

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-256

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO

NDA: 22-256

Submission date: December 18, 2007

Drug: milnacipran

Sponsor: Cypress Bioscience, Inc.

Indication: fibromyalgia

Reviewing Division: Division of Anesthesia, Analgesia and Rheumatology Products

Introductory Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted to be adequate to support approval of milnacipran for the indication described above.

The reviewer and supervisor had recommendations for labeling that differed from the labeling proposed by the sponsor.

The reviewer also had the following recommendation:

It is recommended that the Ames assay be repeated using a clinical batch of milnacipran. This can be completed as a Phase IV requirement, if approved.

The supervisor agreed with this recommendation.

Reproductive and developmental toxicity:

The sponsor, reviewer and supervisor recommend pregnancy category C for milnacipran.

Fertility:

Fertility was assessed in two studies in rats. The sponsor concluded that there was no effect on fertility up to the high dose of 80 mg/kg. The reviewer and supervisor noted that although there were no statistically significant effects, there was an apparent dose-related reduction in fertility index in the rat. These reductions in fertility index occurred at doses that were similar to the human dose based on a mg/m² comparison. The reviewer and supervisor have recommended wording to describe this effect in the fertility section of the labeling.

Embryofetal and peri/postnatal development:

Effects of milnacipran on embryofetal development have been assessed in mice, rats and rabbits. The sponsor described decreased rat pup body weight and viability at postpartum day 4 in the labeling. The reviewer and supervisor noted, in addition, that intrauterine embryoletality occurred in this study at all doses including 5 mg/kg which is approximately 0.25 times the human dose based on a mg/m² comparison. The supervisor and reviewer recommended adding this information to labeling. The reviewer and supervisor also noted that there was an increased incidence of extra single ribs in the rabbit at doses of 15 and 60 mg/kg. They recommend that this information also be included in labeling.

General Toxicity:**Hepatotoxicity:**

The reviewer and supervisor noted that some hepatotoxicity was apparent in the rat at doses that were similar to the human dose based on a mg/m² comparison. These effects were apparent upon histopathological examination of the rat livers. The reviewer and supervisor recommended that a description of the findings be included in the animal toxicology and pharmacology section of labeling.

Ocular toxicity:

The reviewer and supervisor noted that keratitis was observed in the two-year rat carcinogenicity study. They recommend that this be described in the animal toxicology and pharmacology section of labeling.

Genotoxicity:

The sponsor submitted four genetic toxicology studies: Ames assay, mouse lymphoma cell thymidine kinase mutation assay, human lymphocyte chromosomal aberration assay and an in vivo mouse micronucleus assay. All of these assays were negative for genotoxicity. The reviewer noted that the sponsor did not provide a certificate of analysis for the drug used in the Ames assay. Therefore, the reviewer recommended that the Ames assay be repeated as a post marketing study. The supervisor agreed with this recommendation.

Carcinogenicity:

The sponsor conducted three carcinogenicity studies: a two year rat study, a two year mouse study and a 6 month transgenic rasH2 mouse study. The sponsor chose to conduct the 6 month transgenic rasH2 mouse study after the executive carcinogenicity assessment committee concluded that the adequacy of the 2 year mouse study could not be confirmed because a maximum tolerated dose was not achieved nor was adequate pharmacokinetic information available to determine whether 25 fold the human AUC was achieved. The executive carcinogenicity assessment committee concluded that the rat and rasH2 mouse studies were acceptable. The executive carcinogenicity assessment committee concluded that no drug-related tumors were noted in the rasH2 mouse study and that there was a statistically significant increase in thyroid c-cell adenomas and thyroid c-cell adenomas and carcinomas combined in male rats. The reviewer did not recommend that these findings be included in labeling. The supervisor cited the executive carcinogenicity assessment committee findings and recommended that the findings be described in the labeling.

Conclusions and Recommendations:**Reproductive and developmental toxicity:**

I concur that milnacipran could be labeled with pregnancy category C.

Fertility:

While not statistically significant, an effect on fertility was apparent in rats. I agree with the wording as proposed in the supervisory memorandum for the fertility section of labeling.

Embryofetal and peri/postnatal development:

Embryo lethality appears elevated in rats and an increase in extra single ribs occurred in rabbits. In addition, pup weight and viability were reduced at postpartum day 4 in rats. The labeling as proposed in the supervisory memorandum is acceptable.

Genotoxicity:

I do not recommend that the Ames assay be repeated as a post marketing study. The sponsor has provided adequate genotoxicity data and has already completed carcinogenicity evaluation in two species. Additional information from an Ames assay would not be particularly useful at this time and would not result in different recommendations for clinical use considering the other available information.

Hepatotoxicity:

Elevations in hepatic enzymes occurred in only a few patients treated with milnacipran in the clinical studies. These elevations were all considered mild (1-3 times ULN). It appears that the risk of hepatotoxicity in humans from milnacipran has already been described as rare. However, the supervisor notes that the findings in the rat are not easily monitored and occurred at doses that are similar to the clinical dose based on a mg/m^2 comparison. Inclusion of these findings in labeling is acceptable.

Ocular toxicity:

The relevance of keratitis in the 2-year rat study to the clinical use of milnacipran is unclear. It appears that keratitis has not been observed in the clinical studies of milnacipran or in other animal studies including a one year study in cynomolgus monkeys. Keratitis should be readily detected in clinical use, if it occurred. The supervisor has noted that keratitis may be a biologically plausible effect of milnacipran based on the pharmacology of the compound. Inclusion of the keratitis finding in the labeling may be, therefore, acceptable although I believe the utility of such information is limited.

Carcinogenicity:

I agree that the findings of thyroid c-cell adenoma and carcinoma in male rats should be included in the carcinogenicity section of labeling. The wording as proposed in the supervisory memorandum is acceptable.

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

/s/

Paul Brown
10/6/2008 02:11:05 PM
PHARMACOLOGIST

9/24/08



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

**SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION**

NDA NUMBER: 22-256
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: December 18, 2007
PRODUCT: Milnacipran hydrochloride
INTENDED CLINICAL POPULATION: Adult patients with fibromyalgia
syndrome
SPONSOR: Cypress Bioscience, Inc.
REVIEW DIVISION: Division of Anesthesia, Analgesia, and
Rheumatology Products (HFD-170)
PHARM/TOX REVIEWERS: Asoke Mukherjee, Ph.D.
Elizabeth A. Bolan, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob A. Rappaport, M.D.
PROJECT MANAGER: Diana Walker, Ph.D.

Date of review submission to Division File System (DFS): September 24, 2008

Executive Summary

I. Recommendations

A. Recommendation on approvability

The nonclinical pharmacology and toxicology data submitted in support of NDA 22-256 were reviewed by Drs. Asoke Mukherjee and Elizabeth Bolan. Dr. Bolan reviewed the general and safety pharmacology studies, the acute toxicology studies, and the mouse Tg.rasH2 carcinogenicity study. Dr. Mukherjee reviewed the repeat-dose toxicology studies, the genetic toxicology studies, the reproductive and developmental toxicology studies, and the mouse and rat carcinogenicity bioassays. Both reviewers recommended approval of the NDA based on the nonclinical data they reviewed. I concur with this recommendation.

B. Recommendation for nonclinical studies

Dr. Mukherjee has recommended that the Sponsor repeat the Ames bacterial reverse mutation assay, as they are not able to provide adequate documentation regarding the certificate of analysis for the drug substance batch used in the submitted study results. He recommends that the study may be repeated post approval, should the NDA be approved this cycle. I concur with the recommendation that this study should be repeated and this may be conducted as a post marketing requirement.

Although pediatric studies will be deferred at this time, a juvenile animal study should be considered prior to pediatric clinical trials if there are inadequate clinical data from the overseas use of this product to support the safety of the pediatric studies proposed.

C. Recommendations on labeling

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
8. USE IN SPECIFIC POPULATIONS 8.1 Pregnancy	8. USE IN SPECIFIC POPULATIONS 8.1 Pregnancy	

b(4)

4 Page(s) Withheld

 Trade Secret / Confidential (b4)

X Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment

b(4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

General Toxicology. The Sponsor submitted three pivotal chronic toxicology studies to support the NDA, a 6 and 12-month study in the cynomolgus monkey and a 12-month study in the rat. The Sponsor does not have toxicokinetic data from the chronic studies; therefore, exposure margins are based on body surface area comparisons.

The 1-year rat toxicology study demonstrated evidence of hepatocyte vacuolization at a dose of 10 mg/kg in males but not females. The vacuolization was not clearly associated with changes in hepatic enzymes nor was there clear evidence for treatment-related cellular necrosis. The incidence of liver vacuolization in male rats is presented below.

Liver Vacuolization Findings in Males: 1 Year Repeat Dose Toxicity

Organ Findings	0 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Liver					
vacuolation slight	1/14	0/15	0/14	1/15	7/15
vacuolation mild				1/15	3/15
vacuolation total	1/14	0/15	0/14	2/15	10/15
Exposure Margin		0.04	0.1	0.5	1.5

Exposure margins based on mg/m^2 as toxicokinetic data were not obtained for this study. Human dose of 200 mg/60 kg/day = $123 \text{ mg}/\text{m}^2/\text{day}$.

The NOAEL for this study was 3 mg/kg in male rats and 10 mg/kg in female rats, delineated by the liver effect in males and body weight gain reduction in females. Based on a body surface area comparison, these findings occurred at doses equivalent to the proposed clinical doses.

Interestingly, there were additional histopathological findings in liver and other tissues findings in the 2-year rat carcinogenicity study, summarized in the table below:

Incidence of Histological Findings in Rats: 2 Year Carcinogenicity

Organ Findings	Males				Females			
	0 mg/kg	5 mg/kg	15 mg/kg	50 mg/kg	0 mg/kg	5 mg/kg	15 mg/kg	50 mg/kg
Eye Keratitis	2/100 (2%)	0/30	2/35 (6%)	4/48 (8%)	1/105 (1%)	0/29	2/28 (7%)	5/53 (9%)
Liver eosinophilic focus	13/104 (13%)	8/53 (15%)	16/55 (29%)	16/50 (32%)	4/105 (4%)	5/55 (9%)	9/55 (16%)	10/55 (18%)
centrilobular vacuolation	0/104	0/53	3/55 (5%)	13/50 (26%)	0/105	1/55	1/54	0/55
Urinary Bladder Cystitis	2/103 (2%)	0/26 (0%)	3/30 (10%)	4/54 (7%)	0/104	1/22	0/23	0/55
Exposure Margin		0.24	0.7	2.4		0.24	0.7	2.4

Exposure margins based on mg/m² as toxicokinetic data were not obtained for this study. Human dose of 200 mg/60 kg/day = 123 mg/m²/day.

It is not clear what the vacuoles noted in the liver contain. According to the pathology report, the Sponsor was able to determine that the vacuoles do not contain fat; therefore these changes do not suggest steatosis. The Sponsor's NOAEL for liver findings was 5 mg/kg; however, one can argue that there is an increased incidence of eosinophilic focus in even the 5 mg/kg treatment group compared to control incidence in this study. Hepatocyte vacuolation in rats, particularly males, have been reported to be postmortem in a time-dependent manner via plasma influx into the cytoplasm or anoxia (Li et al., 2003). The centrilobular region of liver is particularly sensitive to anoxia. The finding of eosinophilic foci in the liver samples is one of the most prevalent forms of hepatic inflammation noted in the preclinical toxicity studies. In general these findings may be unrelated to treatment or are generally regarded as nonspecific changes subjected to potent pharmaceutical agents given at high doses (Greaves, 2007).

Via a weight-of-evidence evaluation, the findings of hepatocyte vacuolization in the male rats and the eosinophilic foci in the male and female rats, although treatment-related, do not raise significant safety concerns for the following reasons: there is extensive clinical experience with the drug without over hepatotoxicity, the finding of vacuolation in the male rat was not evident in the female rat or the monkey, there is a lack of evidence of necrosis in the surrounding tissue nor were they were correlated with liver enzyme changes. The hepatic vacuolation finding was discussed with the clinical review team and it was concluded that the potential for hepatotoxicity was adequately characterized via the clinical studies and that labeling will include specific language regarding the clinical findings of increased hepatic enzymes with reference to information in the animal toxicology section of the labeling.

Dr. Mukherjee also noted an increased incidence of keratitis in the 2-year rat study that appeared to be dose-related and occurring at clinically relevant doses based on body surface area. Similar findings were not noted in the 6-month rat study, the 1-year rat study, the 6-month primate study, or the 1 year primate study.

In a 6-month repeat dose toxicology study in the monkey, animals were dosed with 0, 5, 15 and 60/40 mg/kg/day milnacipran. In this study, the high dose was reduced to 40 mg/kg due to severe vomiting and mortality which appeared to be due to aspiration of the vomitus, therefore the study provided clear evidence of frank toxicity. The Sponsor reports a NOAEL value of 15 mg/kg. Toxicokinetic data were not obtained in this study. Based on body surface area comparison, the NOAEL dose of 15 mg/kg/day provides an exposure margin of 1.5 times the proposed maximum human daily dose.

