second species.

A total of eight fertility and reproductive safety studies were conducted in rats, mice and rabbits. Treatment with milnacipran reduced fertility in rats at 5 mg/kg (30 mg/m^2) as evidenced by an increase in dead fetuses. The no-effect dose was not established.

Mice and rats treated with milnacipran during organogenicity did not show teratogenicity at 125 mg/kg (375 mg/m^2) and 5 mg/kg (30 mg/m^2), respectively. However, intrauterine deaths were increased at 5 mg/kg (30 mg/m^2) in rats. Milnacipran showed extra single rib in pregnant rabbits at 15 mg/kg (180 mg/m^2). The no effect dose was 5 mg/kg in pregnant rabbits.

Milnacipran had no effect on gestation period and delivery of rats. However, increased post-natal deaths were noted in rats at 5 mg/kg (30 mg/m^2) and higher doses. The effect of milnacipran on fertility and pregnancy was noted at maternally non-toxic dose. Therefore, findings in pregnant animals are considered to be treatment related. Pregnancy Category C is recommended for milnacipran.

Based on above summary of non-clinical findings, it appears that toxicity and side effects of milnacipran were observed in monkeys and rats within 2 fold higher doses than the target maximum recommended dose of 200 mg/day in humans for chronic therapy. The adverse effects were body weight reduction, vomiting, mydriasis, liver centrilobular vacuolation (more prevalence at longer than one year treatment in male rats) and Keratitis in male and female rats at 50 mg/kg. The NDA for milnacipran may be approved based on non-clinical studies reviewed for the NDA at 200 mg/day dose based on no carcinogenic risks of the product. However, monitoring of liver function beyond one year of the treatment is recommended for chronic uses of the drug. Based on incidences of Keratitis in the 2-year carcinogenicity study in rats, it appears patients should be monitored for potential dry-eye like conditions when the treatment is given on a chronic basis. The Ames test needs to be repeated using a clinical batch of milnacipran as a Phase IV requirement if it is approved for the clinical use. The recommendations for the package insert are also provided in the review.

Unresolved toxicology issues (if any): The Ames assay was conducted using a batch that does not have a certificated of analysis acceptable to the review chemist and pharmacologist indicating its purity. Therefore, the Ames assay must be repeated using a clinical supply batch.

Recommendations: Milnacipran is approvable on the basis of non-clinical safety studies reviewed in the NDA.

Suggested labeling: See under individual review section and Executive Summary.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

Appendix/attachments: Nil

C.C:

CDER/OND/DAARP/PM/Diane Walker
CDER/OND/DAARP/Pharmacologist/ Asoke Mukherjee
CDER/OND/DAARP/Pharmacology Supervisor/Daniel Mellon
CDER/OND/DAARP/Pharmacologist/Beth Bolan
CDER/OND/DAARP/Medical Officer/Jane Filie

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

/s/

Asoke Mukherjee
9/17/2008 09:08:57 AM
PHARMACOLOGIST

R. Daniel Mellon
9/17/2008 09:20:43 AM
PHARMACOLOGIST

8/27/08

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-256**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**January 18, 2008**
PRODUCT:	**Milnacipran Hydrochloride**
INTENDED CLINICAL POPULATION:	**Proposed use is for treatment of fibromyalgia syndrome.**
SPONSOR:	**Cypress Bioscience, Inc.**
DOCUMENTS REVIEWED:	**Portions of Module 2 and 4**
REVIEW DIVISION:	**Division of Analgesia, Anesthesia and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Elizabeth A. Bolan, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, PhD.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Diana Walker**

Date of review submission to Division File System (DFS): August 21, 2008

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Based upon review of the pharmacology sections, acute toxicology and mouse Tg rasH2 carcinogenicity studies from this NDA, no outstanding issues were noted that would preclude approval of this NDA. The reader is referred to the review conducted by Dr. Asoke Mukherjee for the remainder of the nonclinical portions of this NDA.

B. Recommendation for nonclinical studies

None.

C. Recommendations on labeling (pharmacology and portions of the carcinogenesis only)

Sponsor's proposed wording	*Reviewer's proposed wording*	*Reviewer's rationale for proposed wording*
[redacted]		

b(4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings (Tg rasH2 mouse carcinogenicity study)

A 26-week carcinogenicity study with milnacipran was conducted in a transgenic rasH2 mouse model. Daily oral treatment with milnacipran at doses up to 125 mg/kg did not result in an increase of neoplastic lesions. The most frequent neoplasms noted included pulmonary tumors, hemangiomas and hemangiosarcomas. A trend test for multiple organ hemangiosarcomas in females was significant: however, no significant pairwise comparisons were seen. The incidence of hemangiosarcomas observed for females was similar to the historical control values provided by the Sponsor. Various neoplasms or pre-neoplastic lesions were observed but all were similar to levels observed in vehicle controls and/or similar to levels observed in the historical controls. The Executive Carcinogenicity Assessment Committee agreed that the study was negative for any statistically significant drug-related neoplasms.

B. Pharmacologic activity

Milnacipran is a selective inhibitor of norepinephrine (NE) and serotonin (5-HT) reuptake with approximately 3-fold higher inhibition of NE over 5-HT. Milnacipran has no affinity for the dopamine transporter. The mechanism of action of milnacipran for the treatment of fibromyalgia is not known.

C. Nonclinical safety issues relevant to clinical use

Based upon review of the pharmacology sections, acute toxicology and mouse Tg rasH2 carcinogenicity studies from this NDA, no nonclinical safety issues relevant to clinical use were identified. The reader is referred to the review conducted by Dr. Asoke Mukherjee for the remainder of the nonclinical portions of this NDA.

[Please limit to 1-3 pages]

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

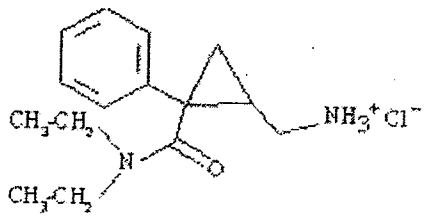
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 22-256
Review number: 000
Sequence number/date/type of submission: 000/January 18, 2008/eCTD
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Cypress Bioscience, Inc. San Diego, CA
Manufacturer for drug substance: Pierre Fabre Medicament Plantes & Industrie Gaillac, France

Reviewer name: Elizabeth A. Bolan, Ph.D.
Division name: Division of Analgesia, Anesthesia and Rheumatology Products (DAARP)
HFD #: 170
Review completion date: August 19, 2008

Drug:

Trade name: NA
Generic name: Milnacipran HCl
Code name: F2207
Chemical name: (1RS, 2SR)-2-(aminomethyl)-N, N-diethyl-1-phenylcyclopropanecarboxamide hydrochloride
CAS registry number: 101152-94-7
Molecular formula/molecular weight: $C_{15}H_{23}ClN_2O$; MW=282.8 g/mol
Structure:



Relevant INDs/NDAs/DMFs: IND 63,736, DMF 11501 (milnacipran); DMF [ ] b(4)
[ ]

Drug class: Milnacipran is a norepinephrine and serotonin reuptake inhibitor.

Intended clinical population: The proposed indication for milnacipran is treatment of fibromyalgia syndrome.

Clinical formulation: The to-be-marketed formulation of milnacipran HCl is an immediate release tablet containing 12.5 mg, 25 mg, 50 mg, and 100 mg of milnacipran. The components of the milnacipran drug product are listed in Table 1. All excipients can be found in approved drug products at equal or greater levels and therefore do not pose any unique toxicological concerns.

Table 1 Composition of Milnacipran HCl tablets

Component	*Function*	*mg/MRHD*
Milnacipran HCl	active	200
[		
]		

b(4)

MRHD= 200 mg

[ ] occurs as a degradant in the drug product. Proposed commercial specification for [ ] in the milnacipran drug product is set at NMT[ ]%. This specification exceeds the threshold for qualification of impurities/degradants in the drug product as per ICH Q3B(R). However, since [ ] specifications set for impurities do not apply. The Sponsor conducted a genetic toxicology battery as well as a 28-day repeat dose toxicology study with [ ] in an attempt to qualify the compound. For a chronically administered drug, a 28-day repeat dose study would be considered inadequate duration for qualification. Since [ ] and was present in the repeat dose toxicology studies, it will be considered qualified. No further studies are needed to support the safety of this compound and a specification of NMT[ ]% in the drug product will be considered adequate.

b(4)

Route of administration: oral

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

Studies reviewed within this submission: The pharmacology sections, acute toxicology and mouse Tg rasH2 carcinogenicity studies from this NDA were reviewed.

Studies not reviewed within this submission: Several studies within this NDA submission were reviewed by Asoke Mukherjee, Ph.D. and are noted as such. The reviews of these studies appear in a separate review under his name.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Milnacipran is a selective inhibitor of norepinephrine (NE) and serotonin (5-HT) reuptake with approximately 3-fold higher inhibition of NE over 5-HT. Milnacipran has no affinity for the dopamine transporter. Several binding screens were conducted to evaluate the affinity of milnacipran for various receptors, channels and enzymes. At 10 μM, milnacipran showed no appreciable affinity toward a panel of 160 receptors and ion channels *in vitro*, with the exception of high affinity binding to the transporters for NE (NET) and 5-HT (SERT; 10 μM: 99% and 104% inhibition, respectively). At 10 μM, milnacipran shows no affinity for the dopamine transporter. Low affinity binding was noted to 5HT2A receptors (10 μM: 61% inhibition) and the PCP-binding site of NMDA receptors (10 μM: 53% inhibition). Milnacipran showed no binding affinity for muscarinic cholinergic, α-adrenergic, β-adrenergic, and histaminergic receptors to which tricyclic antidepressants bind.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Milnacipran has been shown to inhibit NE and 5-HT reuptake. Inhibition of the uptake of NE and 5-HT facilitates neurotransmission of these neurotransmitters. The possible mechanism of facilitation (via monoamine release) was assessed. The ability of milnacipran to cause release of NE or 5-HT was assessed in *ex vivo* preparations using sections of rat hypothalamus (NE and 5-HT) or rat atria (NE only). Negligible effects on release of NE and 5-HT were observed for milnacipran. It is most likely that milnacipran causes its effects by increase of NE and 5-HT concentration at the synapse via inhibition of the synaptic reuptake mechanism.

Drug activity related to proposed indication:
The neurotransmitters NE and 5-HT have been hypothesized to modulate descending inhibitory pain pathways (Crofford, 2008). In animal models of persistent and neuropathic pain, inhibition of NE and 5-HT uptake has been shown to attenuate nociceptive behaviors (Iyengar et al., 2004;Bomholt et al., 2005;Joshi et al., 2006;Pedersen and Blackburn-Munro, 2006). Milnacipran exhibited analgesic activity in the dose range of 3 to 300 mg/kg PO or IP in various rodent models of pain, including spinal nerve ligation (Chung) model, formalin-induced pain, swim-stress induced hyperalgesia, hot plate test, tail clamp test (Haffner), and the acetic acid-induced abdominal writhing test. Milnacipran showed the highest potency in the acetic acid-induced writhing test in rats, exhibiting maximal efficacy at a dose of 3 mg/kg PO. In addition, milnacipran increased the activity of submaximal doses of morphine in the hot plate test in rodents.

2.6.2.3 Secondary pharmacodynamics

The secondary pharmacodynamic effects of milnacipran are mainly due to the increases of NE and to a lesser extent, 5-HT. Milnacipran is selective for NET and SERT and shows no affinity for either DAT or DA receptors. It does not inhibit MAO-A or MAO-B. It shows no effect on AChE activity and does not bind to α1-, α2-, β-adrenergic, muscarinic ACh, or histaminergic receptors.

Milnacipran has been shown *in vivo* to antagonize the activity of the dopaminergic D1/D2 receptor agonist apomorphine in mice. This effect was most likely caused by indirect action through increase of NE and 5-HT, as milnacipran showed no direct effect on the reuptake of dopamine *in vitro* and does not bind to either DA receptors or the DA transporter.

Although milnacipran possesses structural similarities to the MAO inhibitor tranylcypromine, it shows no inhibition for either MAO-A or MAO-B. In *in vitro* studies with homogenates prepared from rat brain (for MAO-A and MAO-B) and human placenta (for MAO-A only) at concentrations up to 10 μM milnacipran did not show appreciable activity against either rat and human MAO-A or rat MAO-B (IC_{50} >10 μM for rat and human MAO-A and rat MAO-B). In *ex vivo* studies in rat after doses up to 100 mg/kg PO, milnacipran did not show appreciable activity against either MAO-A or MAO-B (0% inhibition for both MAO-A and MAO-B). Milnacipran did not exacerbate the pressor effects of tyramine in rat, suggesting a lack of inhibitory effect of milnacipran on MAO activity *in vivo*.

Milnacipran also had no effect on acetylcholine esterase activity *in vitro* in rat brain membranes (IC_{50} >10 μM). Unlike the TCAs, milnacipran displayed no peripheral anticholinergic effects *in vivo*. In contrast, milnacipran antagonized the central effects of the muscarinic cholinergic agonist oxotremorine (i.e., hypothermia, tremor, akinesia) in mice as well as increased pilocarpine-induced salivation in mice and potentiated the pharmacologic effects of direct (bethanechol, oxotremorine) and indirect (neostigmine) muscarinic agonists. However, milnacipran showed no binding affinity toward muscarinic ACh receptors. This activity on the muscarinic cholinergic system is likely due to an increase in NE tone by means of reuptake inhibition of NE.