In the 1-year repeat dose toxicology study in the monkey, animals were dosed with 0, 2.5, 7.9 or 25 mg/kg/day milnacipran. The Sponsor reports a NOAEL of 25 mg/kg/day. Dr. Mukherjee concurs, although notes clinical signs of mydriasis and vomiting at the high dose. Based on body surface area comparison, the dose of 25 mg/kg/day provides an exposure margin of 2.5 the proposed maximum human daily dose.

Reproduction and Developmental Toxicology. The Sponsor conducted the standard battery of tests and proposed a Pregnancy Category C. Dr. Mukherjee also concluded that milnacipran should be considered a Pregnancy Category C drug; however, he did not agree with either the Sponsor's NOAEL values or proposed labeling.

Segment 1 (Fertility and Early Embryonic Development). The Sponsor conducted a dedicated Segment 1 study in the rat model and concluded that there were no treatment related findings at doses of up to 80 mg/kg. Although not statistically significant, the number of fertile males and pregnant females appears to be reduced at the high dose.

Key Fertility Findings in the Dedicated Rat Segment 1 Study:

Parameter	0 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
# fertile males	24/24 (0%)	23/24 (96%)	23/24 (96%)	19/24 (79%)
AUC _{0-∞} (ng•h/mL) males		814	4949	29579
Exposure Margin		0.3	1.9	11.3
# Pregnant females	24/24 (100%)	23/24 (96%)	24/24 (100%)	21/24 (88%)
AUC _{0-∞} (ng•h/mL) females		1100	6051	36661
Exposure Margin		0.4	2.3	14

Exposure margin based on multiple dose PK study in humans at 100 mg/kg BID (study M146): AUC_{0-∞} = 2613 (ng•h/mL). Data from study T092 and Sponsor's toxicology tabulated summary, page 69.

In addition, fertility and early embryonic development were also assessed in the rat via a multigenerational study. Dr. Mukherjee recommends that the labeling state that the low dose tested decreased fertility in the rat model. This recommendation is based on

the findings in the multigenerational study of an apparent dose-related increase in the number of females with dead fetuses in utero. Likewise, although there was also an apparent dose-related decrease in the fertility index (number of pregnant animals divided by the number of paired animals times 100), neither of these findings were statistically significant.

Key Findings from Fertility and Early Embryonic Development in the Rat:

Parameter	0 mg/kg	5 mg/kg	15 mg/kg	60 mg/kg
# females with 100% intra-uterine deaths	0/15 (0%)	0/14 (0%)	0/15 (0%)	1/16 (6%)
# females with dead fetuses in utero at necropsy	0/15 (0%)	2/14 (14%)	3/15 (20%)	8/16 (50%)
# females with live pups on day 21 pp	13/15 (87%)	14/14 (100%)	11/15 (73%)	6/16 (37.5%)
Fertility Index (%)	93.3	90.0	86.2	81.5
Exposure Margin		0.2	0.7	3

Exposure margins based on mg/m² as toxicokinetic data were not obtained for this study. Human dose of 200 mg/60 kg/day = 123 mg/m²/day. Data from multigenerational study T030.

Although not statistically significant, I concur with Dr. Mukherjee that there appears to be a treatment-related embryotoxic effect and a suggestion that milnacipran may reduce fertility based on the fertility index. The clinical significance of these findings; however, is not entirely clear. As the findings occur at clinically relevant exposures, there finding should be included in the labeling. The finding of embryoletality; however, should be included in the pregnancy section of the label.

Segment 2 (Embryo-Fetal Development). Embryofetal development was assessed in the mouse, rat, and rabbit.

In the pivotal rabbit Segment 2 study, rabbits were treated with 0, 5, 15, and 60 mg/kg milnacipran during the period of organogenesis. The Sponsor concluded that the NOAEL for both maternal toxicity and embryo-fetal development was the high dose of 60 mg/kg/day. Dr. Mukherjee notes that there was an increase in the incidence of single extra ribs at the high dose and several dams in the mid dose that have more than one pup with an extra rib. He concluded that the NOAEL for embryo-fetal development was the low dose of 5 mg/kg. Although the pivotal study did not demonstrate maternal toxicity, the assessment in the rabbit was deemed adequate as the dose-range finding study demonstrated maternal deaths at 100 mg/kg/day.

In the pivotal mouse Segment 2 study, mice were treated with 0, 5, 25, or 125 mg/kg/day during the period of organogenesis. The Sponsor concluded that the NOAEL for embryotoxicity and teratogenicity was the high dose of 125 mg/kg and the NOAEL for maternal toxicity was 25 mg/kg based on a 17% decrease in body weight gain from gestation day 6-10 in the high dose group. Dr. Mukherjee concluded that based on a slight (8%) statistically significant decrease in mean fetal weight, the NOAEL for fetal effects was 5 mg/kg.

Embryo-fetal development was also evaluated via the multigenerational rat study. Rats were treated with 0, 5, 15, or 60 mg/kg before and throughout the period of organogenesis. The Sponsor noted that the NOAEL for embryoletality was 5 mg/kg due to the findings of dose-related pup mortality in the 15 and 60 mg/kg/day groups. The Sponsor also included these findings in the proposed drug product labeling. Dr. Mukherjee concluded that the NOAEL was less than 5 mg/kg based primarily on the findings of dead fetuses in utero at necropsy (see table above). I concur that the embryo-fetal lethality noted in this study is treatment related and such information should be included in the drug product labeling.

Segment 3 (Peri-natal and Post-natal Development). A total of three Segment 3 studies were conducted in the rat. The results of the studies suggest that exposure to milnacipran in utero and during lactation resulted in a decreased viability and weight of rat pups at doses of 5 mg/kg/day and greater (0.2 fold the MRHD of 200 mg/day based on body surface area comparison). The Sponsor reports a NOAEL of 2.5 mg/kg, a dose that is less than the doses proposed clinically based on body surface area comparison. The Sponsor conducted a cross-fostering study to determine if the reduced pup weight and survivability were secondary to decreased maternal care during lactation; however, the results were not conclusive. I concur with Dr. Mukherjee that the reduced pup weight and survival may be due to the peri/post-natal exposure to milnacipran.

Mutagenicity. Milnacipran tested negative in the stand battery of genetic toxicity studies; however, the Sponsor did not have a certificate of analysis of the drug substance tested in the in vitro Ames bacterial reverse mutation assay that provided an impurity profile. Dr. Mukherjee recommended that this study be repeated as a Phase 4 requirement. I concur.

Carcinogenicity. The Sponsor conducted a total of three carcinogenicity studies. Rat and mouse 2-year bioassays were conducted without input from the Division or the ExecCAC. Upon review of these study reports, the rat bioassay was deemed acceptable to the ExecCAC; however, the mouse bioassay was not acceptable as it did not test high enough doses to be deemed an adequate assay. Therefore, the Sponsor conducted a transgenic mouse study, which was deemed acceptable and negative by the Division and the ExecCAC.

During the initial review of the rat carcinogenicity study findings, the ExecCAC concluded that the study was negative, with the possible exception of thyroid tumor findings in male rats. To clarify this possible finding, the Sponsor was requested to conduct a complete examination of the low dose and mid dose histopathology slides of the thyroid for male rats in order to complete the statistical analysis of that tissue. The Sponsor completed this assessment and a statistical evaluation was completed by both the Sponsor and Agency. The results and revised incidences are summarized in the table below:

Incidence of Thyroid C-Cell Tumors: Male Rats

	Control I and II	5 mg/kg	15 mg/kg	50 mg/kg	Dose Response p-values	Pairwise p-values
No. Examined	99	53	47	51		
Adenoma	5 5%	6 11%	6 13%	11* 21.5%	0.0020	0.0026
Carcinoma	1 1%	1 2%	0	0	0.6800	1.0000
Adenoma + Carcinoma	6 6%	6 11%	6 13%	11* 21.5%	0.0035	0.0057

* Statistically significant compared to combined control group via pairwise comparison.

The Sponsor concluded that although statistically significant for both trend analysis and pairwise tests and occurring at an incidence greater than the concurrent controls, the incidence is still within the historical control range for () (data from the 8 most recent carcinogenicity studies at the time of the written study report ranged from 4-24%, see below). The Sponsor also noted that the male control groups in this study showed an unusually low incidence of C-cell tumors for that laboratory. The Sponsor's summary table for historical data on thyroid C-cell adenomas from the conducting laboratory compared to controls is reproduced below:

b(4)

Text table 1, Background tumour incidence data of thyroid C-cell adenomas compared with control and high dose groups in this study

Study#	C-cell adenoma Percentage incidence		Termination date Month/Year
	M	F	
A	24	12	11/88
B	20	11	9/88
C	17	15	6/88
D	16	2	2/88
E	18	16	1/88
F	12	8	6/87
G	17	14	6/87
H	11	10	4/87
314/58 group 1	8	22	3/89
314/58 group 4	22	8	
314/58 group 5	4	20	

not all studies have been subjected to quality assurance audit at the time of writing this report

The ExecCAC did not concur with the Sponsor's interpretation. The ExecCAC concluded that the increase in the incidence of thyroid C-cell adenomas and combined adenomas and carcinomas in male rats treated with 50 mg/kg milnacipran were treatment-related and that this finding should be included in the drug product labeling.

The clinical significance of this finding is not entirely clear; however, the tumors were not found in the female rats, were not clearly associated with an increase in thyroid hyperplasia, and there was no signal for treatment-related findings in the mouse transgenic study. Therefore, although this finding should be included in the drug

labeling, they do not raise sufficient concern that would impact the ability to approve the drug.

B. Pharmacologic activity

Milnacipran is a selective reuptake inhibitor of both norepinephrine and serotonin but does not inhibit the reuptake of dopamine. The mechanism of action of milnacipran for the treatment of fibromyalgia is not known. As serotonin and norepinephrine are known to play a role in the descending pain pathways and have also been suggested to play a role in the treatment of depression, it is possible that this drug may treat fibromyalgia via multiple mechanisms of action in the CNS.

C. Nonclinical safety issues relevant to clinical use

Dr. Mukherjee has recommended that the hepatic findings in the rat studies should be considered for inclusion in the package insert and that patients treated with milnacipran for longer than one year be monitored for liver enzyme abnormalities and dry eye conditions based on findings of keratitis in the rat carcinogenicity studies.

According to Greaves, keratitis has been noted as a spontaneous event in the laboratory rat; however, in the 2-year rat study, the keratitis appears to be treatment and dose-related in terms of the incidence. The cause of the keratitis has not been determined to date. Dr. Bolan's review notes that the Sponsor's tissue distribution studies suggested that there was uptake of milnacipran and/or its metabolites into the eye. The keratitis could be due to deposition of drug in lacrimal glands or CNS mediated autonomic effects on lacrimal gland function. Increased ocular sympathetic tone is also consistent with the clinical finding of mydriasis described in the proposed package insert. Given the extensive clinical experience with this drug overseas, the known anti-cholinergic-like side effects reported in the literature, and the lack of reported keratitis in the clinical studies, routine specific monitoring for keratitis in the clinical patient population may not be necessary. However, the ocular finding of mydriasis in the drug product labeling may include a cross reference to the animal toxicology section of the label which should include the finding reported in the rat.

As liver enzyme elevations were noted in the clinical studies, following discussion with the medical review team, standard medical care should be adequate to detect potential liver effects of this drug product. Cross reference to the animal toxicology section of the label will provide additional information to the physician, should hepatic changes be noted during clinical use of the product.

The toxicities noted in the nonclinical reproductive and developmental studies of milnacipran occur at doses that are below the proposed clinical maximum recommended daily dose of 200 mg/day based on a body surface area comparison. As the findings of embryofetal lethality and reduced pup weights and viability appear to be treatment related, the labeling for milnacipran should clearly discourage use of this drug during pregnancy and in breast feeding women. Given the adverse effects noted,

b(4)

Reference List

Greaves P (2007) Histopathology of Preclinical Toxicity Studies. Amsterdam, The Netherlands: Elsevier Science.

Li X, Elwell MR, Ryan AM, Ochoa R (2003) Morphogenesis of postmortem hepatocyte vacuolation and liver weight increases in Sprague-Dawley rats. Toxicol Pathol 31:682-688.

**APPEARS THIS WAY
ON ORIGINAL**

**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
9/24/2008 05:35:59 PM
PHARMACOLOGIST

9/17/08



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: 12/18/2007
PRODUCT: Milnacipran hydrochloride
INTENDED CLINICAL POPULATION: Fibromyalgia Syndrome
SPONSOR: Cypress Bioscience Inc.
DOCUMENTS REVIEWED: M4, Repeat-dose toxicology, Genetic
Toxicity, Reproductive and
Developmental Toxicity and Rat
Carcinogenicity
REVIEW DIVISION: DAARP (HFD-170)
PHARM/TOX REVIEWER: Asoke Mukherjee, Ph.D.
PHARM/TOX SUPERVISOR: Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob Rappaport, M.D.
PROJECT MANAGER: Diana Walker, Ph.D.

Date of review submission to Division File System (DFS): Sept 17, 2008

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	7
2.6.1 INTRODUCTION AND DRUG HISTORY.....	7
2.6.2 PHARMACOLOGY.....	10
2.6.2.1 Brief summary	10
2.6.2.2 Primary pharmacodynamics	10
2.6.2.3 Secondary pharmacodynamics	10
2.6.2.4 Safety pharmacology	10
2.6.2.5 Pharmacodynamic drug interactions.....	10
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	10
2.6.4 PHARMACOKINETICS/TOXICOKINETICS	11
2.6.4.1 Brief summary	11
2.6.4.2 Methods of Analysis	11
2.6.4.3 Absorption	11
2.6.4.4 Distribution.....	11
2.6.4.5 Metabolism	11
2.6.4.6 Excretion.....	11
2.6.4.7 Pharmacokinetic drug interactions.....	11
2.6.4.8 Other Pharmacokinetic Studies.....	11
2.6.4.9 Discussion and Conclusions	11
2.6.4.10 Tables and figures to include comparative TK summary	11
2.6.5 PHARMACOKINETICS TABULATED SUMMARY	11
2.6.6 TOXICOLOGY.....	11
2.6.6.1 Overall toxicology summary	11
2.6.6.2 Single-dose toxicity	13
2.6.6.3 Repeat-dose toxicity	13
2.6.6.4 Genetic toxicology	35
2.6.6.5 Carcinogenicity.....	51
2.6.6.6 Reproductive and developmental toxicology.....	68
2.6.6.7 Local tolerance	125
2.6.6.8 Special toxicology studies	125
2.6.6.9 Discussion and Conclusions	125
2.6.6.10 Tables and Figures.....	125
2.6.7 TOXICOLOGY TABULATED SUMMARY	125
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	126
APPENDIX/ATTACHMENTS	130

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability:

The NDA may be approved at a proposed dose of 200 mg per day based on the review of non-clinical data for repeat dose toxicities, reproductive safety, and carcinogenic risk assessment studies reviewed in this review. The recommendation for the package insert is given in the following section.

B. Recommendation for nonclinical studies:

It is recommended that the Ames assay be repeated using a clinical batch of milnacipran. This can be completed as a Phase IV requirement, if approved.

C. Recommendations on labeling:

Reviewer's recommendation for mutagenicity:

The reviewer agreed on the proposed label for mutagenicity studies. However, the certificate of analysis for Ames assay was not provided. It is recommended that the Sponsor conduct another assay using the clinical batch as a Phase IV commitment.

Reviewer's recommendation for impairment of fertility:

Administration of milnacipran at 5 mg/kg/day (4 times less than MRHD on an mg/m² basis) decreased fertility in rats.

Labeling recommendation by the reviewer for the rat carcinogenicity study:

Reviewer's Proposed label for Pregnancy:

Pregnancy

b(4)

b(4)

There are no adequate and well controlled studies in pregnant women. Milnacipran should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Non-teratogenic effect:

Same as recommended by the Sponsor

Labor and delivery:

Same as recommended by the Sponsor

Nursing mothers:

Same as recommended by the Sponsor

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

Milnacipran is a monoamine re-uptake inhibitor at neuronal endings. The 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/m²). The Sponsor submitted non-clinical studies for pharmacology, safety pharmacology, toxicity, pharmacokinetic and metabolism, genetic toxicity, reproductive toxicity and carcinogenicity. In this review, repeat dose toxicity, genetic toxicity, reproductive toxicity and rat carcinogenicity studies are reviewed. The pharmacology, safety pharmacology, acute toxicology, and mouse transgenic carcinogenicity studies were reviewed by Dr. Elizabeth Bolan in 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 from 60 to 40 mg/kg during the study due to mortality in monkeys. A NOEL was not established in the study. The NOAEL was 40 mg/kg. The dose limiting adverse event was vomiting at all doses. There were no treatment related histopathological changes 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 were noted as dose limiting toxicities; the NOEL was 7.9 mg/kg and the 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 change was noted. The NOEL was 10 mg/kg in male and 3 mg/kg in female rats. The 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. Other expected side effects are weight loss, vomiting and mydriasis.

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 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 of milnacipran.

Two species carcinogenicity studies were conducted 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/m²). 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.

Although the reviewer concluded that thyroid C-cell adenoma was not treatment related, the CAC-EC recommended that the thyroid C-cell data from the carcinogenicity study and the historical control data need 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 the 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 repeated the 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 conducted by Dr. Bolan under a separate review. The review of CD-1 mouse carcinogenicity data is not included in this review because 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/m²) 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/m²) and 5 mg/kg (30 mg/m²), respectively. However, intrauterine deaths were increased at 5 mg/kg (30 mg/m²) in rats. Milnacipran showed extra single rib in pregnant rabbits at 15 mg/kg (180 mg/m²). 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/m²) and higher doses. The effect of milnacipran on fertility and pregnancy was noted at a maternally non-toxic dose. Therefore, findings in pregnant animals are considered to be treatment related. Pregnancy Category C is recommended for milnacipran.

B. Pharmacologic activity: see review written by Dr. Bolan

C. Nonclinical safety issues relevant to clinical use:

Chronic toxicity studies would reflect vomiting and liver vacuolation (male rats) as primary findings of toxicity in monkeys and rats, respectively at 1 to 3 times human doses. Additionally, carcinogenicity study in rats also showed high incidences of Keratitis that could reflect its potential to cause dry eye like conditions.

However, it should be noted that vacuolation in the liver was noted in male rats at 30 mg/kg without an elevation of transaminase activity in the 12-month study. Increased centrilobular enlargement was also noted at 35 and 120 mg/kg in male and female rats at the end of 6-month treatment. The clinical significance of the finding in liver in the absence of transaminase elevation is unknown. The reviewer recommends that the medical review team to consider if it is necessary to indicate the liver vacuolation as a non-clinical finding in the package insert. Also, it is recommended to consider if patients receiving the drug beyond one year duration need to be monitored for the liver enzyme abnormality and dry eye conditions. No other safety issue is evident from the non-clinical studies except reproductive safety that would be addressed in the recommended package insert.

**APPEARS THIS WAY
ON ORIGINAL**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-256

Review number: One

Sequence number/date/type of submission: 000, Dec 18, 2007, 505(b)(1)

Information to Sponsor: Yes (x) No ()

Sponsor and/or agent: Cypress Bioscience Inc., Authorized US agent: Forest Laboratories, New Jersey

Manufacturer for drug substance: Pierre Fabre Medicament, Plantes & Industrie, 16 rue jean Rostand, 81603 gaillac Cedex, France

Reviewer name: Asoke Mukherjee, Ph.D.

Division name: DAARP

HFD #: 170

Review completion date: July 31, 2008

Drug:

Trade name: Not given

Generic name: Milnacipran hydrochloride

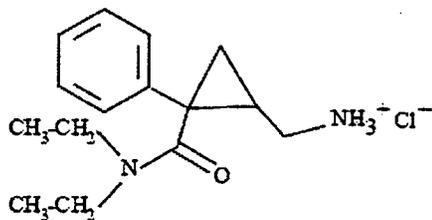
Code name: F2207 and TN-912

Chemical name: (1RS, 2SR)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropanecarboxamide hydrochloride

CAS registry number: 101152-94-7

Molecular formula/molecular weight: C₁₅H₂₃ClN₂O, 282.8

Structure:



Relevant INDs/NDAs/DMFs: IND 63,736, DMF 11501

Drug class: 5-HT and norepinephrine reuptake inhibitor

Intended clinical population: Adult patients with Fibromyalgia

Clinical formulation:

Table 2.3.P.1.1-1. Composition of Milnacipran HCl Tablets

<i>Component</i>	<i>Function</i>	<i>Quality Standard</i>	<i>Theoretical Weight (mg/unit dose)</i>			
			<i>12.5 mg</i>	<i>25 mg</i>	<i>50 mg</i>	<i>100 mg</i>
Milnacipran HCl	Active	Per DMF _a	12.50	25.00	50.00	100.00

b(4)

There are not safety concerns with the proposed drug product formulation. All excipients have been used in previously FDA approved drug products at comparable levels via this route of administration and for the proposed duration.

Route of administration: Oral

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

Studies reviewed within this submission:

A fertility study in rats treated orally with TN-912

F2207 oral gavage fertility study in the rat

F2207, Oral teratology study in the mouse

Preliminary study for a teratology study in the New Zealand white rabbit with F2207

F 2207 Oral (gavage) teratology study in the New Zealand white rabbit

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

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

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

Ames metabolic activation test to assess the potential mutagenic effect of F2207

Clastogenic evaluation of F2207 lot #H7068 in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in purified human lymphocytes

Mutagenicity evaluation of F2207 lot H7068 in the L5178Y TK +/- mouse lymphoma forward mutation assay

Clastogenic evaluation of F2207 lot H7068 in the in vivo mouse micronucleus assay

104-week oral dietary administration carcinogenicity study in the rat

F2207, 26-week oral gastric intubation toxicity study in the cynomolgus monkey

Fifty-two week oral toxicity study of TN-912 in cynomolgus monkeys

A 52-week oral toxicity study of TN-912 in rats

Studies not reviewed within this submission:

┌

b(4)

└

┌

b(4)

All other studies are reviewed by Dr. Bolan under a separate review

└

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary: Reviewed by Dr. Bolan

2.6.2.2 Primary pharmacodynamics: Reviewed by Dr. Bolan

2.6.2.3 Secondary pharmacodynamics: Reviewed by Dr. Bolan

2.6.2.4 Safety pharmacology: Reviewed by Dr. Bolan

2.6.2.5 Pharmacodynamic drug interactions: Reviewed by Dr. Bolan

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Reviewed by Dr. Bolan

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary: Reviewed by Dr. Bolan

2.6.4.2 Methods of Analysis
[see under individual study reviews]

2.6.4.3 Absorption: Reviewed by Dr. Bolan

2.6.4.4 Distribution: Reviewed by Dr. Bolan

2.6.4.5 Metabolism: Reviewed by Dr. Bolan

2.6.4.6 Excretion: Reviewed by Dr. Bolan

2.6.4.7 Pharmacokinetic drug interactions: Reviewed by Dr. Bolan

2.6.4.8 Other Pharmacokinetic Studies: Reviewed by Dr. Bolan

2.6.4.9 Discussion and Conclusions: Reviewed by Dr. Bolan

2.6.4.10 Tables and figures to include comparative TK summary
Reviewed by Dr. Bolan

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Reviewed by Dr. Bolan

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Six-month and twelve month toxicity studies were conducted in cynomolgus monkeys. The six-month study was conducted at oral doses 5, 16 and 60 mg/kg. The high dose was reduced to 40 mg/kg due to mortality due to infections and

other related conditions. Vomiting was noted at all doses. No other treatment related toxicity was noted. A NOEL was not established. The NOAEL was 40 mg/kg.

The fifty two-week oral toxicity study in cynomolgus monkeys was conducted at 2.5, 7.9 and 25 mg/kg. No treatment related mortality was noted. Vomiting and mydriasis was noted at 25 mg/kg. The NOEL was 7.9 mg/kg and the NOAEL was 25 mg/kg.

The fifty-two week oral dose toxicity study was conducted in Wistar rats at 1, 3, 10 and 30 mg/kg. Treatment related change in the body weight gain was noted at 30 mg/kg in male and female rats, and 10 mg/kg in female rats. Liver vacuolation was noted at 30 mg/kg in male rats. The NOEL was 10 mg/kg in male and 3 mg/kg in female rats. The NOAEL was 30 mg/kg.

Genetic toxicology: Milnacipran was negative in the chromosome aberration assay in human peripheral lymphocytes, forward mutation assay in mouse lymphoma cell line L5178Y and mouse micronucleus assay. It was also negative in Ames assay. However, the review of data showed the certificate of analysis was not provided for the batch used for Ames Assay. Accordingly, it is recommended that repeat of Ames assay would be necessary as a Phase IV requirement.

Carcinogenicity: Male and female CD rats were treated at 5, 15 and 50 mg/kg for 104 weeks. Male rats showed statistically significant increase in the thyroid C-cell adenoma. However, due to high incidences in the historical control data during the same period of study in the laboratory, the reviewer concluded that the treatment did not induce milnacipran-related carcinogenicity. CAC-EC recommended that both the thyroid C-cell tumor findings and the historical control data need to be presented in the package insert. The Sponsor also conducted a 104 week carcinogenicity study in CD-1 mice. The CDER CAC-EC concluded that the mouse study was not conducted at maximal tolerated dose. The Sponsor opted to conduct a third carcinogenicity study in a transgenic mouse model as a requirement for the second species. The study would be reviewed under a separate review by Dr. Bolan.