Milnacipran is approved to treat depression in 52 countries outside of the United States. In rodents, milnacipran was shown to be active in the dose range of 2 to 32 mg/kg PO in four models of depression in the rodent: the learned helplessness model, the behavioral despair (Porsolt forced swimming) test, the tail suspension test, and the bulbectomized rat model. Milnacipran also displayed inhibitory activity in the dose range of 30 to 50 mg/kg IP in a rat muricidal model for the evaluation of antidepressant activity. These studies are not relevant to the current indication and will not be reviewed.

A slight mydriatic effect of milnacipran was inhibited by the α1-adrenergic receptor antagonist prazosin. This effect is most likely indirect α1-agonism mediated through the

inhibition of NE uptake rather than a direct agonistic effect on the α1-adrenergic receptor where milnacipran shows no affinity.

Milnacipran (1% solution) showed no local anesthetic properties when directly administered to the eyes of guinea pigs.

2.6.2.4 Safety pharmacology

Safety assessment of neurological, renal, pulmonary, and gastrointestinal effects with milnacipran was conducted.

Neurological effects:
Several Irwin Screens were conducted in mouse with milnacipran. Milnacipran caused alterations in behavior, such as hypoactivity, polypnea, and mydriasis, starting at a dose of 1 mg/kg IP or 30 mg/kg PO. Higher doses (between 64-128 mg/kg IP) produced signs including sedation, ptosis, marked hypothermia and tremors. Akinesia, dyspnea and moderate mydriasis were observed at 100 mg/kg PO. Convulsions and death were observed at doses < 300 mg/kg PO and < 128 mg/kg IP.

Motor coordination was assessed in mouse and rat in the rotarod test with milnacipran. Milnacipran reduced motor coordination in mice (ED_{50}=23.7 mg/kg IP; 133 mg/kg PO) and rats (ED_{50}=31.6 mg/kg IP; 100 mg/kg PO).

In monkeys, higher doses of milnacipran (75-100 mg/kg PO) caused vomiting, piloerection, decreased locomotor activity, and restlessness.

The effect on wakefulness-sleep cycles of milnacipran was assessed in the rat. Sleep pattern (number and duration of sleep phases) in rats was not significantly altered at doses of milnacipran ≤ 15 mg/kg IP. The rapid eye movement (REM) sleep phase was transiently decreased at 30 mg/kg IP. Milnacipran (10 mg/kg IP) caused a significant increase in REM sleep latency without altering the duration of sleep phases. The Sponsor notes that suppression of REM sleep is a common observation with many antidepressant drugs.

Milnacipran lowered body temperature in mice at a dose of 100 mg/kg PO and in rats at doses of ≥ 50 mg/kg PO. In monkeys, hypothermia was observed at doses of ≥ 75 mg/kg PO. In contrast, body temperature was increased in rabbits at a dose of 30 mg/kg PO or ≥ 1 mg/kg IV.

The potential proconvulsant effects of milnacipran were assessed in mouse, rat, and monkey. At doses ≤ 30 mg/kg PO or IP, milnacipran did not show proconvulsant activity in rats with spontaneous epilepsy. In mice, milnacipran did not increase the effects of convulsant stimuli such as bicuculline, pentetrazol, or nicotine up to 100 mg/kg PO. In monkeys, milnacipran infusion (0.3 mg/kg per minute IV) caused a moderate increase in EEG activity starting at 25 to 35 minutes but no changes in the EEG were seen with an infusion rate of 0.1 mg/kg per minute IV over 2 hours.

The effect of milnacipran on learning and memory was assessed in rat. Learning a passive avoidance response was impaired at a higher dose of milnacipran (50 mg/kg IP) without effect on memory retention. Milnacipran showed no anxiolytic or anxiogenic effects in Geller-Seifter or Vogel tests in rats at doses up to 30 mg/kg PO.

Cardiovascular effects:
Several effects on cardiovascular parameters were observed in animal models. It is important to note that milnacipran did not affect cardiac repolarization and did not cause QTc prolongation at the maximum tolerated human dose (300 mg BID) in healthy subjects (see Study MLN-PK-10 and Clinical Review).

Effects of milnacipran on hERG activity:
Milnacipran inhibited tail currents recorded from hERG channels expressed in HEK293 cells in a concentration-dependent manner, with an estimated IC_{20} value of 20.7 μM. The IC_{50} value for milnacipran could not be estimated as the maximum inhibition was 78.7% at the highest concentration tested (30 μM).

Effects of milnacipran on blood pressure:
In conscious rats, increasing doses of milnacipran exhibited biphasic effects on blood pressure (BP). BP increased at doses of up to 9 mg/kg IV but decreased at higher doses. In anesthetized rats, milnacipran (0.5 mg/kg IV) increased BP. In the pithed rat model, BP also increased after IV infusion of milnacipran (≥ 1 mg/kg) as well as potentiated the pressor effects of NE, 5-HT, and electrical stimulation.

Anesthetized dogs showed a biphasic effect on BP with IV administration of milnacipran. Doses ≤ 3 mg/kg IV increased BP, whereas a decrease was seen at 10 mg/kg IV. Milnacipran potentiated the hypertensive effects of NE, epinephrine, or dimethylpiperazinium in anesthetized dogs, whereas the hypertensive effects of tyramine were antagonized. Milnacipran showed no consistently significant activity on the pressor effects of 5-HT, isoprenaline, acetylcholine, histamine, angiotensin, or carotid occlusion in dogs. A biphasic response was also observed in conscious monkeys, with increases in BP at 1 to 4 mg/kg IV and hypotensive effects at doses ≥ 8 mg/kg IV. The Sponsor hypothesizes that the observed BP increase with milnacipran is related to the inhibition of neurotransmitter reuptake, i.e., the proposed mechanism of action of milnacipran. At 10 mg/kg PO, milnacipran had no effect on BP in normotensive and spontaneously hypertensive rats.

Effects of milnacipran on heart rate and ECG:
Changes in the QTc interval on the electrocardiograms (ECG) of dogs and monkeys were minor or not related to milnacipran dosing.

In isolated guinea pig atria, milnacipran exhibited a slight positive chronotropic effect beginning at a concentration of 1 μM and exhibited a maximal increase in heart rate of 12.4% at 10 μM. In isolated guinea pig ventricular fibers, there was a slight prolongation

of the action potential duration (5%-10% at 10 μM). In isolated frog atrial fiber, milnacipran prolonged action potential duration at 10 μM but not at higher concentrations. In contrast, action potential amplitude and V_{max} were depressed only at much higher milnacipran concentrations (100-500 μM).

In vivo, milnacipran showed some effects on ECG parameters in rats, guinea pigs, dogs, and monkeys, although some interspecies variability was observed. In anesthetized rats, an increase in heart rate was detected at a dose of 0.5 mg/kg IV. However, in conscious rats and anesthetized guinea pigs, milnacipran infusion caused bradycardia in a dose-dependent fashion. In anesthetized dogs (open chest or closed thorax), low doses of milnacipran ($\leq$ 0.3-1 mg/kg IV) produced slight tachycardia or bradycardia, whereas bradycardia was observed at higher doses ($\geq$ 3-4 mg/kg IV). This effect may have occurred in response to the observed increase in BP. In the open-chest dog model, QTc and PR intervals were only slightly increased (ΔQTc $\approx$ 25 msec; ΔPR $\approx$ 12 msec; overall change $\approx$ 5%-10%) in the dose range of 1 to 3 mg/kg IV. In a closed-thorax dog model, the QRS complex was moderately widened at doses of 2 to 4 mg/kg IV and further increased at 8 mg/kg IV. In this model, the QT interval was increased by up to 51% at 4 to 8 mg/kg IV. Milnacipran slowed conductions in the atrioventricular node and the His bundle at doses of $\geq$ 2 mg/kg IV and $\geq$ 0.5 mg/kg IV, respectively.

In conscious monkeys, milnacipran increased PR, QT, and QRS intervals by 20% to 27% at a high dose of 20 mg/kg IV in some animals. Variable changes in QTc intervals (range: ± 5-60 msec) were observed at all tested doses of milnacipran and could not be clearly attributed to administration of the compound.

Pulmonary effects:
Respiratory function was evaluated in conscious and anesthetized rats, and conscious cynomolgus monkeys. Milnacipran had no effect on the respiratory rate and amplitude as well as on blood pO_2, pCO_2, or pH at doses of up to 18 mg/kg IV in conscious rats. At 36 mg/kg IV, a slight increase in pCO_2 and a decrease in pH occurred. In anesthetized rats, death from respiratory failure occurred at a cumulative dose of 58 mg/kg IV of milnacipran. This effect was preceded by a decrease in pO_2, an increase in pCO_2, and a decrease in respiratory rate and amplitude. In conscious cynomolgus monkeys, milnacipran had no effect on respiratory rate or amplitude at doses up to 4 mg/kg IV.

Renal effects:
Milnacipran produced a weak dose-dependent diuretic effect at a dose of 5-50 mg/kg PO in rats. High intravenous doses of milnacipran (30 mg/kg IV) in rats also inhibited the micturition cycle during continuous saline perfusion without affecting threshold pressure.

Gastrointestinal effects:
In rats, milnacipran showed no effect on gastric motility at doses $\leq$ 20 mg/kg IP. In mice, milnacipran did not significantly alter intestinal motility at doses $\leq$ 10 mg/kg PO. Gastric secretion was reduced at milnacipran doses of 2 and 32 mg/kg PO in rats. The effect was maximal at 2 mg/kg PO, and no change in acidity was noted at either dose. The Sponsor

notes that since 5-HT itself can inhibit gastric secretion (LePard and Stephens, Jr., 1994), this effect of milnacipran is possibly mediated by inhibition of 5-HT reuptake.

Abuse liability: An evaluation of the abuse potential of milnacipran was conducted by Katherine Bonson, Ph.D. of the Controlled Substance Staff (CSS). CSS concluded that insufficient information was provided by the Sponsor to adequately assess the abuse potential of milnacipran and are requesting several studies as post-marketing agreements to address the deficiencies. The deficiencies noted in the CSS review will not impact the decision to approve/not approve milnacipran. See the CSS review for details and recommendations for labeling.

Other: Not applicable.

2.6.2.5 Pharmacodynamic drug interactions

Interactions of milnacipran with a variety of drugs were studied *in vivo*. Results are summarized in Table 2.

Table 2. Nonclinical drug-drug interactions with Milnacipran (MLN)

MLN plus:	*doses*	*effect*
ethanol	1.5 g/kg PO	≥ 30 mg/kg PO MLN significantly increased motor coordination in the rotarod test in mice
diazepam	2 mg/kg PO	
levomepromazine	1 mg/kg PO	
fluoxetine	32 mg/kg PO	
clomipramine	30 mg/kg PO	57 mg/kg IP MLN significantly increased motor impairment in the rotarod test in mice
amitriptyline	30 mg/kg PO	
imipramine	30 mg/kg PO	
pentobarbital	32 mg/kg PO	30 mg/kg PO MLN significantly increased motor impairment in the rotarod test in mice
haloperidol	2 mg/kg PO	
prazocin	0.03 mg/kg IV	2.5 mg/kg IV MLN did not alter drug induced antihypertensive activity in spontaneously hypertensive rats
clonidine	0.03 mg/kg IV	

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The pharmacokinetic (PK) properties of milnacipran and its enantiomers (*d*-milnacipran and *l*-milnacipran) have been evaluated in mice, rats, rabbits, dogs, and monkeys following intravenous and oral administration.

Milnacipran is metabolized to several phase 1 metabolites and glucuronidated to form *l*- and *d*-milnacipran carbamoyl-O-glucuronide. All metabolites occur in all species tested as well as humans, although at differing levels (Figure 1).

There were no major differences in the pharmacokinetics of both enantiomers. Following administration of various doses of milnacipran, concentrations of *d*-milnacipran and *l*-milnacipran were similar. No enantiomeric bioconversion was observed.

2.6.4.2 Methods of Analysis
Plasma concentrations of milnacipran: LC-MS/MS
Radioactivity measurements: Liquid scintillation counting
Metabolite identification: LC-MS and comparison with standards

2.6.4.3 Absorption
Following oral administration, milnacipran was absorbed rapidly, with T_{max} ranging from 0.17 to 0.5 hours in mice and rats, 0.4 to 0.75 hours in rabbits, approximately 0.8 hour in dogs, and 1.63 to 3.80 hours in monkeys. The absolute bioavailability was 61% in rats. The total clearance (CL/F) in mice ranged from 2.8 to 4.7 L/h•kg (for doses of 5-125 mg/kg), in rats from 1.7 to 6.3 L/h•kg (for doses of 5-120 mg/kg), in dogs from 1.2 to 1.5 L/h•kg (at a dose of 5 mg/kg), and in monkeys from 1.5 to 3.8 L/h•kg (for doses of 5-40 mg/kg).

Elimination half-lives across studies ranged from 0.9 to 1.6 hours at doses of 5-10 mg/kg in mice, from 1.3 to 1.9 hours at doses of 5-10 mg/kg in rats, and from 2.1 to 3.4 hours at doses of 5-40 mg/kg in monkeys. Half-lives increased to 7.6 hours in mice and to 3.9 hours in rats when the administered doses were increased to 125 mg/kg and 120 mg/kg in mice and rats, respectively.