Reproductive toxicology: 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/m²) 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/m²) and 5 mg/kg (30 mg/m²), respectively. However, intrauterine deaths were increased at 5 mg/kg (30 mg/m²) in rats. Milnacipran showed extra single rib in pregnant rabbits at 15 mg/kg (180 mg/m²). 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/m²) 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.

Special toxicology: none

2.6.6.2 Single-dose toxicity: Reviewed by Dr. Bolan

2.6.6.3 Repeat-dose toxicity

1. Study title: F2207 26-week oral gastric intubation toxicity study in the cynomolgus monkey

Key study findings: Vomiting was noted at 5, 15 and 60/40 mg/kg. The NOAEL was 40 mg/kg; a NOEL was not established. The study did not show any histology based toxicity in organs. Proof of absorption was provided in the report. However, PK parameters were not calculated.

Study no.: 314/22

Volume #M4, and page #: 1

Conducting laboratory and location: I b(4)

Date of study initiation: March 19, 1985

GLP compliance: Yes

QA report: yes (x) no ()

Drug: F 2207, lot #D 9032, and % purity: 101%

Methods

Doses: 5, 15 and 60 mg/kg, high dose was reduced to 40 mg/kg from day 40

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): The study design is shown below.

Group number	Number of animals		Group identification	Dose levels (mg/kg/day)	Dose volume (ml/kg/day)
	male	female			
1	5x	5x	Control	0	4
2	5x	4	Low	5	4
3	5x	4	Intermediate	15	4
4*	5x	5x	High	60*/40	4

The control group was given vehicle only.

The Sponsor stated that one animal/sex was added to groups 1 and 5 due to mortalities on week 1.

Route, formulation, volume, and infusion rate: The aqueous solution was administered by gastric intubation once daily for 26 weeks. Water was used as the vehicle for dissolving the drug.

Satellite groups used for toxicokinetics or recovery: No satellite or recovery group was allotted in the study.

Age: Wild caught cynomolgus monkeys were obtained from []
[] However, the age of the animal was not specified in the protocol.

b(4)

Weight: 2.9 to 4.7 kg for male monkeys; 2.6 to 4.6 kg for female monkeys

Sampling times:

Blood samples were taken on days 1, weeks 4, 13, 26 at 2 hour post dose for the determination of milnacipran levels in the plasma. The assay was conducted by a HPLC method using fluorescence dye. Above samples were taken from 2 animal/group.

The Sponsor stated that additional blood samples were taken at 2 hour predose and 2 hour postdose on week 24 from all animals. Serial blood samples were taken on week 26 from all animals at 15, 30, 60, 120 min post dose.

Unique study design or methodology (if any): None

Observations and times:

Mortality: Once daily

Clinical signs: All animals were observed for clinical signs for at least once a day.

Body weights: The body weight was recorded once weekly before dosing, and during the dosing period and at necropsy.

Food consumption: The food consumption was recorded daily before, during the treatment and at necropsy.

Ophthalmoscopy: Eye examinations were conducted at predose, on week 13 and on week 26.

EKG: ECG, BP, and QTc were recorded in all animals before the treatment. Further recordings were made for animals from groups 1 and 4 on weeks 4, 13, and 26 at two hour post dose. Cardiovascular parameters were also recorded on all high dose animals at 15, 30, 60, 90 and 120 min after administration on week 8.

Hematology: Blood samples were collected for hematology and blood chemistry from all animals at predose, during weeks 4, 13 and 26 from overnight fasted animals before the treatment. Standard hematological and coagulation parameters were examined.

Clinical chemistry: Clinical chemistry parameters were investigated from blood samples obtained during the hematological investigation. GOT and GPT were investigated for all animals on week 16. In addition, alkaline phosphatase activity was determined from all animals on week 19 and 22.

Following clinical chemistry parameters were determined.

3.7.2 Clinical chemistry

The following analyses were performed on serum:

glutamate oxalacetate transaminase (GOT)
glutamate pyruvate transaminase (GPT)
alkaline phosphatase
sodium ions (Na^+)
potassium ions (K^+)
chloride (Cl^-)
total protein (TP)
albumin
albumin/globulin ratio (A/G ratio)
glucose
blood urea (BU)
cholesterol
triglycerides

Urinalysis: 24-hour urine samples were collected at predose, during weeks 4, 13 and 26 following a water load. The method did not specify if pooled or individual samples were collected. The following parameters were determined.

APPEARS THIS WAY
ON ORIGINAL

3.7.3 Urine analysis

The following measurements were performed:

pH
volume
specific gravity

The following semiquantitative estimations were made:

protein
blood
glucose
ketones
bilirubin
urobilinogen
reducing substances
colour
microscopy of centrifuged deposits

Gross pathology: All surviving animals were sacrificed by overdose of hexobarbitone and exsanguination. The necropsy was conducted over 8 day period. Animals were examined macroscopically for internal organs and external body surface.

Organ weights (specify organs weighed if not in histopath table): See the histopathology inventory below.

Histopathology: Adequate Battery: yes (), no (x)—explain, several tissues as listed in the table below were not collected for histological examinations.

Peer review: yes (), no (x)

Protocol specified tissues were preserved in 10% formalin and prepared for histopathological examinations.

Results

Mortality: The following table would indicate mortality during the study.

Group	sex	Animal #	Replacement	Day of death	Cause
1	M	126	28665	48	Infection
1	F	26684	26818	55	Enteritis
1	F	24998		156	Enteritis
2	M	114	28694	49	Infection
3	M	130	27060	70	Infection

Group	sex	Animal #	Replacement	Day of death	Cause
3	M	27060		101	Enteritis
4	M	129	24985	25	Infection
4	M	124		129	Asphyxia
4	F	26972	63	33	Asphyxia

Above study suggested that some of monkeys had infections and replaced animals were treated for another six months.

The Sponsor stated that deaths in two monkeys at the high dose could be related to the treatment. However, in the absence of histopathological evidence, it is unclear if the deaths were due to the treatment or due to the procedures. High incidences of vomiting were observed in the high dose animals as mentioned in the clinical sign section.

Clinical signs: Vomiting and diarrhea were observed in treated animals. Vomiting was more severe for high dose group at 60-40 mg/kg and started as early as first week of the treatment. The incidence of vomiting is presented from the Sponsor's table below.

APPEARS THIS WAY
ON ORIGINAL

Vomiting associated with treatment could be observed in all treated groups and was dose-related as follows:

Dose level	Animal no.	Day
5 mg/kg	118 M	122
	759 F	124, 125
	24965 F	78, 94, 97, 98, 104, 121, 123, 126, 169
15 mg/kg	117 M	112
	130 M	15
	25003 F	44, 47, 48
	26962 F	120
60 to 40 mg/kg	120 M	4, 10, 17, 44, 47, 49, 50, 52, 53, 55, 74, 76, 78, 86, 90, 92, 94, 97, 105, 111, 114, 118, 120, 125, 128 - 130, 132 - 135, 139, 144, 145, 151, 152 - 154, 160 - 162, 164, 170, 173
	124 M	124, 129
	129 M	23, 24
	24985 M	121
	25004 F	21, 34, 35, 37, 45, 55, 62, 66, 71, 105, 108, 122, 130, 131, 133, 147, 154, 178, 181
	26803 F	108, 176
	26919 F	124
	26972 F	33
	63 F	92

Vomiting after dosing could be observed in group 2 and 3 animals on isolated occasions only but in the high dose group vomiting could be repeatedly observed in one male (120 M) and one female (25004 F) during the first weeks of the study. One animal (26972 F) showed vomiting on day 33 and came to a very sudden death when it had probably inspired vomitus.

Based on the data vomiting was present at 5 mg/kg dose also.

Body weights: A decrease in the body weight (kg) gain was noted at mid and high doses especially in male monkeys e.g. 50% reduction in the weight gain at high dose male.

Group	Predose, M	Predose, F	Week 26, M	Week 26, F
1	3.4	3.4	4.2	3.3

2	3.2	3.4	4.2	3.1
3	3.6	3.4	4.1	3.3
4	3.5	3.2	3.9	3.0

Food consumption (g/wk):

Group	Predose, M	Predose, F	Week 26, M	Week 26, F
1	689	700	700	642
2	681	700	613	498
3	700	700	642	540
4	700	691	642	548

Food consumption (g) per week was slightly reduced in the drug-treated monkeys.

Ophthalmoscopy: The Sponsor stated that the treatment had no effect on ophthalmological examinations.

EKG: ECG data did not show treatment related prolongation of QTc and blood pressure. Blood pressure at the end of the treatment was comparable between control and the high dose animals.

Hematology: There were no treatment-related changes in hematology parameters examined.

Clinical chemistry: There was no treatment-related change in the clinical chemistry parameters including transaminase activity in male and female monkeys.

Urinalysis: The Sponsor stated that the treatment had no effect on the urine chemistry. The pH of urine was between 8 and 9.

Gross pathology: The Sponsor stated that that there was no treatment related gross changes.

Organ weights (specify organs weighed if not in histopath table): There was no treatment related change in the absolute organ weight.

Histopathology: Adequate Battery: yes (x), no ()—explain
Peer review: yes (), no (x)

No treatment related histological changes were noted. Several tissues showed leukocyte foci in the control and treated groups. However, its biological significance is unknown.

Toxicokinetics: Plasma levels of milnacipran are shown in the table below from the Sponsor's submission.

Group	N ^o	Sex	O 1		W 4		W 13	
			Before	After -	Before	After -	Before	After -
G 1 (Control)	122	M		ILD	ILD	1088	ILD	ILD
	126	M		ILD	ILD	ILD		
	116	M					ILD	ILD
	745	F		ILD	ILD	ILD	ILD	ILD
	24998	F		ILD	ILD	ILD	ILD	ILD
G 2 (5)	118	M		638	33	s 600	52	156
	119	M		64	ILD	98	ILD	ND
	25006	F		131	ND	88	ND	45
	759	F		42	ILD	37	ILD	45
G 3 (15)	117	M		794	ILD	706	ILD	s 1000
	130	M		496	ILD	ILD		
	115	M					ILD	95
	26962	F		3326	ILD	984		
	25003	F					ILD	363
	850	F		847	s 1000	ILD	ILD	915
G 4 (60 → 40)	120	M		2216 [#]	36	1764 [#]	11	2019 [#]
	129	M		3784 [#]	137	1346 [#]		
	124	M					252	739 [#]
	25004	F		1411 [#]	83	2360 [#]	35	1837 [#]
	26972	F		1600 [#]	191	4987 [#]		
	26919	F					31	ND

* Levels greater than the concentrations from the standard curve

ILD : Less than the detectable limit
 ND : Not determined

→ Blood sampling was performed 2 H after dosing.

APPEARS THIS WAY
 ON ORIGINAL

Group	Dose mg/kg/d	N ^o	Sex	W 24		W 26				
				0	2.00.	0	0.25 ^(a)	0.50 ^(a)	1.00 ^(a)	2.00 ^(a)
G 1 (Control)		116	M	ILD	ILD	15	-	-	-	ILD
		122	M	ILD	ILD	ILD	-	-	-	ILD
		127	M	ILD	ILD	ILD	-	-	-	ILD
		23	F	ILD	ILD	ILD	-	-	-	ILD
		745	F	ILD	ILD	ILD	-	-	-	ILD
G 2 (5)		118	M	26	377	52	82	30	157	319
		119	M	ILD	117	ILD	72	166	186	139
		121	M	ILD	359	ILD	31	62	294	224
		759	F	ILD	93	11	273	20	76	103
		779	F	12	213	25	243	27	71	104
		26965	F	ILD	178	112	97	114	181	203
G 3 (15)		25006	F	ILD	130	ILD	21	76	95	135
		115	M	14	1409 [#]	10	189	685	800	1325 [#]
		117	M	27	1729 [#]	28	105	189	905 [#]	1001 [#]
		123	M	42	1503 [#]	19	57	223	951	1057 [#]
		850	F	12	1239 [#]	28	345	892	912	1230 [#]
		25003	F	ILD	945	ILD	48	146	884	1362 [#]
G 4 (60 → 40)		26962	F	ILD	1496 [#]	21	422	712	770	898
		26964	F	ILD	883	28	1023 [#]	1591 [#]	722	1200 [#]
		120	M	14	2133	25	43	14	142	387
		128	M	108	3947	251	415	549	2657	5237
	25004	F	48	3356	24	1304	1912	1911	4123	
	26803	F	27	4389	26	1304	2186	1278	863	
	26919	F	39	3810	54	459	1353	2490	3403	

* Levels greater than the concentration from the standard curve, the linearity of the curve being controlled up to 5000.