Milnacipran shows non-linear PK in mice, rats, and monkeys. As the dose for mice (from 25 to 125 mg/kg) and rats (from 10 to 120 mg/kg) was increased, the AUC of milnacipran increased more than proportionally. In monkeys, both the AUC and C_{max} of milnacipran increased more than proportionally when the dose was increased from 5 to 40 mg/kg.

After 14 days of repeated milnacipran exposure in rats and monkeys, no accumulation was seen. Following daily oral administration of 5 mg/kg for 27 days in rats, exposure on Day 27 was slightly greater than the exposure observed on Day 1.

2.6.4.4 Distribution
Tissue distribution of milnacipran was assessed in mouse, rat, and monkey. In studies with [^{14}C]-labeled milnacipran HCl, radioactivity was detected in most body tissues and organs except bones for all three species. Milnacipran was distributed in equal amounts in plasma and red cells, and protein binding was mainly the result of albumin binding. Milnacipran crosses the blood-brain barrier. There was no accumulation of milnacipran or its metabolites in various glands (thyroid, pituitary, pancreas, thymus, and adrenal).

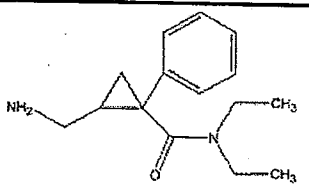
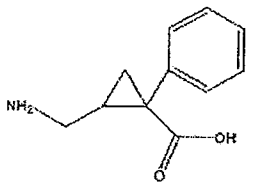
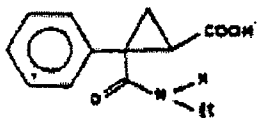
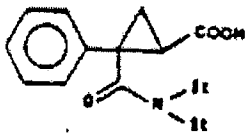
Uptake of milnacipran and its metabolites into the eye was observed, suggesting the affinity for melanin-containing tissues.

Relative to the administered dose, a small fraction of milnacipran and/or its metabolites can cross the placental barrier.

2.6.4.5 Metabolism

The majority of milnacipran is excreted in its unchanged form in the urine in mouse, rat, monkey and human (Figure 1; unchanged milnacipran: human: 55%; monkey: 42%; rat: 72%; mouse: 79%). The major metabolite in human is *l*- and *d*- milnacipran carbamoyl O-glucuronide. Humans produce considerably more glucuronidated milnacipran than mouse, rat or monkey (racemic mixture of milnacipran carbamoyl O-glucuronide: human: 19%; monkey: 2%; rat: < 1%; mouse: 1%) but since conjugation is a detoxification mechanism and all species tested show some exposure (albeit at low levels) to the glucuronide conjugates, the species employed in the toxicological testing can be considered appropriate for the safety qualification of milnacipran. The N-desethyl metabolite (F2800) is produced in all species tested at varying levels (F2800: human: 8%; monkey: 10%; rat: 13%; mouse: 1%). Several other minor metabolites (Table 3) have been observed in all species tested at low levels.

Table 3. Metabolites of milnacipran

name		*reaction*	*structure*
parent	Unchanged F2207	NA	
M1	F1567	Oxidative deamination of the alkylamine side chain of F2941	
M2	-	Oxidative deamination of the alkylamine side chain of F2800	
M3	-	Oxidative deamination of the alkylamine side chain of F2207	

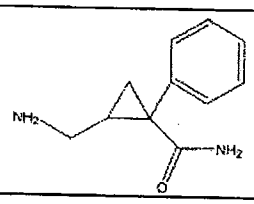
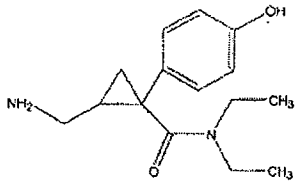
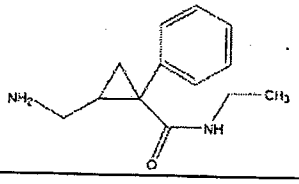
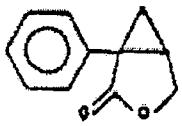
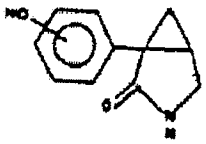
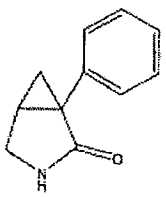
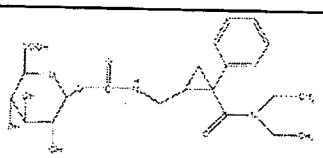
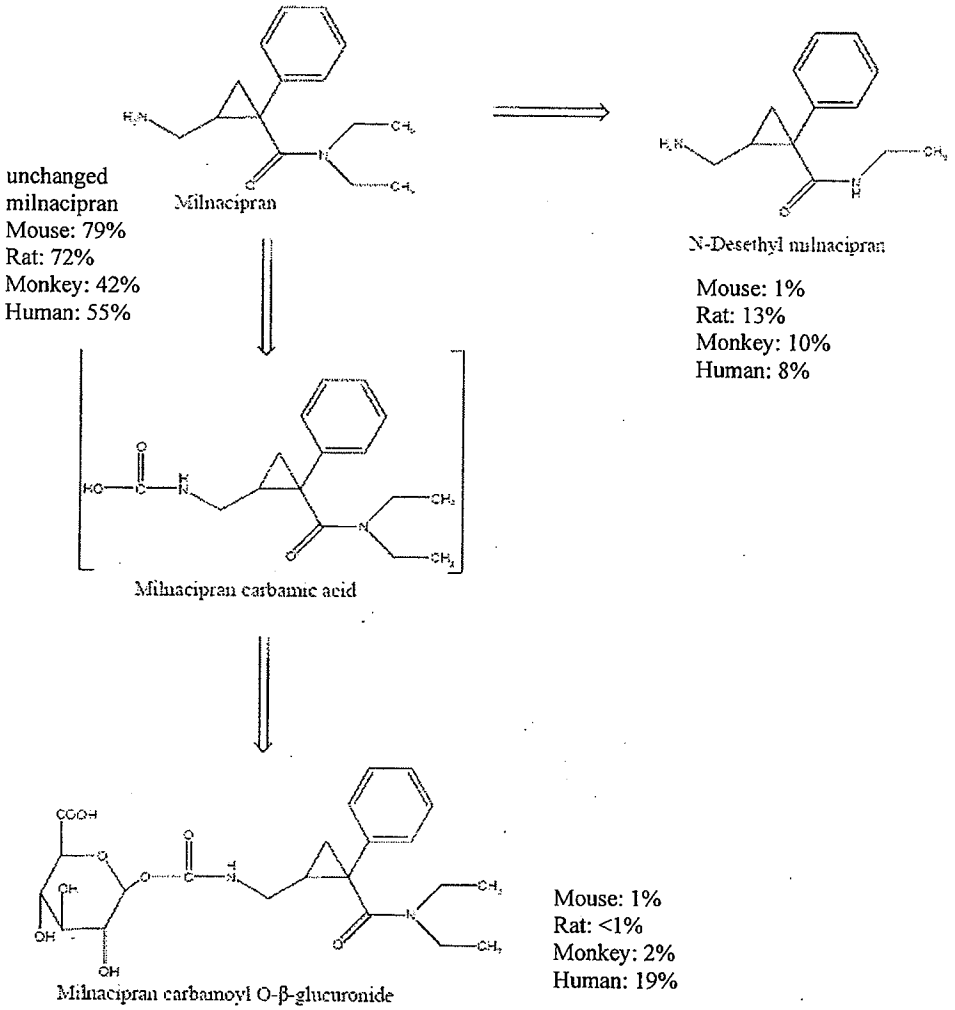
M4	F2941	-	
M5	-	Mono ethyl F2800	-
M6	F2782	p-hydroxy 2207	
M7	F2800	N-desethyl 2207	
M8	-	Cyclized metabolite of 2207 (F1612 -H)	
M9	-	Cyclized metabolite of 2207 (F1612+N)	
M10	F1612	Cyclized metabolite of 2207	
-	-	*l*- and *d*-milnacipran carbamoyl-O-glucuronide	

Figure 1. Proposed metabolic pathway of milnacipran (a racemic mixture of the *d*-isomer and *l*-isomer)



In Vitro Metabolism: The biotransformation of milnacipran by human hepatic microsomes and hepatocytes was slow.

Inhibition of CYP450 Enzymes: The IC_{50} of milnacipran on the activity of cytochrome P450 isozymes was always greater than 50 μM, indicating that it is likely that therapeutic usage of milnacipran will not inhibit the activity of these isozymes.

Milk levels of milnacipran and/or its metabolites at C_{max} were approximately three times higher than plasma levels in lactating rats. Elimination from milk was below the detection limit at 48 hours postdose.

2.6.4.6 Excretion
Following oral administration, milnacipran is predominantly excreted in urine as unchanged milnacipran (60% of the dose in mice, 67% of the dose in rats, and 40% of the dose in monkeys).

2.6.4.7 Pharmacokinetic drug interactions
No nonclinical pharmacokinetic drug interactions were examined.

2.6.4.8 Other Pharmacokinetic Studies
Not applicable.

2.6.4.9 Discussion and Conclusions
The majority of orally administered milnacipran is excreted unchanged in the urine in all species tested as well as in humans. The major metabolites in humans are *l*- and *d*-milnacipran carbamoyl O-glucuronide. Humans produce considerably more glucuronidated milnacipran than mouse, rat or monkey but since glucuronide conjugation is a detoxification mechanism and all species tested show some exposure to the glucuronide conjugates the nonclinical models will be considered appropriate for evaluation in the toxicology studies. The N-desethyl metabolite (F2800) is produced in all species tested at varying levels and is a minor metabolite in humans. Although many other metabolites are formed, they are found at low levels and at varying concentrations in the different species tested.

2.6.4.10 Tables and figures to include comparative TK summary
Not applicable.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY
Not applicable.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: With the exception of the acute toxicology studies (summarized below) the general toxicology studies were reviewed by Asoke Mukherjee, Ph.D.

Acute toxicology studies were conducted with the racemic mixture of milnacipran (PO and IV administration) and the individual *d*- and *l*- isomers of milnacipran (PO administration only) in mouse and rat. In mice, similar clinical signs of hypoactivity, cyanosis, prostration, and convulsions were observed at high doses for the three compounds tested. With IV administration of milnacipran, hypoactivity, piloerection, prostration and convulsions were observed. LD_{50} values were similar in males and

females with PO and IV administration. With PO administration, LD_{50} values for milnacipran and the *d-* and *l-* isomers were all $\geq$ 206 mg/kg (M and F combined) in the two strains of mice tested. The LD_{50} in mice with IV milnacipran administration was 36 mg/kg (M and F combined).

In rats, similar clinical signs of hypoactivity, prostration, convulsions and congested lungs were observed at the higher doses with PO administration for the three compounds tested. Milnacipran with IV administration caused tremors, apathy, gasping and decreased respiration at higher doses. LD_{50} values were similar in males and females with both PO and IV administration in rat. With PO administration, LD_{50} values for milnacipran and the *d-* and *l-* isomers were all $\geq$ 228 mg/kg (M and F combined). The LD_{50} in rats with IV administration was 51 mg/kg (M and F combined).

Genetic toxicology: Studies reviewed by Asoke Mukherjee, Ph.D.

Carcinogenicity: A 26-week carcinogenicity study with milnacipran was conducted in a transgenic rasH2 mouse model. Daily oral treatment with milnacipran at doses up to 125 mg/kg did not result in an increase of neoplastic lesions. The most frequent neoplasms noted included pulmonary tumors, hemangiomas and hemangiosarcomas. A trend test for multiple organ hemangiosarcomas in females was significant: however, no significant pairwise comparisons were seen. The incidence of hemangiosarcomas observed for females was similar to the historical control values provided by the Sponsor. Various neoplasms or pre-neoplastic lesions were observed but all were similar to levels observed in vehicle controls and/or similar to levels observed in the historical controls. The Executive Carcinogenicity Assessment Committee agreed that the study was negative for any statistically significant drug-related neoplasms.

The Sponsor also conducted a two-year rat carcinogenicity study and a two-year mouse carcinogenicity study. These studies were reviewed by Asoke Mukherjee, Ph.D.

Reproductive toxicology: Studies reviewed by Asoke Mukherjee, Ph.D.

Special toxicology: Milnacipran does not appear to have antigenic potential with subcutaneous administration. Guinea pigs sensitized with milnacipran did not show a systemic anaphylactic reaction after challenge with milnacipran.
The possible phototoxic properties of milnacipran were evaluated in guinea pig. After oral administration of milnacipran, depilated skin was exposed to an infra-erythematous dose of UVA and UVB. Examination did not reveal any skin reaction due to a phototoxic effect of milnacipran.

2.6.6.2 Single-dose toxicity

Single dose toxicity studies were conducted in two strains of mice (Swiss and [redacted]/OF1) and rats (Sprague-Dawley) with milnacipran (F2207), and its two enantiomers, the *d-* isomer, (F2695) and the *l-*isomer (F2696). All three compounds were administered by oral gavage and F2207 was also administered intravenously. b(4)

Clinical signs observed in mice were similar for the three compounds with oral administration. Hypoactivity, cyanosis, prostration, and convulsions were observed at the higher doses. The major clinical signs with IV F2207 were hypoactivity, piloerection, prostration and convulsions. LD_{50} values in mice were similar between males and females for all compounds and were combined (Table 4). Toxicokinetics were not performed in any of the studies.

Clinical signs observed in rats were also similar for the three compounds. Hypoactivity, prostration, convulsions and congested lungs were observed at the higher doses with oral dosing (Table 4). Tremors, apathy, gasping and decreased respiration were observed for IV F2207. LD_{50} values in rat were similar between males and females for all compounds and were combined (Table 4). Toxicokinetics were not performed in any of the studies.

Table 4. Acute toxicity and LD_{50} values for F2207, F2695, and F2696 in mice and rats					
Species	***Strain***	***Compound***	***ROA***	***LD_{50} values (M and F combined), mg/kg***	***Findings***
Mouse	Swiss	2207	oral gavage	240	>140 mg/kg: cyanosis ≥140 death, hypoactivity, convulsions ≥ 182 mg/kg: prostration
		2695		270	≥140 death, hypoactivity, convulsions 236 mg/kg: prostration
		2696		251	182 mg/kg: cyanosis ≥182 hypoactivity ≥ 236 mg/kg: death, convulsions, prostration
		2207	IV	36	≥ 31.5 mg/kg: piloerection ≥ 34.7 mg/kg: death, prostration, hypoactivity ≥ 38.1 mg/kg: convulsions
	C 3/OF1	2695	oral gavage	206	147-178 mg/kg: transient subdued behavior, prostration ≥ 190 mg/kg: death, subdued behavior, prostration, convulsions
		2696		239	178 mg/kg: transient subdued behavior ≥ 224 mg/kg: death, subdued behavior, prostration
Rat	Sprague-Dawley	2207	oral gavage	228	≥ 215 mg/kg: death, prostration, congested lungs (decedents) ≥ 237 mg/kg: clonic convulsions
		2695		238	215 mg/kg: decreased BW (F) ≥ 215 mg/kg: death, prostration, subdued behavior, congested areas in lungs and intestines (decedents) ≥ 261 mg/kg: tremors ≥ 464 mg/kg: clonic convulsions
		2696		231	≥ 100 mg/kg: subdued behavior 178 mg/kg: decreased BW (M) ≥ 178 mg/kg: prostration 224 mg/kg: tremors ≥ 224 mg/kg: death, congested areas in lungs and intestines (decedents)
		2707	IV	51.2	50 mg/kg: death, tremors, apathy, gasping, decreased respiration ≥ 63 mg/kg: 100% death

b(4)

2.6.6.3 Repeat-dose toxicity
Studies reviewed by Asoke Mukherjee, Ph.D.

2.6.6.4 Genetic toxicology
Studies reviewed by Asoke Mukherjee, Ph.D.

2.6.6.5 Carcinogenicity
Two-year rat and mouse carcinogenicity studies reviewed by Asoke Mukherjee, Ph.D.

Study title: Twenty-six week repeated dose oral carcinogenicity study in Tg-rasH2 mice.

Key study findings:

- A significant trend test for multiple organ hemangiosarcomas was observed for females. No significant pairwise comparisons were present and the incidence was similar to historical control values.
- Decreases in body weights in high dose males were observed (-5.2 to -8.6%)
- Decreases in food consumption in high dose females were observed (-16.3 to -32.1%)

Adequacy of the carcinogenicity study and appropriateness of the test model:
The study was adequately conducted and analyzed. Urethane, the positive control, produced tumors consistent with what is found in the literature and is considered an appropriate positive control for this study. The test model was appropriate for assessment of the carcinogenic potential of milnacipran HCl.

Evaluation of tumor findings: The most frequent neoplasms noted included pulmonary tumors, hemangiomas and hemangiosarcomas (Tables 12 and 13). A trend test for multiple organ hemangiosarcomas in females was significant; however, no significant pairwise comparisons were seen. The incidence of hemangiosarcomas observed for females was similar to the historical control values provided by the Sponsor. Various neoplasms or pre-neoplastic lesions (Tables 11, 12, and 13) were observed in the treated groups with incidences similar to vehicle controls and/or similar to levels observed in the historical controls.

Study no.: Forest study number: CSIMMLNPCTX13; [ ] study number AA98KN.7G8R.01[ ]
Volume #, and page #: eCTD 4.2.3.4.2.1
Conducting laboratory and location: [ ] b(4)
Date of study initiation: November 21, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Milnacipran HCl, lot #L0000734, 99.4%
CAC concurrence: Yes, see ECAC report (Appendix 1), ECAC minutes (Appendix 2) and ECAC minutes and protocol review by Asoke Mukherjee, Ph.D. (Appendix 3).

Methods

Doses: Doses of 25, 50 and 125 mg/kg/d of milnacipran in sterile water were administered to the mice in the main study. The vehicle used was sterile water. A positive control group was administered urethane in saline, at a dose of 1000 mg/kg in three intraperitoneal injections (one each on Day 1, 3 and 5) at a dosage volume of 10 mL/kg.

Basis of dose selection (MTD, MFD, AUC etc.): The dose selection was based on findings from a 28-day dose range finding study (reviewed by Asoke Mukherjee). The protocol for this study was approved by the ECAC and the minutes are attached as Appendix 3.

Species/strain: Tg rasH2 mice were used in the main study, CByB6F1 hybrid mice (Tg rasH2 non-transgenic littermates) were used for TK (all mice from [ ] b(4)

Number/sex/group (main study): 25/sex/group (Table 5)

Route, formulation, volume: oral gavage, 10 mL/kg of body weight

Frequency of dosing: daily for 26 weeks

Satellite groups used for toxicokinetics or special groups: TK groups: 10/sex vehicle control, 58/sex each low, med and high dose groups (Table 5)

Age: 9-11 weeks at initiation of dosing

Animal housing: Animals were individually housed during quarantine and during the study in polycarbonate cages.

Restriction paradigm for dietary restriction studies: N/A

Drug stability/homogeneity: Drug uniformity and stability were confirmed in study # PRD-RPT-00052. The results were within the acceptance criteria of ±10%.

Dual controls employed: A vehicle control as well as a positive control were used.

Interim sacrifices: none

Deviations from original study protocol: On one occasion (Day 8) 23 mice were dosed according to their Day 1 body weights. The erroneous doses fell within ±5% of the target volumes. This deviation was not expected to affect the outcome of the study.

Histopathology Inventory

Study Number	*AA98KN.7G8R.01.[ ]*		
Species	*Tg rasH2 mice*		
Adrenals	X	Nasal cavity	X
Aorta	X	Optic nerves	
Bone Marrow (femur and sternum)	X	Ovaries	X*
Bone (femur and sternum)	X	Pancreas	X
Brain	X*	Parathyroid	X
Cecum	X	Peripheral nerve	
Cervix		Pharynx	
Colon	X	Pituitary	X
Duodenum	X	Prostate	X
Epididymis	X	Rectum	X
Esophagus	X	Salivary gland	X

b(4)

Eye	X	Sciatic nerve	X
Fallopian tube		Seminal vesicles	X
Gall bladder	X	Skeletal muscle	X
Gross lesions	X	Skin	
Harderian gland	X	Spinal cord	X
Heart	X*	Spleen	X
Ileum	X	Sternum	
Injection site	NA	Stomach	X
Jejunum	X	Testes	X*
Kidneys	X*	Thymus	X
Lachrymal gland		Thyroid	X
Larynx		Tongue	
Liver	X*	Trachea	X
Lungs and bronchi	X	Urinary bladder	X
Lymph nodes, mediastinal	X	Uterus	X
Lymph nodes mandibular	X	Vagina	X
Lymph nodes, mesenteric	X	Zymbal gland	
Mammary Gland with adjacent skin	X		

*organ weighed

Table 5. Experimental Design for Carcinogenicity and Toxicokinetics of Milnacipran in Mice (Table is reproduced from the Sponsor's submission)

Dose Group and Treatment	Number of Animals			
	Main Study (Tg.rasH2)		TK Study (CByB6F1)	
	Male	Female	Male	Female
Group 1 Vehicle Control	25	25	10	10
Group 2 Positive Control. Urethane*	25	25	-	-
Group 3 Low dose (25 mg/kg/day)	25	25	58	58
Group 4 Middle dose (50 mg/kg/day)	25	25	58	58
Group 5 High dose (125 mg/kg/day)	25	25	58	58
Total	125	125	184	184

* The Positive Control animals were administered a total of 3 intraperitoneal injections on Days 1, 3, and 5.

Observation times (stated in the Results section)

Results

Mortality: All animals were observed twice daily for moribundity and mortality.

In the male mice, one animal each in the control group and 50 mg/kg group and three animals in 125 mg/kg group were found dead or sacrificed under moribund conditions prior to terminal sacrifice (Table 6). The cause of death for the control male was hemangiosarcoma involving multiple organs. The cause of death for the 50 mg/kg male and one 125 mg/kg male was skin hemangiosarcoma. The causes of death for two 125 mg/kg males were nasal cavity sarcoma and spleen hemangiosarcoma. In the female mice, two animals each in the control group and 125 mg/kg group and one animal in the 50 mg/kg group were found dead or sacrificed under moribund conditions prior to terminal sacrifice (Table 6). The cause of death for one female each in the control group was spleen lymphoma and the other in the control group was undetermined. The cause of death for the 50 mg/kg female was salivary gland hemangiosarcoma and spleen lymphoma. The cause of death for one of the 125 mg/kg females was skin hemangiosarcoma and the other was spleen lymphoma. The Sponsor states that none of the male or female deaths (in the test article groups) were statistically significant when compared to the male or female control group (respectively).

Table 6. Summary of main study mortality

dose, mg/kg	*Males*		*Females*	
	deaths/ total	*cause of death (study day)*	*deaths/ total*	*cause of death (study day)*
0	1/25	Hemangiosarcoma, mult. organs (154)	2/25	Spleen lymphoma, malignant, 1° (87) Undetermined (114)
25	0/25	-	0/25	-
50	1/25	Skin hemangiosarcoma, malignant, 1° (144)	1/25	Salivary gland hemangiosarcoma and spleen lymphoma, both malignant and 1° (137)
125	3/25	Spleen hemangiosarcoma, malignant, 1° (41) Nasal cavity sarcoma, malignant, 1° (86) Skin hemangiosarcoma, malignant, 1° (118)	2/25	Skin hemangiosarcoma, malignant, 1° (102) Spleen lymphoma, malignant, 1° (170)

Clinical signs: A detailed hands-on examination was performed on test day 1 and weekly thereafter.
No treatment-related clinical signs were observed during the study. In the positive control group, clinical signs of toxicity in males and females associated with urethane treatment included rapid and shallow breathing and thin appearance.

Body weights: Body weights were measured once weekly beginning on test day 1 of the study through week 13 and biweekly thereafter. Body weights were also measured on the date of death or unscheduled sacrifice.
The weekly group mean body weights of the high dose males were statistically significantly decreased when compared to the control group at all but two weight collections taken between Day 71 and Day 183. These statistically significant decreases ranged between 5.2-8.6% (Table 7). In the females, there were no statistically significant differences in any of the weekly group mean body weights. Percent decreases for the females ranged from 0.1-2.4% at the high dose (Table 8).

Male weekly group mean body weight gains were statistically significantly decreased compared to the vehicle control group on five occasions each in the mid dose and high dose mice between Week 6 and Week 21. Weekly group mean body weight gain was statistically significantly decreased compared to the vehicle control group once in the low dose females (between Day 85 and Day 99). However, there were no statistically significant differences in absolute weight gain (from Day 1 to Day 183) in either sex when the test article treatment groups were compared to the vehicle control.
The Sponsor states that the body weight effects in the high dose males suggest that the high dose was at or near the MTD.