ILD : Less than the detectable limit

^(a) Time of blood sampling after dosing.

The PK data demonstrated absorption of the drug. However, the exposure to each dose was not determined. The assay sensitivity was 2 ng/g of plasma.

Conclusion of the 6-month monkey toxicity study:

Cynomolgus monkeys were treated at 5, 16 and 60 mg/kg orally for 26 weeks. The high dose was reduced to 40 mg/kg due to mortality and its relationship to the treatment is difficult to establish due to infections in wild caught monkeys. The treated animals showed vomitus especially in the high dose. The clinical pathology and histopathology data did not show any treatment related toxicity. However, on the basis of tolerance to the treatment, the Sponsor concluded that the animals in the high dose were treated up to

a MTD. Considering vomiting as the tolerance criteria to the treatment, the NOEL was not established and the NOAEL was 40 mg/kg.

2. Study title: Fifty-two week oral toxicity study of TN-912 in cynomolgus monkeys

Key study findings: The NOEL was 7.9 mg/kg and the NOAEL was 25 mg/kg for the 52-week monkey toxicity study. Mydriasis and vomiting was noted at 25 mg/kg.

Study no.: T088

Volume # M4, and page #: 1

Conducting laboratory and location: [redacted] b(4)

Date of study initiation: May 21, 1991

GLP compliance: Yes

QA report: yes (x) no ()

Drug: TN-912, lot # [redacted] 006, and % purity: 99.9% b(4)

Methods

Doses: 2.5, 7.9, 25 mg/kg once daily at 4 mL/kg by oral route. The doses were selected on the basis of a 3-month dose finding study in which vomiting and mydriasis was noted as dose limiting side effects.

Species/strain: Cynomolgus monkeys purchased from [redacted] b(4)

Number/sex/group or time point (main study): 4 animals/sex/group

Route, formulation, volume, and infusion rate: Aqueous solution of the drug was administered orally at 4 mL/kg.

Satellite groups used for toxicokinetics or recovery: N/A

Age: 3-7 years old

Weight: At the initiation of administration, the body weight was 3.33-5.08 kg for male and 2.34- 4.15 kg for female monkeys.

Sampling times: See below

Unique study design or methodology (if any): Monkeys were injected with heptosulfarein dye intravenously at 5 mg/kg before dosing, weeks 12, 25 and 51. The dye concentration was determined by a spectrophotometer at 565 nM. The dye injection was given for the determination of hepatic function.

Observations and times:

Mortality: All animals were observed 3 times a day during the administration period for mortality and clinical signs.

Clinical signs: see above

Body weights: The body weight was recorded once per week during predose, dosing period and on the day of necropsy.

Food consumption: The Sponsor stated that appetite conditions were observed visually as complete food intake or half food intake or no food intake once prior to dosing or once a week during the dosing period.

Ophthalmoscopy: Ophthalmological examinations were conducted once before the dosing, weeks 13, 26 and 52 under ketamine anesthesia at 5-10 mg/kg IM. Eyes were dilated by Mydrin and examined by slit lamp and fundus camera. Electroretinogram was recorded at predose, weeks 12, 25 and 51 under topical anesthesia by 0.4% Benoxyl.

EKG: ECG was recorded under ketamine anesthesia for all animals in the control and high dose at predose, weeks 12, 25, and 51 using leads I, II, III and aVr, aVL and aVf bipolar and unipolar leads. The average blood pressure was also recorded at the same time points.

Hematology: Blood samples were collected from the jugular vein at predose, weeks 13, 26 and 52 to measure standard hematology and coagulation parameters. The Sponsor did not mention how much blood/sample was collected.

Clinical chemistry: Samples collected for hematology were also used for the serum chemistry parameters at predose, weeks 13, 26 and 52. Standard hematology parameters were analyzed.

Urinalysis: Two-hour urine or 18 hour urine samples were collected from all animals at predose, weeks 13, 26 and 52 weeks. Standard parameters of urine chemistry were analyzed.

Gross pathology: Bone marrow was collected at necropsy from sternum from all animals. Bone marrow smears were prepared and stained by Giemsa stain for the determination of erythroid and myeloid ratios. Animals were sacrificed by sodium pentobarbital and exsanguination at the end of dosing period. Gross changes were recorded.

Organ weights (specify organs weighed if not in histopath table): organ weight of protocol specified tissues were recorded.

Histopathology: Adequate Battery: yes (x), no ()—explain
Peer review: yes (), no (x)

Tissues were fixed in 10% formalin. Eye tissues were fixed in formalin-glutaraldehyde mixtures. The Sponsor stated that the arch of the aorta from selected animals was stained by PAS stain. Other tissues were stained by hematoxylin-eosin stain.

Results

Mortality: No treatment related mortality was reported in the study.

Clinical signs: Mydriasis was observed during the first week at 25 mg/kg in male and female monkeys. The effect was tolerated and mydriatic response disappeared in following weeks. Occasional vomiting and salivation was also observed at 25 mg/kg in male monkeys.

Body weights: The body weight (kg) data are shown in the table below. Data suggest that there was no treatment related effect on the body weight gain in male and female monkeys. However, the body weight (kg) gain in control animals was minimal during one year period.

Dose, mg/kg	Predose, M	Predose, F	Week 26, M	Week 26, F	Week 52, M	Week 52, F
Control	4.4	2.9	4.7	3.0	4.5	3.0
2.5	4.4	2.9	4.8	3.1	4.8	2.9
7.9	4.2	3.0	4.6	3.0	4.7	3.0
25	4.3	3.2	4.2	3.2	4.3	3.1

Food consumption: Male and female monkeys showed normal appetite at all treated animals.

Ophthalmoscopy: No treatment related changes in the anterior, posterior chambers of the eye and electroretinograph was noted. However, clinical signs of mydriasis were noted as described under the clinical signs.

EKG: ECG data did not show a treatment related change in the QTc in male and female monkeys. The blood pressure data also did not show treatment-related change in male and female monkeys on chronic administration. However, these data do not reflect any transient change in the blood pressure at the beginning of the treatment.

Hematology: A slight reduction in WBC counts and lymphocyte counts was noted in the male monkeys at 2.5 mg/kg and 25 mg/kg at the end of treatment period. However, the counts appeared to be within the normal range and do not reflect as a treatment effect. Data are shown below.

Male monkeys:

Parameter	Control, Pre	Control, Wk 52	2.5 mg/kg, Pre	2.5 mg/kg, Wk 52	7.9 mg/kg, Pre	7.9 mg/kg, Wk 52	25 mg/kg, Pre	25 mg/kg, Wk 52
WBC, $10^2/mm^3$	146	152	155	84	129	110	154	102
Lymph, $10^2/mm^3$	72	73	69	40	61	68	72	55

Bone marrow smears did not show treatment related suppression in the myeloid and erythroid cells. Basophil cell counts in the bone marrow were 0.25% in the control and 65% at 25 mg/kg. However, total granulocyte counts in the bone marrow were unaffected by the treatment. Biological significance of the change in basophil is not known.

Female monkeys did not show any treatment related changes in hematology.

Clinical chemistry: Male and female monkeys did not show any treatment related changes in clinical chemistry. Hepatic function test did not show any treatment related change.

Urinalysis: Urine chemistry data showed pH was between 8.5 to 9 for most of the treated and control animals. Treatment related changes in the urine volume and specific gravity were not noted.

Gross pathology: No treatment related changes were noted in the gross pathological examinations at necropsy.

Organ weights (specify organs weighed if not in histopath table):

Organ weight data for liver in male monkeys are shown below.

Organ	Control	2.5 mg/kg	7.9 mg/kg	25 mg/kg
Liver (g)	67.3	89.5*	81	86.7*

*statistically significant

In the absence of any biochemical or histological changes or any dose effect relationship, relevance of the change in liver weight is unknown.

Female monkeys did not show any treatment related changes in the organ weights.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (), no (x)

Male and female monkeys did not show any histopathological changes related to the treatment.

Toxicokinetics: No toxicokinetic data were obtained in the study.

Conclusion of the 52 week oral toxicity study in cynomolgus monkeys:

Treatment at 2.5, 7.9 and 25 mg/kg per oral for 52 weeks did not show any treatment related changes. The highest dose tolerated was 25 mg/kg. Transient mydriasis was noted at 25 mg/kg dose during the first week of treatment. Occasional vomiting and salivation was noted at 25 mg/kg during the treatment. No treatment related changes in the ECG and laboratory parameters were noted. Histopathology data did not show any treatment related lesion. The six-month toxicity data in monkeys showed vomiting as a clinical sign. Based on the finding in the six-month study and the present study, the high dose was considered to be adequate for the 52-week study. The Sponsor did not

determine the exposure level for milnacipran in the 52-week study. The NOEL was 7.9 mg/kg and NOAEL was 25 mg/kg for the 52-week monkey toxicity study.

3. Study title: A 52-week oral toxicity study of TN-912 in rats

Key study findings: Based on the data, the NOEL was 10 mg/kg/day in male and 3 mg/kg/day in female rats. The NOAEL was 30 mg/kg. Liver vacuolation was noted at 30 mg/kg in male rats.

Study no.: T087

Volume #M4, and page #: 1

Conducting laboratory and location:

Date of study initiation: Feb 6, 1991

GLP compliance: Yes

QA report: yes (x) no ()

Drug: TN912, lot # 006, and % purity: 99.9%

b(4)

b(4)

Methods

Doses: The study design is shown below.

Test Groups	Dose Levels (mg/kg)	Concentration of Test Solution (%)	Sex	No. of Animals	Animal No.
Control	0	0	M	15	1001 - 1015
			F	15	1101 - 1115
Low	1	0.04	M	15	2001 - 2015
			F	15	2101 - 2115
Intermediate	3	0.12	M	15	3001 - 3015
			F	15	3101 - 3115
High	10	0.4	M	15	4001 - 4015
			F	15	4101 - 4115
Highest	30	1.2	M	15	5001 - 5015
			F	15	5101 - 5115

The dose levels were determined on the basis of a 13-week toxicity study in rats at 5, 10, 20 mg/kg. The Sponsor stated that vacuolation in the liver was noted at 10 mg/kg and above doses. The high dose was chosen to characterize the frank toxicity to the treatment over a 52-week period. It should be noted that a 26-week oral toxicity study conducted in

CD rats at 10, 35 and 120 mg/kg also showed minimal centrilobular enlargement at 35 and 120 mg/kg without any treatment related mortality.

Species/strain: Wistar rats

Number/sex/group or time point (main study): See study design above

Route, formulation, volume, and infusion rate: The test substance was dissolved in water for injection and administered orally by gavage daily at 2.5 mL/kg once a day. The control animals received the water for injection.

Satellite groups used for toxicokinetics or recovery: None

Age: six weeks after quarantine

Weight: 144-172 g for male and 113-129 g for female rats at the start of drug administration.

Sampling times: Blood samples were collected at necropsy for hematology and blood chemistry

Unique study design or methodology (if any): None

b(4)

Observations and times:

Mortality: See clinical signs

Clinical signs: Animals were observed three times daily during the treatment period for toxicity and mortality.

Body weights: The body weight was recorded twice a week up to 13 weeks and once a week thereafter.

Food consumption: The cumulative food consumption was measured over a week period before dosing period and once a week thereafter. The average food consumption per day per animal was calculated.

Ophthalmoscopy: Ophthalmological examinations were conducted at predose, months 6 and 12 by ophthalmoscope. Eyes were dilated with Mydrine before the examination. Only 10 animals/sex/group were examined during the treatment period. Fundus of control and high dose animals were photographed on months 6 and 12.

EKG: Not recorded

Hematology: Blood samples were collected from the ether anesthetized fasted rats from the abdominal aorta at necropsy for the hematology and for the determination of hematological parameters. Standard parameters were analyzed were.

Clinical chemistry: Standard plasma chemistry parameters were analyzed from the blood samples collected at necropsy.

Urinalysis: Urine analysis was conducted on months 6 and 12 from 24 hour urine collected from a metabolic cage. Animals were deprived of food for first 4 hours of the collection. Standard urine chemistry parameters were determined. The Sponsor did not mention if pooled samples were analyzed in the method section. However, samples from all animals were collected for the urine chemistry.

Gross pathology: Animals were sacrificed by exsanguination under ether anesthesia. Any macroscopic change in the external surface and internal organs was recorded.

Organ weights (specify organs weighed if not in histopath table): The organ weight for protocol specified tissues was recorded.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (), no (x)

Protocol specified tissues were preserved in 10% formalin. Eyes, Harderian glands and optic nerve were fixed in 4% glutaraldehyde. Tissues were stained with hematoxylin and eosin for microscopic examinations. Part of liver and kidney cortex from 2 animals/sex/group were fixed in 0.5% glutaraldehyde, 1.5% formaldehyde and 1% osmic acid for electron microscopic examinations. The Sponsor stated that histopathology was conducted in tissues from control and the high dose groups. In addition, macroscopic changes, liver and prostate from male rats were examined for all animals.

Results

Mortality: One male (1004) from the control group died on day 194, the cause of death was not specified. Male # 3011 at 3 mg/kg died on day 263, the cause of death was reported to be gavage accident based on esophageal rupture. Pituitary hyperplasia was also observed for male # 3011.

Clinical signs: There was no treatment related changes in the male rats. Opacity of the eyeball was noted in one animal each from control, 1, 3 and 30 mg/kg after 40 weeks of dosing.

Opacity of eyeball in one female each from control and 30 mg/kg groups was also observed from week 29 onwards. However, the change was considered un-related to the treatment.

Body weights: The body weight of male rats (g) is shown below. The high dose group showed about 8% reduction in the body weight gain at the end of 364 days.

Day	Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
0	157	156	157	157	157
91	376	373	372	367	355
182	427	431	431	425	410
364	479	485	484	470	454
Gain 0-364	322	328	327	313	297
% Control		101%	101%	97%	92%

The body weight (g) of female rats is shown in the table below. Female rats showed 10 and 18% reduction in the body weight gain at 10 and 30 mg/kg, respectively.

Day	Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
0	120	121	120	121	120
91	209	203	203	200	198
182	230	223	222	219	215
364	269	266	260	255	243

Gain	148	144	140	134	122*
% Control		97%	94%	90%	82%

*Statistically significant

Food consumption (g/rat/day):

Male rats showed about 4% reduction in the food consumption at the end of 364 days at 30 mg/kg compared to the control. Data are shown below.

Day	Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
0	18.3	17.9	18.7	18.5	18.5
182	17.7	18.2	18.6	18.9	18.6
364	19.0	19.0	19.5	19.3	18.4
% control					4%

Female rats showed about 9% reduction in the food consumption at the end of treatment at 30 mg/kg as shown in the table below.

Day	Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
0	13.8	13.8	13.4	14.7	14.7
182	13.0	12.4	12.9	12.8	12.5
364	14.0	14.0	13.5	13.2	12.8*
% control					9%

* statistically significant

Based on the data, the effect of the drug on body weight at 30 mg/kg was due to a reduction of food consumption.

Ophthalmoscopy: One male animal (#5001) showed incidental changes in the eye, e.g. unilateral arterial-venous narrowness at 30 mg/kg during 6 and 12 months of the treatment. The incidence was considered an isolated incidence in the absence of a similar finding in female rats and a single incidence. As discussed in the clinical sign section, few animals in the control and treated groups showed opacity that could be incidental. Data from male rats are shown below from the Sponsor's table.

**APPEARS THIS WAY
ON ORIGINAL**

Table 4-3 A 52-week oral toxicity study of TN-912 in rats
Ophthalmology (12 months)
Male

Findings	Dose (mg/kg)	0	1	3	10	30
	No. of animals	9	10	10	10	10
No abnormality		7	8	8	8	9
External appearance						
Opacity						
Unilateral		1	1	1	0	0
Anterior portion						
Focal opacity						
Unilateral		0	1	1	0	0
Bilateral		1	0	0	2	0
Transparent body						
Focal opacity						
Unilateral		0	1	0	0	0
Fundus oculi						
Retina						
Arterial narrowness						
Unilateral		0	0	0	0	1
Venous narrowness						
Unilateral		0	0	0	0	1
Chorioides						
Atrophy						
Unilateral		0	0	0	0	1

EKG: Nil

Hematology: There were no treatment related changes in the hematology parameters.

Clinical chemistry: Clinical chemistry parameters did not show treatment related abnormalities that could be due to the organ system toxicity. Male rats showed a decrease in the GOT and LDH activity at 30 mg/kg. Female rats showed a slight increase in the BUN at 10 and 30 mg/kg. However, clinical significance of the magnitude of change is unknown. Data are shown from the Sponsor's table below.

Blood chemistry

Female

Dose mg/kg	No.		T. billi- rubin mg/dl	Glucose mg/dl	BUN mg/dl	Crea- tinine mg/dl	UA mg/dl	Na mEq/l	K mEq/l	Cl mEq/l	Ca mg/dl	P mg/dl
0	15	Mean	0.09	119.3	20.2	0.61	1.14	144.8	4.6	120.2	9.9	3.9
		S.D.	0.02	12.9	1.9	0.07	0.24	1.3	0.4	2.2	0.3	1.1
1	15	Mean	0.09	117.4	21.1	0.62	1.17	145.1	4.5	119.6	9.7	4.1
		S.D.	0.02	13.3	3.1	0.05	0.29	1.0	0.3	2.3	0.3	1.4
3	15	Mean	0.08	116.3	21.5	0.64	1.25	144.6	4.8	120.3	9.8	4.4
		S.D.	0.02	11.6	1.7	0.10	0.26	1.2	0.3	1.8	0.2	1.2
10	15	Mean	0.08	115.9	22.5 ^a	0.62	1.31	144.2	4.7	119.8	9.9	4.0
		S.D.	0.02	12.9	1.6	0.06	0.27	1.7	0.3	2.6	0.2	1.1
30	15	Mean	0.07	115.4	22.7 ^{aa}	0.62	1.38	144.9	4.6	119.9	10.0	4.1
		S.D.	0.01	11.9	1.2	0.08	0.29	1.9	0.2	2.0	0.2	1.2

^a : p<0.05 ; ^{aa} : p<0.01 (Significant difference from control)

Urinalysis:

Male:

Male rats showed pH between 7 and 8 in the control and treated animals at the end of 12 months. Traces of protein in the urine were also observed in control and treated rats. Urine volume was slightly increased at 10 and 30 mg/kg in male rats at the end of 12 months. Data are shown from the Sponsor's table below.

Male

Dose mg/kg	No.		Urine volume ml/24hrs	S.G.	Electrolytes		
					Na mEq/24hrs	K mEq/24hrs	Cl mEq/24hrs
0	14	Mean	11.6	1.055	1.21	2.61	1.46
		S.D.	5.5	0.010	0.28	0.51	0.27
1	15	Mean	11.8	1.068	1.26	2.90	1.60
		S.D.	2.7	0.009	0.28	0.53	0.31
3	14	Mean	12.7	1.060	1.41	3.29*	1.78
		S.D.	2.9	0.008	0.35	0.52	0.33
10	15	Mean	14.6**	1.059	1.38	3.07	1.53
		S.D.	3.3	0.008	0.27	0.46	0.26
30	15	Mean	13.9*	1.057	1.33	3.01	1.55
		S.D.	2.9	0.009	0.22	0.44	0.21

* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Female:

Female rats did not show any treated related change.

It was concluded that the treatment showed an increase in the urine volume at 10 and 30 mg/kg in male rats.

Gross pathology: There were no apparent treatment-related changes in gross pathology endpoints.

Organ weights (specify organs weighed if not in histopath table):

Male rats:

A statistically significant change was noted in the weight of spleen at 30 mg/kg, epididymides and seminal vesicles at 10 and 30 mg/kg and prostate at 30 mg/kg. Body weight normalized organ weight data also showed a decrease in several organs. Salivary glands showed an increase in the normalized weight. Data are shown in the table below.

Organ	Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Spleen (g)	0.82	0.76	0.78	0.78	0.66*
Spleen (g%)	0.18	0.16	0.17	0.17	0.15*
Epididymis (g), Right	507	476	486	428*	436*
Epididymis	112	104	106	97*	102*

Organ	Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
(mg%), R					
Epididymis (g), Left	517	505	500	437*	448*
Epididymis, L (mg%),	114	111	109	99*	104*
Seminal vesicle (g)	1.2	1.09	1.11	0.94*	0.84*
Seminal vesicle, g%	0.26	0.24	0.24	0.21*	0.20*
Salivary gland, Right mg%	66	65	66	75*	76*
Salivary gland, Left, mg%	64	64	65	73*	74*
Prostate, g	0.93	0.86	0.88	0.86	0.77*

* Statistically significant

Female rats:

Organ weight data showed a decrease in the absolute weight of liver, spleen and kidney in female rats as shown below.

Organ	Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Liver (g)	6.13	6.0	5.85	5.7	5.6*
Spleen (g)	0.49	0.45	0.45	0.39*	0.38*
Kidney (g), R	0.83	0.82	0.80	0.80	0.78*

The normalized organ weight data are shown below.

Organ	Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Salivary, R, mg%	78	79	86*	86*	92*
Salivary gland, L, mg%	77	78	84	85	91*
Spleen, g%	0.19	0.18	0.19	0.15*	0.17*

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (), no (x)

Male rats:

Some of the histopathological findings are shown below. Sponsor's table 11-1 provided histology of liver and prostate in male rats at 1, 3, 10 and 30 mg/kg among surviving animals. Sponsor's table 11-2 provided histology data from several organs at control and 30 mg/kg. Composite data are shown below.

Organ	Control, n=14	1 mg/kg, n=15	3 mg/kg, n=14	10 mg/kg, n=15	30 mg/kg, n=15
Liver, focal necrosis	0	1=mild	1=slight	1=slight, 1=mild	1=slight
Liver, vacuolation	1, slight			1=slight, 1= mild	7=slight, 3=mild
Prostatitis	2=slight, 2=mild	5=slight, 3= mild	2=slight, 4=mild	4=slight, 5=mild	4=slight, 2=mild
Testes, interstitial cell hyperplasia	6, mild				12, mild

Liver and testes showed increased lesions in male rats. Vacuolation in the liver at 30 mg/kg was considered treatment related based on incidences and severity. Focal necrosis in the liver could be incidental due to a lack of dose response, small number of incidences and unaltered severity between doses. The incidence of prostatitis increased in the treatment groups compared to controls. However, it is unlikely that incidences were related to the treatment because there was no dose response among treated groups with respect to incidences and severity. Also, control rats showed high incidences of prostatitis.

Due to high incidences of inflammatory conditions in testes in control animals, the effect of the drug in treated groups is questionable. Based on the histological data in male rats, vacuolation in the hepatocytes was treatment related at 30 mg/kg.

Female rats:

Female rats did not show any treatment related change histologically in lung, liver, pituitary, adrenal, kidney, uterus, eye, optic nerve, sternum and femur in control and 30 mg/kg dose among 15 animals per group. Based on the data, female rats did not show any treatment related histological change.

Histopathological data for individual rats for gross lesions and electron microscopic lesions were provided. Other than a summary table 11, histopathology findings for individual rat were not provided. However, results of the study did not show any unknown change than that observed in the 6-month and 24 month treatment. The reviewer conceded that the report can be accepted to determine non-clinical safety on chronic administration of milnacipran.

Toxicokinetics: The sponsor did not determine blood level of milnacipran in the study.

Other: None

Conclusion of 12-month toxicity study in Wistar rats:

Wistar rats were treated at 1, 3, 10 and 30 mg/kg/oral for 52 weeks. No treatment related mortality was reported in the study. The body weight gain was reduced by 8% and 18% at 30 mg/kg in male and female rats, respectively, due to a reduction in the food consumption. Female rats showed about 10% reduction in the body weight gain at 10 mg/kg. No treatment related change in hematology and plasma chemistry was noted. Histopathology data showed increased vacuolation in hepatocytes associated with a decrease in LDH and GOT activity at 30 mg/kg. No treatment related histopathological change was noted in female rats. The study was conducted at MTD and acceptable.

Based on the data, NOEL was 10 mg/kg/day/oral in male and 3 mg/kg/day/oral in female rats. NOAEL was 30 mg/kg. Liver vacuolation was noted at 30 mg/kg in male rats.