Table 7. Group mean body weights, males: Percent change from control

Dose	*Study day*																			
mg/kg	*1*	*8*	*15*	*22*	*29*	*36*	*43*	*50*	*57*	*64*	*71*	*78*	*85*	*99*	*113*	*127*	*141*	*155*	*169*	*183*
Uret	-0.5	**-6.6***	-1.7	-0.9	-1.3	1.7	2.8	2.2	**4.9***	3.2	4.5	4.7	**6.3***	**6.6***	5.8	NA	NA	NA	NA	NA
25	-1.6	-1.8	-0.7	-2.0	-2.8	-2.1	-2.0	-2.7	-0.8	-2.6	-3.2	-4.2	-3.3	-3.0	-3.8	-4.2	-3.9	-3.9	-4.5	-3.7
50	-1.6	-1.6	-2.2	-1.2	-1.9	-0.3	-2.2	-0.9	1.1	-1.3	-3.0	-3.8	-2.5	-3.7	-3.4	-3.2	-2.7	-4.3	-3.7	-3.5
125	-1.7	-2.4	-3.0	-2.5	-3.1	-1.9	-3.7	-3.3	-1.1	-4.2	**-5.2***	**-6.0***	-3.8	-4.7	**-6.9***	**-7.1***	**-7.9***	**-8.5***	**-8.6***	**-7.6***

Uret= total of 3 doses of 1000 mg/kg Urethane as positive control
NA= not applicable
Group means significantly different from control group: *$p \leq 0.05$ (Dunnett's t-Test)

Table 8. Group mean body weights, females: Percent change from control

Dose	*Study day*																			
mg/kg	*1*	*8*	*15*	*22*	*29*	*36*	*43*	*50*	*57*	*64*	*71*	*78*	*85*	*99*	*113*	*127*	*141*	*155*	*169*	*183*
Uret	-2.4	**-6.4***	-1.5	-2.4	-0.5	0.2	1.0	3.0	2.3	0.9	1.4	1.8	2.5	3.9	NA	NA	NA	NA	NA	NA
25	-1.9	-1.4	-2.2	-1.9	-2.2	-2.6	-2.1	-1.1	-0.8	-2.1	-1.7	-2.3	-0.8	-2.9	-2.3	-2.2	-1.4	-1.6	-1.6	-1.9
50	-0.6	-1.0	-0.6	-0.6	0.5	1.0	0.0	1.5	0.3	0.6	-0.5	0.0	0.0	-0.4	0.8	1.2	1.6	2.2	1.6	-0.4
125	-1.3	-0.3	-0.6	0.5	-0.1	-0.3	-1.5	0.5	0.0	-0.8	-2.3	-0.5	-0.6	-1.0	-0.6	-2.3	-1.7	-0.3	-2.4	0.2

Uret= total of 3 doses of 1000 mg/kg Urethane as positive control
NA= not applicable
Group means significantly different from control group: *$p \leq 0.05$ (Dunnett's t-Test)

Food consumption: Food consumption was measured weekly throughout the duration of the study.
Several statistically significant decreases in both sexes for weekly food consumption in the test article-treated groups were seen throughout the study (Figures 2 and 3, Tables 9 and 10). High dose females showed several significant decreases in weekly food consumption at the high dose ranging from -16.3 to -32.1%, mostly occurring later in the study. Quite a bit of variability is observed in the consecutive weekly food consumption measurements, for example, week 22 the high dose females had a decrease in food consumption of 2.7% and the following week the decrease was 20.5% (Table 10). Although the decreases may reach statistical significance on several occasions, the data may not accurately reflect an effect of the drug on food consumption. Total food consumption (Day 1-183) was significantly decreased for high dose females (-11.1%). No other significant differences for the total food consumption were observed for treated groups.

Table 9. Weekly Food Consumptions, males: Percent change from control

Dose	*Week*												
mg/kg	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Uret	**-23.2***	**20.8***	**30.9***	**13.5***	**19.2***	**17.7***	**13.4***	**24.9***	6.3	**11.4***	5.6	**20.2***	**16.0***
25	2.2	-10.7	14.0	-2.5	**17.4***	-0.9	-0.6	4.8	-8.2	-4.1	-1.1	2.3	2.9
50	-5.1	-11.6	10.4	-2.2	4.8	-0.6	-0.6	1.8	**-10.4***	**-11.7***	-8.3	-0.3	-2.6
125	-8.1	0.3	7.6	-2.5	12.6	1.7	-0.3	4.5	-6.0	**-12.0***	**-11.3***	2.0	-3.2

Table 9. Weekly Food Consumptions, males: Percent change from control, Continued

Dose	*Week*												
mg/kg	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*
Uret	**10.4***	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	-2.7	-3.8	-3.7	-3.7	-3.4	-7.3	-2.1	-6.5	-8.0	-3.7	**-6.9***	-5.0	-1.3
50	-4.9	-2.4	-5.9	-3.1	-1.5	-4.8	-1.2	**-9.4***	-15.7	-2.2	**-6.6***	-3.4	4.1
125	-1.5	-3.8	**-11.5***	-5.2	-2.8	-1.2	-3.0	-5.1	-16.2	-3.7	**-8.7***	-2.8	0.0

Uret= total of 3 doses of 1000 mg/kg Urethane as positive control
NA= not applicable
Group means significantly different from control group: *$p \leq 0.05$ (Dunnett's t-Test)

Table 10. Weekly Food Consumptions, females: Percent change from control													
Dose	*Week*												
mg/kg	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Uret	-19.5	**-21.2***	13.6	8.7	11.0	**27.1***	6.9	11.1	**19.1***	4.0	15.7	**24.1***	16.2
25	0.8	-14.7	4.2	-10.4	-2.2	12.3	-2.6	-15.0	-2.4	-10.8	-4.3	3.3	-6.8
50	-4.3	12.5	0.2	-4.8	5.8	-0.2	-3.8	-12.0	-3.2	-9.9	-2.7	-5.1	-2.0
125	-3.3	-19.9	-1.0	-5.8	-3.6	-7.5	-10.0	-17.9	-10.1	**-23.0***	**-18.9***	-3.3	-9.7

Table 10. Weekly Food Consumptions, females: Percent change from control, Continued													
Dose	*Week*												
mg/kg	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*
Uret	9.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	-2.9	-6.7	**-20.5***	0.0	-8.9	**-12.0***	-12.7	-7.6	-2.1	-10.0	-3.4	-3.8	**-13.7***
50	-2.6	-9.6	**-20.0***	-4.8	-4.7	-9.0	-4.5	-6.2	-5.7	-12.2	-3.1	-7.8	**-13.4***
125	-11.7	**-17.1***	**-32.1***	**-14.3***	**-21.5***	**-16.3***	-14.3	1.1	-2.7	**-20.5***	-9.3	-10.4	**-14.6***

Uret= total of 3 doses of 1000 mg/kg Urethane as positive control
NA= not applicable
Group means significantly different from control group: *$p \leq 0.05$ (Dunnett's t-Test)

Figure 2. Group Mean Food Consumption in Main Study Males

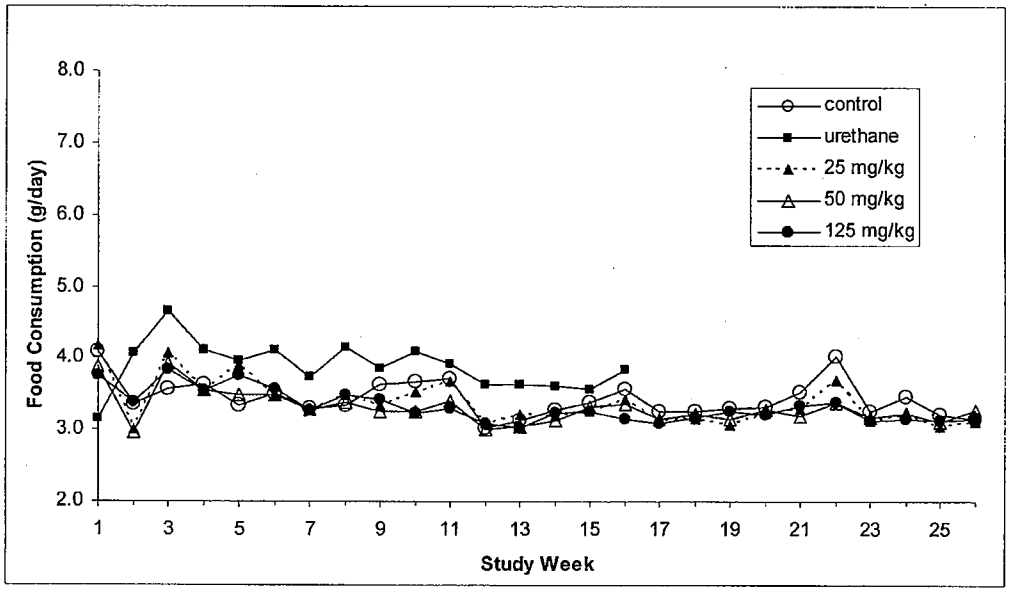
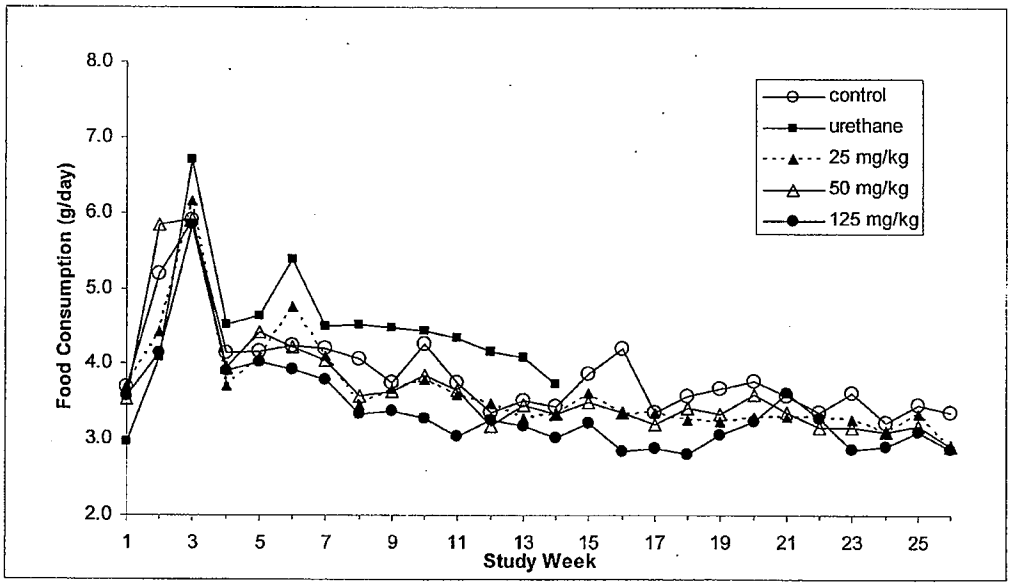


Figure 3. Group Mean Food Consumption in Main Study Females



Gross pathology: No treatment-related gross lesions were observed. The urethane positive control showed pulmonary and splenic lesions that were related to the urethane treatment.

Organ Weights: The following organs were weighed at the scheduled necropsy of all Main Study animals: brain, heart, liver, kidneys and testes or ovaries.

No treatment-related changes in organ weights were observed.

Histopathology: Peer review: yes ( ), no (X) See the Histopathology Inventory table in the Methods section for a listing of organs. Representative portions of all protocol-specified tissues (including gross lesions) from all Main Study animals, i.e., test article treated, vehicle control, all early Main Study deaths and selected tissues from Positive Control animals (lungs and spleen, and any other gross lesions) were embedded in paraffin and sectioned at 6 microns or less. All sections were stained with hematoxylin and eosin.

Statistically significant increased frequencies of neoplastic lung and spleen lesions in the Positive Control animals were observed. This demonstrates the validity of the test system.

Non-neoplastic: Marked proliferation of vessels lining the serosa of the uterus was observed in one mouse each in the control, low, mid and high dose groups. This lesion is considered by the pathologist to be a pre-neoplastic lesion of hemangiomas or hemangiosarcomas. Since the incidence of this lesion was similar in the vehicle and test article treated groups, the lesion is not considered to be test article related.

Neoplastic: A variety of tumors were observed in this study. These tumors occurred with a low incidence that was not statistically significantly different when compared to the incidence of the vehicle control (Table 11). None of these tumors were considered to be treatment-related. Pulmonary tumors and hemangiosarcomas/hemangiomas are discussed below.

Pulmonary tumors: Both sexes of the urethane treated mice had a significantly ($p \leq 0.05$) higher incidence of pulmonary tumors (which included adenomas and carcinomas) when compared with the vehicle group and when compared to the test article treated groups (Table 12). Single pulmonary adenomas were observed in all dose groups and seen in both sexes at levels similar to that of the vehicle groups (Table 12). One pulmonary carcinoma was seen in a high dose male. Pulmonary carcinomas were not observed in either male or female vehicle group. Pulmonary tumors are spontaneous tumors noted in this strain of mouse and their presence in low numbers in the treated dose groups is not considered to be treatment-related. No historical control data for pulmonary tumors was provided by the Sponsor.

Hemangiosarcomas: Both sexes of the urethane treated mice had a significantly ($p \leq 0.05$) higher incidence of hemangiosarcomas when compared with the vehicle group and when

compared to the treated groups (Table 13). Test article treated females showed a significant positive trend (p=0.0185) in multiple organ vascular lesions but did not show any significant increases in any of the treated groups (Table 13). The observed lesions were spread across various tissues in a nonspecific fashion and are common in this strain of female transgenic mice and are similar to historical control values (Table 14). This apparent dose-response in the females is considered to be due to biological background variations. Two hemangiosarcomas of the testes were observed at the mid dose in males. This incidence was similar to historical control values and is considered not test article-related (Table 15).