Histopathology inventory (optional) :

Study	104 weeks		26 weeks	52 weeks	52 week
	Rats	LD	Cynomolgus monkey	Cynomolgus monkeys	Wistar rat
Adrenals*, **, #	X		X	X	X
Aorta			X	X	X
Bone Marrow smear			X	X	X
Bone (femur)				X	
Bone (sternum)					
Brain**#	X		X	X	X
Cecum #	X		X	X	X
Cervix					
Colon	X		X	X	X
Diaphragm				x	
Duodenum	X		X	X	X
Epididymis**, #			X	X	X
Esophagus	X		X	X	X
Eye	X		X	X	X
Fallopian tube					
Gall bladder			X	X	
Gross lesions			X		X
Harderian gland					X
Heart*, **, #	X		X	X	X
Ileum	X		X	X	X
Injection site					
Jejunum	X		X	X	X
Kidneys*, **, #	X		X	X	X
Lachrymal gland				X	
Larynx					
Liver*, **, #	X		X	X	X
Lungs**, #	X		X	X	X
Lymph nodes, cervical					X
Lymph nodes mandibular**	X		X	X	

b(4)

Study	104 weeks	26 weeks	52 weeks	52 week
Species	Rats [♂] JCD	Cynomolgus monkey	Cynomolgus monkeys	Wistar rat
Lymph nodes, mesenteric	X	X		X
Mammary Gland	X		X	X
Nasal cavity				
Optic nerves				X
Ovaries*, **, #	X	X	X	X
Pancreas*	X	X	X	X
Parathyroid			X	
Peripheral nerve				
Pharynx				
Pituitary*, **, #	X	X	X	X
Prostate*, **, #	X	X	X	X
Rectum		X	X	X
Salivary gland #	X	X		X
Sciatic nerve		X	X	X
Seminal vesicles**, #	X	X	X	X
Skeletal muscle		X	X	X
Skin	X	X	X	X
Spinal cord		X	X	X
Spleen*, **, #	X	X	X	X
Sternum	X			X
Stomach	X	X	X	X
Testes*, **, #	X	X	X	X
Thymus*, **	X		X	X
Thyroid*, **	X		X	X
Tongue	X		X	X
Trachea	X		X	X
Urinary bladder	X		X	X
Uterus**, #	X		X	X
Vagina	X		X	X
Zymbal's gland				
Standard List				
Tissue masses				

b(4)

X, histopathology performed

* Organ weights were recorded for 6-month monkey toxicity

** Organ weights were recorded for 12-month monkey study

Organ weights were recorded for 52-week rat study

2.6.6.4 Genetic toxicology

Study title: Ames metabolic activation test to assess the potential mutagenic effect of F2207

Key findings: Milnacipran was negative in Ames assay of reverse mutation in the absence and presence of S-9 liver mixtures. The Sponsor did not provide purity data for the test substance.

Study no.: T010

Volume #M4, and page #: 1

Conducting laboratory and location: C

Date of study initiation: June 22, 1982

GLP compliance: Yes

QA reports: yes (x) no ()

Drug: F2207, lot # CA 6/174, and % purity: Not provided

b(4)

Methods

Strains/species/cell line: TA 1535, TA 1537, TA 1538, TA 98 and TA 100 of *Salmonella typhimurium*

Doses used in definitive study: 50, 150, 500, 1500 and 5000 ug/plate

Basis of dose selection: Preliminary cytotoxicity study was conducted at 5, 50, 500 and 5000 ug/plate in TA 1535, TA 1537, TA 1538, TA 98 and TA 100 in the presence and absence of S-9 rat liver homogenates. The highest dose was non-toxic and 5000 ug/plate was chosen as the highest dose for the mutagenicity assay.

Negative controls: Sterile distilled water

Metabolic activation system (S-9) from C treated Sprague Dawley rat liver homogenates and cofactors for mixed function oxidase generating system was used to simulate metabolic conditions of the drug substance in S-9 + test systems.

b(4)

Positive controls: Positive controls used in the assay are shown in the Sponsor's table below along with revertant colonies observed in the assay.

APPEARS THIS WAY
ON ORIGINAL

TABLE 3

Test 1

Mutability and sterility tests with S. typhimurium strains
TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Compound	Concentration of compound (µg/plate)	Metabolic activation	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	Sodium azide	5	-	375	80.7	452,291,382
TA 1537	9-amino-acridine	20	-	219	28.5	244,188,225
TA 1538						
TA 98	2-nitro-fluorene	10	-	681	102.4	733,747,563
TA 100						
TA 100	Sodium azide	5	-	375	31.8	370,409,346
TA 1535	2-amino-anthracene	2	+	137	10.7	139,125,146
TA 1537						
TA 1538						
TA 98						
TA 100						
-	S-9 mix	500 µl	-	0		0
-	F 2207	5000	-	0		0

- Absence
+ Presence
SD Standard deviation

APPEARS THIS WAY
ON ORIGINAL

TABLE 5

Test 2

Mutability and sterility tests with *S. typhimurium* strains
TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Compound	Concentration of compound (µg/plate)	Metabolic activation	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	Sodium azide	5	-	499	74.3	585,452,461
TA 1537	9-amino-acridina	20	-	739	116.1	802,605,810
TA 1538				732		
TA 98	2-nitro-fluorene	10	-	533	22.1	549,508,543
TA 100				545		
TA 1535	2-amino-anthracene	2	-	160	8.5	168,151,160
TA 1537			+	47	8.1	56,42,42
TA 1538			+	203	7.0	211,200,198
TA 98			+	1063	124.4	1153,921,1115
TA 100			+	262	109.6	305,343,137
-	S-9 mix	500 µl	-	0		0
-	F 2207	5000	-	0		0

- Absence
+ Presence
SD Standard deviation

Incubation and sampling times: The assay was carried out in triplicates. About 0.1 mL of tester strains, 0.1 mL of test compound at appropriate concentrations in the absence of S-9 mixtures were mixed in 2 mL histidine deficient agar medium overlaid on plates containing 15 mL minimal agar and incubated at 37 for 72 hours. Revertant colonies were counted at the end of incubation period. Colonies were counted using automatic counters.

b(4)

In separate set of experiments, 0.5 mL of S-9 liver homogenates and mixed function oxidase systems was added for metabolic activation. Rest of the procedure was similar to that described above.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Mean number of mutant colonies was counted. A compound was deemed positive if a statistically significant and dose related increase in revertant colonies was observed.

Study outcome: Two mutagenicity assays were conducted in the absence and presence of S-9 mixtures. There was no increase in revertant colonies in tester strains used in both studies. It was concluded that milnacipran was negative in Ames reverse mutagenicity assay. Data are shown from Sponsor's table below.

TABLE 4

Test 2

F 2207 - revertant colony counts obtained per plate using S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Dose level (µg/plate)	Metabolic activation	Mean revertant colony counts	SD	Individual revertant colony counts	
TA 1535	5000	-	13	2.5	16,11,13	
	1500	-	9	4	9,13,5	
	500	-	16	1.2	17,15,17	
	150	-	20	6	26,14,19	
	50	-	12	3.5	15,8,12	
	0	-	12	3.1	9,15,13	
	5000	+	14	3	14,11,17	
	1500	+	10	4	8,8,15	
	500	+	14	3.8	17,16,10	
	150	+	21	5.8	14,24,24	
	50	+	11	2.5	13,8,11	
	0	+	11	3.8	9,15,8	
	TA 1537	5000	-	13	3.8	16,9,15
		1500	-	12	0.6	12,11,12
500		-	10	2.6	9,13,8	
150		-	17	0.6	17,17,16	
50		-	11	1.2	12,12,10	
0		-	15	3.5	17,11,17	
5000		+	22	7.6	15,20,30	
1500		+	13	3.8	9,16,15	
500		+	15	6.4	8,19,19	
150		+	17	4.4	12,19,20	
50		+	19	5	14,19,24	
0		+	13	8.1	4,18,18	
TA 1538		5000	-	6	2.3	9,5,5
		1500	-	10	2.5	7,12,10
	500	-	8	2	8,10,6	
	150	-	9	1	10,8,9	
	50	-	9	3	9,12,6	
	0	-	8	1.5	10,8,7	
	5000	+	22	9.5	29,11,25	
	1500	+	18	0	18,18,18	
	500	+	13	6.6	20,7,12	
	150	+	22	7.6	19,31,17	
	50	+	14	2	16,12,14	
	0	+	16	5.9	14,12,23	

- Absence
 + Presence
 SD Standard deviation

TABLE 4
(continued)

Strain	Dose level (µg/plate)	Metabolic activation	Mean revertant colony counts	SD	Individual revertant colony counts	
TA 98	5000	-	37	6.6	31, 36, 44	
	1500	-	36	2.1	38, 34, 37	
	500	-	32	10.8	40, 20, 37	
	150	-	36	6.6	42, 37, 29	
	50	-	32	6.5	32, 39, 26	
	0	-	36	4.5	36, 40, , 31	
	5000	+	36	4.6	31, 39, 39	
	1500	+	34	7.9	28, 43, 31	
	500	+	41	6.6	40, 35, 48	
	150	+	34	1.5	35, 32, 34	
	50	+	36	1.5	38, 35, 36	
	0	+	29	4	25, 28, 33	
	TA 100	5000	-	82	2.5	80, 82, 85
		1500	-	93	12.5	81, 92, 106
500		-	94	6.4	97, 99, 87	
150		-	97	5	98, 102, 92	
50		-	105	11.4	102, 96, 118	
0		-	89	12.9	75, 100, 93	
5000		+	90	2.6	88, 89, 93	
1500		+	111	8	112, 119, 103	
500		+	103	11	91, 112, 107	
150		+	93	9	98, 99, 83	
50		+	90	5.6	85, 89, 96	
0		+	99	10	88, 107, 103	

- Absence
+ Presence
SD Standard deviation

Study title: Clastogenic evaluation of F2207 lot # H7068 in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in purified human lymphocytes

Key findings: Milnacipran did not induce chromosomal aberration in vitro in the absence and presence of S-9 mixtures in human peripheral blood lymphocytes.

Study no.: T016

Volume #M4, and page #: 1

Conducting laboratory and location: []

Date of study initiation: Nov 11, 1985

GLP compliance: Yes

QA reports: yes (x) no ()

Drug: F2207, lot # H7068, and % purity: 100.6%, certificate of analysis provided in report # T018.

b(4)

Methods: Human peripheral blood from a single donor was obtained for the experiment. Human lymphocytes from the donor was prepared. Lymphocytes were activated by phytohemagglutinin (PHA) in the culture. The experiment was conducted in the absence or presence of rat liver metabolic activation systems. Cells were incubated for 46 hours at several concentrations of milnacipran in the absence of S-9 mixtures. Cell mitosis was arrested with the addition of Colcemide two hours before the cell harvesting. Cells were harvested, air dried and stained with Giemsa stain. Culture medium was used as the vehicle control. For the aberration assay, metaphase cells were treated with hypotonic KCl solution and fixed with methanol and acetic acid before preparing slides. Duplicate cultures were used for each concentration.

In another set of experiment, lymphocytes were incubated for one hour in the presence of test article and S-9 mixtures from rat liver at 37° C. Cells were washed and incubated for additional 48 hours. Colcemide was added two hour before termination of incubation for arresting the cell cycle in metaphase. Cells were prepared for chromosome aberration assay using the method described above.

Metabolic activation systems were prepared from Arochlor treated rat liver homogenates and cofactors.

Strains/species/cell line: See above

Doses used in definitive study: Cytogenetics of following doses was conducted:

In the absence of S-9 mixtures:

25, 50, 100 and 150 ug/ml.

In the presence of S-9 mixtures:

250 ug/ml, 500 ug/ml, 1 mg/ml and 2 mg/ml

Basis of dose selection: A preliminary assay was conducted in the absence and presence of S-9 mixtures at several concentrations. The cells were incubated with the test substance for 24 hours. Cells were fixed on the 64-72 hours after the exposure to the test substance and cell cycle kinetics was determined. BrdUrd was added to monitor cell cycle. Cells were stained with Giemsa stain. Doses were selected from the inhibition of mitosis in the preliminary experiment. Data for the range finding assay in the absence of S-9 mixtures showed about 80% of the cell was in metaphase up to 100 ug/mL. The Sponsor indicated that cytotoxicity was noted at 333.3 ug/mL. Aberration assay was done up to 250 ug/mL concentration of the test substance. However, highest concentration at which cytogenetic analysis was done indicated above.

In the presence of S-9 mixtures, data for metaphase cells in M1 cycle were about 75% of total cell up to 100-333 ug/mL. Complete cytotoxicity was noted at 10 mg/mL. The

aberration assay with metabolic activation was conducted up to 5 mg/mL. Concentrations and time of incubation were chosen to obtain adequate number of metaphase cells. However, the highest concentration used for the cytogenetic analysis is indicated above.

Negative controls: The assay was done with untreated control and 10 uL/mL culture medium as untreated and solvent control, respectively.

Positive controls: Ethylmethanesulfonate (EMS) and Mitomycin C (MMC) were used as positive controls in the absence of S-9 mixtures. Cyclophosphamide (CP) was used in the presence of S-9 mixtures.

Incubation and sampling times: See above procedures.

One hundred metaphase cells were assessed for cell cycle kinetics. About 50 cells for each duplicate culture for at least four concentrations of the test substance or controls were analyzed for chromosome aberrations. However, only 25 metaphase cells were scored for one of the positive controls as a deviation.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Some of the criteria for evaluation of a positive response were provided as follows:

1. Overall chromosomal aberration frequencies
2. % of aberrant cells
3. % of cells with more than one aberration
4. A positive dose response
5. Number of breaks
6. Increase in aberrant cells from 1 to 7% among 100 metaphase cells.