Table 11. Various tumors (excluding pulmonary and hemangiosarcomas)

		vehicle		*25 mg/kg*		*50 mg/kg*		*125 mg/kg*	
		M	*F*	*M*	*F*	*M*	*F*	*M*	*F*
Organ	*tumor type n=*	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Nasal cavity	*Sarcoma: malignant, primary*	-	-	-	-	-	-	1	-
Harderian glands	*Hyperplasia*	-	-	-	1	1	1	-	-
	Adenoma: benign, primary	-	-	1	1	-	-	-	-
	Carcinoma: malignant, primary	-	-	-	-	-	1	-	-
Mandibular lymph node	*Hyperplasia; lymphoid*	-	2	-	1	-	1	-	-
Skin	*Squamous cell carcinoma, malignant, primary*	-	-	-	-	-	-	2	-
	Papilloma; benign, primary	-	-	-	1	-	-	-	-
Spleen	*Lymphoma: malignant, primary*	-	1	-	-	-	1	-	1
Thymus	*Lymphoma: malignant, primary*	-	-	-	-	-	-	-	1

Table 12. Pulmonary tumors										
Tumor type	vehicle		urethane		25 mg/kg		50 mg/kg		125 mg/kg	
	M	F	M	F	M	F	M	F	M	F
n	25	25	25	25	25	25	25	25	25	25
Adenoma single	0	1	0	0	2	1	0	1	1	2
Adenoma multiple	1	1	20	16	0	0	0	0	0	0
Carcinoma	0	0	5	9	1	0	0	0	0	0
Total	1	2	25*	25*	3	1	0	1	1	2

*p<0.05 (Fisher's Exact Test): total tumors for each treated group compared to vehicle

Table 13. Multiple organ hemangiomas and hemangiosarcomas											
Tumor type		vehicle		urethane		25 mg/kg		50 mg/kg		125 mg/kg	
		M	F	M	F	M	F	M	F	M	F
	n	25	25	25	25	25	25	25	25	25	25
Hemangio-sarcoma	Bone, calvarium	0	0	0	0	0	0	0	0	1	0
	Nasal cavity	0	0	0	0	1	0	0	0	0	0
	Liver	1	0	0	0	0	0	0	0	0	0
	Lung	1	0	0	0	0	0	0	0	0	0
	Testes	0	NA	0	NA	0	NA	2	NA	0	NA
	Skin	0	0	0	0	0	0	1	0	1	1
	Spleen	1	0	25*	23*	0	0	0	0	1	0
	Mediastinal lymph node	0	0	0	0	0	0	0	1	0	0
	Mandibular lymph node	0	0	0	0	0	0	0	0	0	1
	Salivary gland	0	0	0	0	0	0	0	1	0	0
	Ovaries	NA	0	NA	1	NA	0	NA	0	NA	0
	Vagina	NA	0	NA	0	NA	0	NA	0	NA	1
	Total	2	0	25*	23*	1	0	3	2	3	3
Hemangioma	Liver	0	0	0	1	0	0	0	0	0	0
	Ovaries	NA	0	NA	1	NA	0	NA	0	NA	0
	Total	NA	0	NA	2	NA	0	NA	0	NA	0
Total Hemangiosarcoma and Hemangioma		2[a]	0	25*[a]	23*[a]	1	0	3	2	3	3

[a]Multiple organ sites for each animal
*p<0.05 (Fisher's Exact Test): treated group compared to vehicle
NA= not applicable

Table 14. Historical Controls: Vascular Lesions in Females					
study number	*1*	*2*	*3*	*4*	*total*
n	*25*	*25*	*25*	*25*	*100*
Hemangioma	0	0	0	0	0
Hemangiosarcoma	2	3	2	3	**10**

Table 15. Historical Controls: Vascular Lesions in Males					
study number	*1*	*2*	*3*	*4*	*total*
n	*25*	*25*	*25*	*25*	*75*
Hemangiosarcoma, testes	**NE**	**1**	**0**	**0**	**1**

NE= not examined

Toxicokinetics: 4/sex/dose/timepoint were bled on Day 1 of dosing and during Week 26 after 182 days of daily treatments. Test article-treated TK animals were bled pre-dose and at 0.5, 1, 2, 4, 10 and 24 hours post-dose on each bleed day. Vehicle control TK animals (4/sex) were bled once at 0.5 hour after dosing (approximate T_{max}) on Day 1 and during Week 26 after 182 days of daily treatment.

Milnacipran was rapidly absorbed with a T_{max} of 0.5 h. Overall, female mice had higher exposure (AUC and C_{max}) to milnacipran than male mice. Systemic exposure (AUC) to milnacipran was similar on Day 1 (following once daily dosing) and on Day 182 for male mice, indicating no accumulation in male mice. Systemic exposure (AUC) to milnacipran was slightly higher on Day 182 than on Day 1 for females. The toxicokinetic data are provided in Table 16 which was reproduced from the NDA submission.

Exposure comparisons between mice and humans at the Cmax and steady state AUC for the maximum proposed dose of milnacipran (200 mg) are provided in Table 17.

Table 16.

Toxicokinetic Parameters of Milnacipran (Free Base) Following Oral Administration of 25, 50, and 125 mg/kg/day of Milnacipran HCl to Male and Female Mice

	Dose (mg/kg)	Cmax (ng/mL)		AUC_{0-24} (h*ng/mL)		Tmax (h)		T½ (h)	
		Male	Female	Male	Female	Male	Female	Male	Female
Day 1	25	4492.37	6517.81	6657.13	8302.47	0.5	0.5	1.31	1.24
	50	6691.36	9901.42	14354.35	17499.73	0.5	0.5	1.25	2.08
	125	13335.28	13839.09	44864.05	52356.39	0.5	0.5	2.07	2.46
Day 182	25	3686.71	4492.22	6151.83	8518.50	0.5	0.5	2.62	3.13
	50	5485.76	6748.38	13235.60	21687.20	0.5	0.5	2.89	2.68
	125	11119.08	12872.41	39902.76	59646.12	0.5	0.5	2.87	3.60

Table 17. Exposure Comparison Between Mouse and Human (200 mg dose)			
	Dose, mg/kg	***Mouse/human C_{max}, (ng/mL)***	***Mouse/human AUC_{0-24}, (h.ng/mL)***
Male	***25***	3.4	0.9
	50	5.1	2.0
	125	10.3	6.0
Female	***25***	4.2	1.3
	50	6.2	3.3
	125	11.9	9.0

Human combined male and female AUC= 6650 h.ng/mL
Human combined male and female C_{max}= 1078 ng/mL

Summary and Evaluation:
The high dose of milnacipran used in this study was 125 mg/kg. This dose was recommended by the ECAC on the basis of being half of the lethal dose (250 mg/kg) observed in the 28-day dose range finding study. The low dose of 25 mg/kg is approximately equal to a human daily dose of 200 mg, which is the highest dose proposed to be marketed in this NDA. The doses (25, 50 and 125 mg/kg) used in this study and the use of urethane as a positive control were recommended by the ECAC (see Appendix 3).

Toxicity observed in this study was minimal, however, some minor changes in body weights in males and food consumption in females were observed. Decreases in body weights in high dose males were statistically significant (-5.2 to -8.6%). There were no significant changes in female body weights. High dose females showed several significant decreases in weekly food consumption at the high dose ranging from -16.3 to -32.1%, mostly occurring later in the study, although the changes in food consumption from week to week were highly variable. Total food consumption in females at the high dose (-11.1%) was also significantly decreased as compared to the vehicle controls. There were no treatment-related changes in food consumption in males. No other findings of drug-related toxicity were observed in the study.

Daily treatment with milnacipran HCl up to 125 mg/kg did not result in an increase of neoplastic lesions. The most frequent neoplasms noted included pulmonary tumors, hemangiomas and hemangiosarcomas. A trend test for multiple organ hemangiosarcomas in females was significant: however, no significant pairwise comparisons were seen. The incidence of hemangiosarcomas observed for females was similar to the historical control values provided by the Sponsor. Various neoplasms or pre-neoplastic lesions were observed but all were similar to levels observed in vehicle controls and/or similar to levels observed in the historical controls.

2.6.6.6 Reproductive and developmental toxicology
Studies reviewed by Asoke Mukherjee, Ph.D.

2.6.6.6 Local tolerance
No local tolerance studies were conducted.

2.6.6.8 Special toxicology studies

The structure of milnacipran was submitted to the Computational Toxicology Consult Service in CDER OPS. *MC4PC* analysis was performed using MultiCASE, Inc. software and *MDL-QSAR* analysis was performed using Symyx, Inc. software.

The results summary received from the Computational Toxicology Consultation dated May 30, 2008 is reproduced verbatim below.

> The test compound, Milnacipran, was predicted to be positive for rodent carcinogenicity by MDL-QSAR but not by MC4PC. In general, compounds with the highest carcinogenic potential have a consensus positive prediction in multiple QSAR platforms and models. In this case, consideration of both the MC4PC and MDL-QSAR results suggest weak/equivocal carcinogenic potential for the test compound.

2.6.6.9 Discussion and Conclusions

The acute toxicology studies and the Tg rasH2 mouse carcinogenicity study are evaluated in this review for NDA 22-256. All other toxicology studies were reviewed by Asoke Mukherjee, Ph.D. in a separate review for this NDA.

Acute toxicology studies were conducted with the racemic mixture of milnacipran (PO and IV administration) and the *d*- and *l*- isomers of milnacipran (PO administration only) in mouse and rat. In mice, similar clinical signs of hypoactivity, cyanosis, prostration, and convulsions were observed at high doses for the three compounds tested. With IV administration of milnacipran, hypoactivity, piloerection, prostration and convulsions were observed. LD_{50} values were similar in males and females with PO and IV administration. With PO administration, LD_{50} values for milnacipran and the *d*- and *l*- isomers were all $\geq$ 206 mg/kg (M and F combined) in the two strains of mice tested. The LD_{50} in mice with IV milnacipran administration was 36 mg/kg (M and F combined).

In rats, similar clinical signs of hypoactivity, prostration, convulsions and congested lungs were observed at the higher doses with PO administration for the three compounds tested. Milnacipran with IV administration caused tremors, apathy, gasping and decreased respiration at higher doses. LD_{50} values were similar in males and females with both PO and IV administration in rat. With PO administration, LD_{50} values for milnacipran and the *d*- and *l*- isomers were all $\geq$ 228 mg/kg (M and F combined). The LD_{50} in rats with IV administration was 51 mg/kg (M and F combined).

A 26-week carcinogenicity study with milnacipran was conducted in a transgenic rasH2 mouse model. Daily oral treatment with milnacipran at doses up to 125 mg/kg did not result in an increase of neoplastic lesions. The most frequent neoplasms noted included pulmonary tumors, hemangiomas and hemangiosarcomas. A trend test for multiple organ hemangiosarcomas in females was significant: however, no significant pairwise comparisons were seen. The incidence of hemangiosarcomas observed for females was similar to the historical control values provided by the Sponsor. Various neoplasms or pre-neoplastic lesions were observed but all were similar to levels observed in vehicle controls and/or similar to levels observed in the historical controls. The Executive Carcinogenicity Assessment Committee agreed that the study was negative for any statistically significant drug-related neoplasms.

The structure of milnacipran was evaluated by the Computational Toxicology Consult Service using the MDL-QSAR and MC4PC software programs. Milnacipran was predicted to be positive for rodent carcinogenicity by MDL-QSAR but not by MC4PC which suggests weak/equivocal carcinogenic potential for the test compound.

2.6.6.10 Tables and Figures
Not applicable.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The studies evaluated in this review support the approval of NDA 22-256. No nonclinical approval issues were identified during this review. Please note that the majority of the toxicology studies were reviewed by Asoke Mukherjee, Ph.D. in a separate review for this NDA.

Unresolved toxicology issues (if any): There are no unresolved toxicology issues in the portions of the NDA evaluated in this review.

Recommendations: From a pharmacology/toxicology perspective, based upon evaluation of the nonclinical data contained within this review, NDA 22-256 may be approved.

Suggested labeling: The table below contains the draft labeling submitted by the sponsor and the changes proposed by this reviewer. Please note that the table only reflects labeling changes for the portions of the NDA evaluated in this review.

Sponsor's proposed wording	*Reviewer's proposed wording*	*Reviewer's rationale for proposed wording*

b(4)

Signatures (optional):

Reviewer Signature Elizabeth A. Bolan, Ph.D.

Supervisor Signature R. Daniel Mellon, Ph.D. Concurrence Yes X No

Reference List

Bomholt SF, Mikkelsen JD, Blackburn-Munro G (2005) Antinociceptive effects of the antidepressants amitriptyline, duloxetine, mirtazapine and citalopram in animal models of acute, persistent and neuropathic pain. Neuropharmacology 48:252-263.

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Iyengar S, Webster AA, Hemrick-Luecke SK, Xu JY, Simmons RM (2004) Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats. J Pharmacol Exp Ther 311:576-584.

Joshi SK, Hernandez G, Mikusa JP, Zhu CZ, Zhong C, Salyers A, Wismer CT, Chandran P, Decker MW, Honore P (2006) Comparison of antinociceptive actions of standard analgesics in attenuating capsaicin and nerve-injury-induced mechanical hypersensitivity. Neuroscience 143:587-596.

LePard KJ, Stephens RL, Jr. (1994) Serotonin inhibits gastric acid secretion through a 5-hydroxytryptamine1-like receptor in the rat. J Pharmacol Exp Ther 270:1139-1144.

Pedersen LH, Blackburn-Munro G (2006) Pharmacological characterisation of place escape/avoidance behaviour in the rat chronic constriction injury model of neuropathic pain. Psychopharmacology (Berl) 185:208-217.

APPENDIX/ATTACHMENTS

Appendix 1: Report of study results to the Executive Carcinogenicity Assessment Committee for the Tg rasH2 mouse carcinogenicity study

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

P/T REVIEWER(s): **Elizabeth A. Bolan**
DATE: **July 1, 2008**

IND/NDA: **NDA 22-256**
DRUG CODE#: **N/A**
CAS#: **101152-94-7**
DIVISION(s): **Division of Anesthesia, Analgesia, and Rheumatology Products**
DRUG NAME(s): **Milnacipran HCl**

SPONSOR: **Cypress Bioscience, Inc.**
LABORATORY: [redacted] **b(4)**
CARCINOGENICITY STUDY REPORT DATE: **October 26, 2007**

THERAPEUTIC CATEGORY: **antidepressant**
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: **NE and 5-HT reuptake inhibitor**
MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay):
Negative: Ames Test
Negative: *in vitro* chromosomal aberration in purified human lymphocytes
Negative: L5178Y TK +/- mouse lymphoma forward mutation assay
Negative: *in vivo* mouse micronucleus assay

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc.):
Twenty-six week repeated dose oral carcinogenicity study in Tg-rasH2 mice

MOUSE STUDY DURATION (weeks): **26 weeks**
STUDY STARTING DATE: **November 21, 2005**
STUDY ENDING DATE: **September 7, 2006**
MOUSE STRAIN: **Tg.rasH2 mice were used in the main study, CByB6F1 hybrid mice (Tg.rasH2 non-transgenic littermates) were used for TK**
ROUTE: **oral gavage**
DOSING COMMENTS: **The doses have been approved by the eCAC (October 18, 2005).**

NUMBER OF MICE:
- Control-1 (C1): **25/sex**
- Control-2 (C2):
- Low Dose (LD): **25/sex**
- Middle Dose (MD): **25/sex**
- High Dose-1 (HD1): **25/sex**
- High Dose-2 (HD2):

MOUSE DOSE LEVELS* (mg/kg/day):
- Low Dose: **25 mg/kg**
- Middle Dose: **50 mg/kg**
- High Dose-1: **125 mg/kg**
- High Dose-2:

(*Dose adjusted during study)

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible):
The basis for dose selection was MTD from a 28-day dose range finding study. Mortality was observed at 250 mg/kg in males and females in the 28-day study.

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date):
The dose range finding study and protocol were reviewed by Asoke Mukherjee (DAARP). The doses used in the study were recommended by the eCAC (October 18, 2005).

MOUSE CARCINOGENICITY (conclusion: negative; positive; MF; M;F):
Negative in both males and females

MOUSE TUMOR FINDINGS (details):
The most frequent neoplasms noted included pulmonary tumors, hemangiomas and hemangiosarcomas. A trend test for multiple organ hemangiosarcomas in females was significant: however, no significant pairwise comparisons were seen. The incidence of hemangiosarcomas observed for females was similar to the historical control values provided by the Sponsor. Various neoplasms or pre-neoplastic

lesions were observed in the treated groups with incidences similar to vehicle controls and/or similar to levels observed in the historical controls.

MOUSE STUDY COMMENTS: NONE

Appendix 2: Executive Carcinogenicity Assessment Committee minutes: Study results of Tg rasH2 mouse carcinogenicity study

Executive CAC
Date of Meeting: July 1, 2008

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Anne Pilaro, Ph.D., DBOP, Alternate Member
R. Daniel Mellon, Ph.D., DAARP, Team Leader
Elizabeth Bolan, Ph.D., DAARP, Presenting Reviewer

Author of Minutes: Elizabeth Bolan, Ph.D., DAARP

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 22-256
Drug Name: Milnacipran Hydrochloride
Sponsor: Forest Laboratories, Inc.

Background:

Milnacipran hydrochloride is a non-tricyclic antidepressant which inhibits the reuptake of norepinephrine and serotonin. It is approved for the treatment of depression in several countries at a maximum dose of 50 mg BID. The indication sought for this NDA is treatment of fibromyalgia syndrome with a maximum dose of 100 mg BID.

A 26-week Tg.rasH2 mouse carcinogenicity study was submitted as part of the NDA submission. The formal review of the carcinogenicity study can be found in the NDA review. The basis for dose selection for the 26-week Tg.rasH2 study was MTD from a 28-day dose range finding study. The high dose of milnacipran used in the 26-week study was 125 mg/kg. This dose was recommended by the eCAC (October 18, 2005) on the basis of its being half of the lethal dose (250 mg/kg) observed in the 28-day dose range finding study. The low dose of 25 mg/kg is approximately equal to a human daily dose of 200 mg, which is the highest dose proposed to be marketed in the NDA.

Milnacipran was found to be negative in the Ames Test, *in vitro* chromosomal aberration assay in purified human lymphocytes, L5178Y TK +/- mouse lymphoma forward mutation assay, and in the *in vivo* mouse micronucleus assay.

Tg.rasH2 Mouse Carcinogenicity Study

The doses (25, 50 and 125 mg/kg) used in this study and the use of urethane as a positive control were recommended by the eCAC. The vehicle used was sterile water.

Daily treatment with milnacipran HCl up to 125 mg/kg did not result in an increase of neoplastic lesions. The most frequent neoplasms noted included pulmonary tumors, hemangiomas and hemangiosarcomas. A

trend test for hemangiosarcomas (multiple organs combined) in females was significant: however, no significant pairwise comparisons were seen. The incidence of hemangiosarcomas observed for females was similar to the historical control values provided by the Sponsor and those previously seen in studies submitted to the FDA. Various neoplasms or pre-neoplastic lesions were observed but all were similar to levels observed in vehicle controls and/or similar to levels observed in the historical controls.

Executive CAC Recommendations and Conclusions:

Tg.rasH2 mouse final study:

- The Committee agreed that the study was adequate, noting prior Executive CAC concurrence with the doses used.
- The Committee agreed that the study was negative for any statistically significant drug-related neoplasms

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
NDA 22-256/Division File, DAARP
R. Daniel Mellon/Supervisor, DAARP
Elizabeth Bolan/Reviewer, DAARP
Diana Walker/CSO/PM, DAARP
Adele Seifried, OND IO

Appendix 3: Executive Carcinogenicity Assessment Committee protocol review and meeting minutes for Tg rasH2 mouse carcinogenicity study (Reviewed by Asoke Mukherjee, Ph.D.)

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals

Application (IND/NDA) number: 63,736
Submission date and number: Sept 13, 2005, serial # 118
Division: DAARP
Project manager: Jane Dean
CAS#: 86181-08-0
Drug name: Milnacipran hydrochloride
Pharmacological Classification: NE and 5-HT uptake inhibitor
Sponsor/Applicant: Forest Laboratories
Sponsor/Applicant contact name: Michael K. Olchaskey, Pharm.D.
Sponsor/Applicant telephone and fax number: Tel: 201-386-2142, Fax 201-524-9711
Date submitted (stamp date): Sept 15, 2005
45-day date (from submission stamp date): Oct 30, 2005
P/T Reviewer(s): Asoke Mukherjee
Date Review Completed: Oct 4, 2005

Date of CAC review: Oct. 18, 2005
CAC members: David Jacobson-Kram, Abby Jacobs, Joe Contrera, Jasti Choudary, and Dan Mellon

Summary of Proposal for Review:

Species/strain: Transgenic mice, CB6F1Jic-TgrasH2@Tac
Number/sex/dose: 25/group/sex
Route: Oral gavage

	male	female
Doses proposed:	25, 75, 150_mg/kg_	25, 75, 150__mg/kg___
Basis of dose selection:		
MTD	___x__	___x__
AUC ratio	___8__	___8__
saturation	_____	_____
MFD	_____	_____
PD	_____	_____
other	_____	_____
	_____	_____
Kinetics submitted:	**rodent**	**human**
pharmacokinetics	_yes__	yes___
metabolism	_yes__	yes___
protein binding	_19.1%__	15.4%_

Notable design features: Nil

Summary of Recommendations to CAC: The sponsor selected doses on the basis of MTD in wild type non transgenic strain. Mortality was noted at 250 mg/kg in a 28-day study.

	male	female
Doses recommended by reviewer:	_25, 75, 200_mg/kg	25, 75, 200 mg/kg_____

Basis for recommendation (details): Mortality in the 28-day dose range finding study.

CAC Concurrence (y/n):

CAC Recommendations:

Comments:

APPEARS THIS WAY
ON ORIGINAL

Memo To:

IND 63,736 Div File

Dated: Sept 21, 2005

From:

Asoke Mukherjee, Ph.D., DAARP
Pharmacologist

Through: Dan Mellon, Ph.D., DAARP
Team Leader

Re: Special protocol assessment, Mouse Carcinogenicity study for IND 63,736, milnacipran hydrochloride, serial # 118, Sept 13, 2005

Forest Laboratories Inc. (New Jersey) previously conducted 2-year bioassays in both the rat and mouse model. The study protocols, however, were not reviewed by the eCAC. Following review of the study results on June 15, 2004, the eCAC concluded that the rat bioassay was acceptable, however, the doses chosen for the mouse bioassay failed to reach an MTD. The eCAC minutes from June 15, 2004 reflect the following recommendations:

> The Committee could not conclude that the study was adequate because an MTD had not been reached, based on absence of effects on the mortality, body weight or other signs of toxicity at the high dose, nor had pharmacokinetic (PK) data been provided to demonstrate a 25-fold multiple of the maximum human exposure. In order to support acceptability of the study, the committee suggested that the sponsor conduct a PK study in the same strain of mice to see if the exposure at 100 mg/kg would have provided 25 times human exposure at the maximum recommended human dose. Alternately, the sponsor could conduct a carcinogenicity study in Tg*ras*H2 transgenic mice or repeat a conventional 2-year mouse study at higher doses. If the multiple of the human exposure is not at least 25-fold the maximum human exposure, then another carcinogenicity study is needed. For either a traditional or alternative carcinogenicity study, a dose range-finding study with PK parameters would have to be conducted in the same strain of mouse for selection of a high dose. The sponsor should submit its selection of doses for the mouse carcinogenicity study for concurrence by the exec-Carcinogenicity Assessment Committee.

The sponsor elected to submit a protocol for a mouse carcinogenicity study in Tg.rasH2 mice (mice-transgenic strain) in response to the recommendation of eCAC dated June 15, 2004.

Background:

Milnacipran hydrochloride is a non-tricyclic antidepressant that inhibits re-uptake of norepinephrine and serotonin. It is approved for the treatment of depression in several countries outside the USA at 50 mg BID doses. The IND was submitted for the treatment of fibromyalgia.
Milnacipran is not mutagenic in the Ames assay, mouse lymphoma assay, or mouse micronucleus test. The maximum dose proposed in the ongoing 12-week trial (FMS-021) clinical trial is 200 mg daily. The dose will be titrated according to tolerability of the drug. According to DrugDex Database, the most effective dose of milnacipran in depression is 50 mg BID. Doses as high as 100 mg BID did not show evidence of greater efficacy.

The sponsor provided tolerability and PK data in humans in healthy subjects. Maximum doses were escalated to 200 mg per day. The sponsor provided PK data at 200 mg/day on day 9 of the treatment (page 15, vol. 1) as shown in the table below.

PK parameter	Milnacipran, n=6
AUC, ng.hr/ml	3325
C_{max}, ng/ml	538
C_{av}, ng/ml	277
C_{min}, ng/ml	115
T_{max}, hr	2.6
$T_{1/2}$, hr	7.3

The sponsor stated that 75, 150 and 200 mg/kg doses used in the 28-day repeat/TK study in CByB6F1 mice (non-transgenic) were approximately 4, 8 and 11 times the steady state human AUC at 100 mg BID dose for 12 days (page 22, vol. 1). The kinetic data in mice on day 25 of the treatment are shown below.

28-day toxicity/TK study	Dose, mg/kg	C_{max}, ng/ml		AUC, ng.h/ml		Mouse to/human exposure ratio	
		Male	Female	Male	Female	Male	Female
Day 25	25	NA	NA				
Day 25	75	7449	6607	24805	21079	4	3

nd female CByB6F1 hybrid strain of mice obtained by cross-breeding wild type C57BL/6 and BALB/cBy (non-transgenic littermates). Mice will be 6-11 weeks old and will weigh 15-20

Day 25	150	9867	9109	55956	52173	8	8
Day 25	200	10354	12913	67852	80603	10	12

NA= not available
Human data for AUC were 6649 ng.hr/ml at 100 mg BID dose for 12 days.

1. Protocol for the mouse carcinogenicity study (page 25, vol. 1):

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The study will be conducted at [redacted] according to GLP. Male and female CB6F1Jic-TgrasH2@Tac hemizygous transgenic mice will be used in the study. The transgenic strain will be obtained by cross-breeding C57BL/6 mice with BALB/c by knock-in mouse carrying human c-Ha-ras gene. The pharmacokinetic study will be carried out in male a g at the beginning of the treatment.

The purpose of the study is to evaluate tumorigenic potential of milnacipran in Tg.rasH2 mice given orally by gavage for 26 weeks. Toxicokinetic study will be conducted in CByB6F1 mice (non-transgenic littermate) strain. The study design is shown in the table below.

Dose group	Dose (mg/kg/day)	Tg.rasH2 mice (n for main study)		CByB6F1 mice (n for TK study)	
		Male	Female	Male	Female
1. Vehicle control	0	25	25	10	10
2. Positive control (urethane)	1000*	25	25	-	-
3. Low Dose	25	25	25	58	58
4. Mid Dose	75	25	25	58	58
5. High Dose	150	25	25	58	58

*three ip injections will be given on days 1, 3 and 5.

Doses will be administered daily by oral gavage at 10 mL/kg volume for 26 weeks. Drug substance will be dissolved in sterile water for injection. The control animals will receive sterile water for injection. Animals in the TK group will be treated similarly.

Blood samples for toxicokinetics will be taken from 4 animals/sex/group at predose, and 1, 2, 4, 10 and 24 hours post dose on days 1 and week 16. Each animal will be bled once or twice for the collection of blood samples and will be sacrificed at the end of blood collection.

Doses of 25, 75 and 150 mg/kg/day for milnacipran were equivalent to 22, 66 and 132 mg/kg of free base. The doses were selected on the basis of a 28-day dose finding study in CByB6F1 (non-transgenic littermates) at 75, 150 and 250 mg/kg. Mortality was observed at 250 mg/kg in male and female mice. Centrilobular hypertrophy of the liver was observed in all treated animals.

Body weights will be recorded once weekly up to week 13 and biweekly thereafter. Food consumption of main study animals will be measured weekly. Clinical signs and mortality will be observed twice a day.

Dead animals will be refrigerated before necropsy and will be necropsied within 8 hours. Moribund animals will be sacrificed by carbon dioxide asphyxiation and necropsy will be performed. Surviving animals will be sacrificed at the end of the treatment by carbon dioxide asphyxiation on days 183-184. Gross changes will be recorded. Organ weights for following organs will be recorded.

Brain, heart, liver, kidneys, testes and ovaries.

The sponsor provided a standard list of tissues that will be fixed in 10% neutral buffered formalin. The sponsor stated that tissues from the control and high dose groups will be examined histologically. Protocol specified tissues from early deaths and moribund animals will also be examined for histological changes. Lungs, spleen and any other gross changes from animals treated with urethane will also be examined histologically.

If macroscopic changes are observed in any tissue, histological examination will be conducted for the tissue from all animals.

If an increase incidence of rare or common tumor is seen in the high dose, the histological examination of the tissue will be conducted at lower doses.

If excessive loss of body weight or survival is observed in the study, histological examination of protocol specified tissues will be conducted at lower doses.

Histological examination of tissues will be conducted following standard procedures. Tissues will be stained with hematoxylin and eosin.

The list of tissues is shown below.

Adrenal glands, aorta, bone (femur and sternum), bone marrow (femur and sternum), brain, epididymides, esophagus, eyes, gall bladder, gross lesions, Harderian glands, heart, kidneys, intestine, liver, lungs, lymph nodes, mammary gland, skin, nasal cavity, ovaries, pancreases, parathyroid glands, pituitary gland, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urinary bladder, uterus and vagina.

2. 28-Day repeat dose oral toxicity and toxicokinetic study in CByB6F1 hybrid mice with a preliminary range finding study: Study # MLNTX12000 Page 43, vol. 1

The study was conducted by [redacted] according to GLP. The study was initiated on Sept 23, 2004. A 5-day dose range finding study was conducted at 50, 100, 200, 300 and 400 mg/kg by oral gavage. Mortality was observed at 300 and 400 mg/kg in male and female rats. The major clinical sign was lethargy. Based on the data, 75, 150 and 250 mg/kg were selected for the 28-day toxicity study. The study design is shown in the table below.

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Group	Dose, mg/kg/day	Main Study	TK study

		Male	Female	Male	Female
1. Vehicle control	0	10	10	10	10
2. Low Dose	75	10	10	58	58
3. Mid dose	150	10	10	58	58
4. High Dose	250	10	10	58	58

The drug substance was dissolved in sterile water for injection. Main study animals were treated for 28 days daily by oral gavage at 10 mL/kg volume. Vehicle control groups were treated with sterile water for injection. Some of the animals in the TK group were treated with one dose for the determination of single dose pharmacokinetics. The rest of the animals in the TK group were treated up to 25 days daily by oral gavage.

Body weights of animals were recorded on days 1, 8, 15, 22, 29 and 30. The body weight of TK study animals were recorded on days 1, 8, 15 and 22. Animals were observed twice daily for moribund conditions and mortality. Clinical signs were observed once daily within 12 hours post dose. Blood samples were collected from main study animals on day 30 following overnight fasting. Serum samples from 5 mice/sex/group were collected for clinical chemistry and whole blood was collected from 5 mice/sex/group for hematology. Animals from the main study group were sacrificed on day 30 by CO_2 asphyxiation for necropsy. Gross changes and organ weights were recorded. The sponsor stated that weights of brain, heart, liver, kidney, testes and ovaries were recorded at necropsy. All gross lesions and protocol specified tissues from the main study high dose groups and vehicle groups were processed for histological examinations following hematoxylin and eosin staining. The sponsor stated that liver from all animals were examined histologically.

Four animals/sex/dose/time point from the TK group were bled at pre dose, 0.5, 1,2,4,10 and 24 hours after the first dose or 25 days of the treatment for pharmacokinetic measurements. Five mice/sex from the vehicle control group of the TK satellite animals were bled once at 0.5 hour after the vehicle treatment on day 1 and day 25. Blood samples were collected from the retro-orbital sinus following CO_2/O_2 exposure to immobilize the animal. Plasma levels of the drug were determined.

The TK study was repeated due to mortality at 250 mg/kg. The study design for the repeat TK study is shown below.

Group	Dose (mg/kg/day)	Number of animals	
		Male	Female
2	75	58	58
3	150	58	58
4	200	58	58

The experimental procedure for the repeat TK study was similar to the initial TK study.

Results:

A total of 3 male and 5 female mice were found dead at 250 mg/kg in the main study animals. A total of 11 male and 17 female mice were found dead at 250 mg/kg in TK group. One male at 200 mg/kg and one female at 150 mg/kg in the repeat TK study were found dead on days 2 and 5, respectively. The mortality in the repeat TK study was not due to gavage error. Lethargy and prostration were clinical signs at 250 mg/kg.

Mortality

Group	Dose, mg/kg/day	Main Study		TK study	
		Male	Female	Male	Female
1. Vehicle control	0	10	10	10	10

2. Low Dose	75	10	10	58	58
3. Mid dose	150	10	10	58	1/58
4. High Dose	250	10	10	11/58	17/58

The mean body weight (g) on day 1 and day 30 did not show substantial change due to the treatment as shown in the table below.

Mean Body Weights (g)

Group	Day 1, male	Day 1, female	Day 30, male	Day 30, female
1	26.07	20.40	24.82	19.62
2	26.78	20.28	24.87	19.63
3	25.79	20.85	25.14	20.39
4	26.22	20.55	24.90	21.16

The total food consumption (g) in female mice was not affected by the treatment. However, male mice showed statistically significant reduction in the total food consumption as shown in the table below.

Food Consumption (g)

Group	Day 1-29	Day 1-29
1	107.3	110.8
2	99.8	11.7
3	99.7	102.3
4	95.4*	103.3

* P<0.05 compared to group 1?

Clinical pathology data showed a reduction in the percent reticulocyte in male mice. Data were 1.52, 0.7, 0.68, 0.75% for groups 1, 2, 3 and 4, respectively. The historical control data were 2.4-6%. No other treatment related changes were observed in male and female mice.

Organ weight data showed an increase in the relative weight of liver in male and female mice. Average data are shown in the table below.

Organ Weights

	Gr 1, M	Gr 2, M	Gr 3, M	Gr 4, M	Gr 1, F	Gr 2, F	Gr 3, F	Gr 4, F
Liver wt (g)	0.96	1.01	1.01	1.02	0.77	0.79	0.85	0.88
% BW	3.91	4.08*	4.03	4.12*	3.97	4.05	4.21*	4.21*

*P < 0.05

No treatment related macroscopic changes were noted in either male and female mice. However, histological data showed minimal centrilobular hypertrophy in the liver. Histology data for 10 animals/treatment group are provided and number of incidences per group is shown below.

Histology

Lesion	Gr 1, M	Gr 2, M	Gr 3, M	Gr 4, M	Gr 1, F	Gr 2, F	Gr 3, F	Gr 4, F
Liver,Centrilobular hypertrophy, Focal, minimal	0	10	10	7	0	5	8	5

Pharmacokinetic data:

The day-1 pharmacokinetic data from the 5-day dose range-finding experiment is shown in the table below.

Dose, mg/kg	C_{max} (ng/ml)		AUC_{0-24} (ng.hr/ml)		T_{max} (hr)	
	Male	Female	Male	Female	Male	Female
75	94-9	8885	29644	20986	0.5	0.5
150	12845	14021	57220	51699	0.5	0.5
250	20832	19921	103196	102515	0.5	0.5

The PK data for the repeat experiment is shown in the table below.

Day	Dose, mg/kg	C_{max} (ng/ml)		AUC_{0-24} (ng.hr/ml)		T_{max} (hr)	
		Male	Female	Male	Female	Male	Female
1	75	10310	8189	23129	20962	0.5	0.5
1	150	13485	14747	54474	57887	1.0	0.5
1	200	18436	15993	76746	74721	0.5	0.5
25	75	7449	6607	24805	21079	0.5	1.0
25	150	9867	9109	55956	52173	2.0	1.0
25	200	10354	12913	67852	80603	1.0	1.0

The above data suggest that the exposure increased dose dependently and was not altered significantly between the first and last doses. There were no evident gender differences in the kinetics.

Evaluation:

The sponsor's proposed doses a 6-month carcinogenicity study in TgrasH2 transgenic mice are 25, 75 and 150 mg/kg based on the dose range-finding study in wild type non-transgenic litter mates. Based on the TK data, a dose of 25 mg/kg should be similar to the human exposure at 100 mg/day. The reviewer recommends that the high dose be 200 mg/kg in order to optimize the exposure. Centrilobular hypertrophy in the liver was also observed in all treated mice. Perhaps, xenobiotics of the drug in hepatocytes were responsible for the change. The high dose proposed by the sponsor is about 8 times higher than the maximum recommended human dose.

Although the dose range-finding study was not conducted in the transgenic mice, it is expected that the pharmacodynamic effect of the drug in the wild type nontransgenic litter mates will mimic that of the transgenic species. There is no need to allot a satellite group for the TK study in vehicle treated animals.

Recommendation to the sponsor:

The reviewer recommends 25, 100 and 200 mg/kg for the carcinogenicity study in transgenic mice. The sponsor may chose not to add an extra group of vehicle treated animals for the TK study.

C.C:

IND 63,736 Div File/DAARP
Jane Dean/PM/DAARP
Dan Mellon/Team Leader/DAARP
Asoke Mukherjee/Pharmacologist/DAARP

IND63736mouseCAprotocol09212005.doc

ECAC meeting minutes for protocol review

Executive CAC
Date of Meeting: October 18, 2005
Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Jasti Choudary, B.V.Sc., Ph.D., DGP, Alternate Member
Daniel Mellon, Ph.D., Team Leader , DAARP
Asoke Mukherjee Ph.D. DAARP, Presenting Reviewer

Author of Draft: Asoke Mukherjee

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the carcinogen bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 63,736
Drug Name: Milnacipran Hydrochloride
Sponsor: Forest Laboratories

Background:

Milnacipran is a tricyclic antidepressant currently under development for the treatment of fibromyalgia. It is approved in several countries outside the United States at 50 mg BID doses for the treatment of depression. The sponsor conducted a 2-year mouse carcinogenicity study in [redacted] CD-1 mice dosed up to 100 mg/kg per day. The ECAC concluded that the high dose did not reach maximum tolerated dose. The ECAC further recommended that the sponsor either perform a mouse carcinogenicity study in the TgRasH2 transgenic mouse model or provide data to demonstrate that the exposure in the 2-year study was 25 times higher than the human exposure. b(4)

Mouse Carcinogenicity Study Protocol and Dose Selection

The sponsor submitted a protocol for 6-month carcinogenicity study in TgRasH2 mice. The doses proposed were 25, 75 and 150 mg/kg/day by oral gavage. Urethane would be used as a positive control at 1000 mg/kg by IP injection on days 1, 3 and 5. The high dose was selected on the basis of a 28-day toxicity study in wild type mice (CByB6F1 strain) at 75, 150 and 250 mg/kg. Due to mortality observed in 3 male and 5 female mice at 250 mg/kg, the sponsor considered 150 mg/kg as the highest dose for the carcinogenicity study.

Executive CAC Recommendations and Conclusions:

The ECAC recommended that the high dose be about half of the lethal dose identified in the 28-day toxicology study, which is considered an MTD for the 6-mo transgenic study. Accordingly, doses recommended were 25, 50 and 125 mg/kg. Urethan is an acceptable positive control provided it induces tumors at a rate consistent with the published literature and the historical values for the performing laboratory. The committee also recommended that sponsor conduct histopathological examinations of all tissues treated with milnacipran and urethane.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:
Division File, IND 63,736/DAARP
Dan Mellon/Team Leader/DAARP
A. Mukherjee/Reviewer/ DAARP
Jane Dean/PM/DAARP
ASeifried/ OND IO

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

/s/

Elizabeth Bolan
8/27/2008 02:54:11 PM
PHARMACOLOGIST

R. Daniel Mellon
8/27/2008 03:03:15 PM
PHARMACOLOGIST

1/31/08

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: 22-256 **Applicant: Cypress (Forest)** **Stamp Date: 18-Dec-2007**

Drug Name: Milnacipran **NDA/BLA Type: 505(b)(1)**

On **initial** overview of the NDA/BLA application for RTF: **Filable**

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	--	--	Not applicable. Sponsor did not have a preNDA meeting with PharmTox
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed	x		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

	Content Parameter	Yes	No	Comment
	in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?			
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		
11	Has the applicant addressed any abuse potential issues in the submission?	--	--	This is a CSS issue.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	--	--	Not applicable.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes___

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None at this time.

Asoke Mukherjee and Beth Bolan
Reviewing Pharmacologist Date

Dan Mellon
Team Leader/Supervisor Date

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

/s/

R. Daniel Mellon
1/31/2008 12:06:28 PM
PHARMACOLOGIST