Study outcome:

The chromosome aberration assay in the absence of S-9 mixtures showed cytotoxicity at 200 ug/mL. Number of aberrant cells was not increased up to 150 ug/mL. Number of cells in metaphase was low and comparable to the control. That restricted collections of adequate metaphase cells for analysis. However, no aberration due to the treatment was noted. Data from the Sponsor's table are shown below.

**APPEARS THIS WAY
ON ORIGINAL**

Table 2B CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES (PURIFIED)
 Results pooled from replicate cultures
 Assay No.: E-9442 Lab Code: 021285 Without Activation
 Compound: F 2207 Dosing Date: December 3, 1985 Trial No.: I

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION														NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS		
		CHROMATID							CHROMOSOME											
		TB	ID1	F	TR	UR	CR	SB	AF	U	R	MT	PU	E	CI				Other	
CONTROLS UNTREATED AND SOLVENT:	a	100	1															0.01	1.0	0.0
POSITIVE: Mitomycin C 100 ng/ml		25	10				5			2								0.72	64.0*	8.0
25 µg/ml		100							5									0.05	2.0	2.0
50 µg/ml		100	2															0.02	2.0	0.0
100 µg/ml	b	100	2						2									0.04	3.0	1.0
150 µg/ml	c	100																0.00	0.0	0.0

* Significantly greater than untreated and solvent control, p<0.05.
 Mitotic index: a: 2.83
 b: 1.33
 c: 1.23

TB: Chromatid break
 CR: Quadriradial
 AF: Acentric fragment
 ID: Interstitial deletion

MILNACIPRAN HYDROCHLORIDE 25 mg and 50 mg
 IN D. MUTAGENIC POTENTIAL

3

Chromosome aberration in the absence of S-9 mixtures was scored up to 2 mg/mL. Data are shown from the Sponsor's table below.

APPEARS THIS WAY
 ON ORIGINAL

Table 38 **CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES (PURIFIED)**
 Results pooled from replicate cultures
 Assay No.: E-9442 S9-Batch no. 05081
 Compound: F 2207 Lab Code: 021285 Activation
 Dosing Date: December 3, 1985 Trial No.: 1

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION														NO. OF ABERRA-TIONS PER CELL	% CELLS WITH ABERRA-TIONS	% CELLS WITH >1 ABERRA-TIONS		
		CHROMATID							CHROMOSOME											
		TB	ID	F	TR	QR	CR	SB	AF	D	R	RI	PU	E	GY				Other	
CONTROLS UNTREATED AND SOLVENT:	a	100	1															0.01	1.0	0.0
POSITIVE: Cyclophosphamide 25.0 µg/ml		25	7			1	1											0.36	28.0*	8.0
250 µg/ml		100																0.00	0.0	0.0
500 µg/ml		100	1							4						ID1		0.06	3.0	1.0
1 mg/ml	b	100																0.00	0.0	0.0
2 mg/ml	c	84								2						ID1		0.04	3.5	0.0

*Significantly greater than untreated and solvent control, p<0.05.
 Mitotic index: a: 4.9%
 b: 4.2%
 c: 1.7%

TB: Chromatid break
 TR: Triradial
 QR: Quadriradial
 AF: Acentric fragment
 ID: Interstitial deletion

MILNACIPRAN HYDROCHLORIDE 25 mg and 50 mg
 IILD MUTAGENIC POTENTIAL

Due to a reduction of number of cells in metaphase only 75 cells were scored at 2 mg/mL and there were no treatment related aberrations.

Positive controls showed increased aberrations in the absence and presence of S-9 mixtures.

Based on the data, milnacipran did not show any treatment related aberration according to the criteria of the assay. Milnacipran was considered to be negative in the absence and presence of chromosome aberration in human peripheral lymphocytes in vitro. The study was conducted at optimum concentration based on the cytotoxicity data. The certificate of analysis to confirm the purity of the drug substance was not provided in the report. However, the certificate was provided for the same batch used in another study.

Study title: Mutagenicity evaluation of F2207 lot H7068 in the L5178Y TK +/- mouse lymphoma forward mutation assay

Key findings: F2207 is not mutagenic in the absence and presence of S-9 liver mixtures.

Study no.: T018

Volume #M4, and page #: 1

Conducting laboratory and location: []

b(4)

Date of study initiation: Nov 12, 1985

GLP compliance: Yes

QA reports: yes (x) no ()

Drug: F2207, lot #H7068, and % purity: 100.6% (certificate of analysis was provided)

Methods

Strains/species/cell line: Mouse lymphoma cell line LY5178Y TK^{+/+} was used in the assay. To reduce spontaneous mutation, cells were treated with methotrexate.

Doses used in definitive study: In the absence and presence of metabolic activation systems, the test substance was tested at doses 35 to 750 ug/mL.

Basis of dose selection: The concentrations for the mutagenicity assay were chosen on the basis of cytotoxicity of the drug. The test substance showed cytotoxicity at 630 ug/mL in the absence and presence of S-9 liver mixtures. Based on the data the highest dose for mutagenicity study was 750 ug/mL. The cytotoxicity data are shown from the Sponsor's table below.

NONACTIVATION			ACTIVATION		
TEST CONDITION	CULTURE DENSITY ^a	RELATIVE SURVIVAL (PERCENT) ^b	TEST CONDITION	CULTURE DENSITY ^a	RELATIVE SURVIVAL (PERCENT) ^b
SOLVENT CONTROL ^c	28.9	100.0	SOLVENT CONTROL ^c	25.6	100.0
TEST COMPOUND mg/ml			TEST COMPOUND mg/ml		
0.02	33.8	117.0	0.02	23.2	90.6
0.04	25.1	86.9	0.04	23.2	90.6
0.08	13.2	45.7	0.08	23.5	91.8
0.16	18.9	65.4	0.16	17.4	68.0
0.31	10.8	37.4	0.31	4.7	18.4
0.63	0.1	0.3	0.63	0.0	0.0
1.25	0.0	0.0	1.25	0.0	0.0
2.50	-	-	2.50	-	-
5.00	-	-	5.00	-	-
10.00	-	-	10.00	-	-

Negative controls: DMSO was used as a negative control.

Positive controls: Ehylmethane sulfonate (EMS) was used as positive control in the absence of S-9 liver homogenate mixtures. Methylcholanthrene (MCA) was used as a positive control for the metabolic activation assay.

Incubation and sampling times: For the non-activation and activation assays, cells were incubated with the drug substance and metabolic activation system for four hours. Recovery period for the growth was 2 to 3 days. The expression of mutant cells in the presence of 3 ug/mL TFT was carried out for 10 days.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Some of the assay criteria are shown below.

1. Absolute cloning efficiency in the solvent control system should be between 60 and higher.
2. Suspension growth for 2 days was 8
3. Mutant frequency was considered to be between 10×10^{-6} to 110×10^{-6}
4. Highest dose of the test substance to reach 10 to 20% of the growth for non-mutagenic compound unless solubility affects the assay.
5. The Sponsor stated that up to 5 mg/mL concentration would be tested if possible for a relative non-toxic compound.

Study outcome: Due to cytotoxicity, cells were cloned up to 450 ug/mL in the absence of S-9 liver homogenate mixtures. The relative growth was 14.6% compared to the solvent at the highest concentration for the cloning of cells. No mutagenicity was observed in the absence of S-9 mixtures when the assay criteria were considered. Data from Sponsor's table are shown below.

APPEARS THIS WAY
ON ORIGINAL

B. SOLVENT: Fischers' medium
 C. SELECTIVE AGENT: 3 µg/ml TFT
 D. TEST DATE: December 3, 1986

TABLE 2

TEST CONDITION	DAILY CELL COUNTS CELLS/ML 10 ⁸ UNITS		SUSP. GROWTH	AVERAGE SOLV. CONTR.	TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	% CLONING EFFICIENCY	RELATIVE GROWTH(%)	MUTANT FREQUENCY 10 ⁻⁸ UNITS
	1	2							
NONACTIVATION									
Solvent Control	14.0	12.0	18.7	19.6	59	380	63.3		31.0
Solvent Control	14.9	11.1	18.4		62	416	69.3	61.0	29.8
Solvent Control	14.1	11.6	18.2		65	306	51.0		42.5
Solvent Control	14.4	14.6	23.4		58	362	60.3		32.0
Positive Control									
0.25 µl/ml EMS	11.9	7.4	9.8		928	486	81.0	66.1	381.9
0.25 µl/ml EMS	11.9	11.0	14.5		716	341	56.8	69.0	419.9
0.40 µl/ml EMS	9.6	7.9	8.4		854	242	40.3	28.4	705.8
0.40 µl/ml EMS	9.6	10.1	10.8		845	244	40.7	36.6	692.6
TEST COMPOUND									
				RELATIVE TO SOLV. CONTR. (%)			RELATIVE TO SOLV. CONTR. (%)		
35 µg/ml	11.8	12.1		80.8			NOT CLONED		
35 µg/ml	13.1	13.4		99.3			NOT CLONED		
50 µg/ml	15.9	10.8		97.1	65	448	122.4	118.9	29.0
50 µg/ml	14.1	9.6		76.6	75	532	145.4	111.3	28.2
70 µg/ml	11.6	12.8		84.0	50	425	116.1	97.5	23.5
70 µg/ml	12.4	11.9		83.5	31	333	91.0	75.9	18.6
100 µg/ml	9.7	12.7		69.7	98	271	74.0	51.6	72.3
100 µg/ml	12.1	11.6		79.4	56	398	108.7	86.3	28.1
150 µg/ml	9.3	11.1		58.4	61	427	116.7	68.1	28.6
150 µg/ml	9.8	12.7		70.4	54	613	167.5	117.9	17.6
225 µg/ml	9.0	10.8		55.0	43	452	123.5	67.9	19.0
225 µg/ml	9.5	13.5		72.5	64	413	112.8	81.9	31.0
300 µg/ml	5.8	12.2		40.0	62	532	145.4	58.2	23.3
300 µg/ml	7.5	12.0		50.9	74	405	110.7	56.3	36.5
450 µg/ml	3.3*	9.2		15.7	40	360	98.4	15.4	22.2
450 µg/ml	3.1*	8.1		13.7	73	390	106.6	14.6	37.4
600 µg/ml	0.2*	0.6		1.0			NOT CLONED		
600 µg/ml	0.6*	0.2		0.3			NOT CLONED		
750 µg/ml	0.1*	0.0		0.0			NOT CLONED		
750 µg/ml	0.0*	0.0		0.0			NOT CLONED		

For evaluation of Table see page 15

13

Total mutant colonies were not increased compared to the control in the presence of S-9 liver mixtures. Due to cytotoxicity of the drug substance, cells were cloned up to 225 µg/mL concentration. Data from the Sponsor's table are shown below.

APPEARS THIS WAY
ON ORIGINAL

B. SOLVENT: Fischers' medium
 C. SELECTIVE AGENT: 3 µg/ml TFT
 D. TEST DATE: December 18, 1985

TABLE 3

S9 BATCH : 05081

TEST CONDITION	DAILY CELL COUNTS CELLS/ML 10 ³ UNITS		SUSP. GROWTH	TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	% CLONING EFFICIENCY	RELATIVE GROWTH(%)	MUTANT FREQUENCY 10 ⁻⁶ UNITS
	1	2						
ACTIVATION								
Solvent Control	7.6	9.3	7.9	84	452	75.3		37.2
Solvent Control	7.2	10.6	8.5	9.4	60	419	74.9	28.6
Solvent Control	19.5	9.6	11.2	70	399	64.8		36.0
Solvent Control	8.3	10.7	9.9	81	537	69.5		30.2
Positive Control.								
2.5 µg/ml MCA	3.2*	8.5	2.8	604	457	76.2	30.9	264.3
2.5 µg/ml MCA	4.0*	11.6	3.9	510	377	62.8	34.7	270.6
4.0 µg/ml MCA	3.8*	11.4	3.8	490	321	53.5	29.0	305.3
4.0 µg/ml MCA	6.1	9.6	6.5	436	388	64.7	60.1	224.7
TEST COMPOUND								
			RELATIVE TO SOLV. CONTR. (%)			RELATIVE TO SOLV. CONTR. (%)		
35 µg/ml	7.3	9.2	79.8	41	595	132.4	105.7	13.8
35 µg/ml	6.5	11.3	87.3	48	429	95.5	83.3	22.4
50 µg/ml	8.9	8.1	85.7	52	423	94.2	80.7	24.6
50 µg/ml	7.0	12.6	104.8	29	192	42.7	44.8	30.2
70 µg/ml	7.7	10.1	92.4	16	272	60.5	56.0	11.8
70 µg/ml	5.5	13.2	86.3	61	419	93.3	80.5	29.1
100 µg/ml	4.9	11.2	65.2	67	538	119.8	78.1	24.9
100 µg/ml	6.1	11.1	80.5	61	396	88.1	70.9	30.8
150 µg/ml	7.2	12.4	106.1	56	474	105.5	111.9	23.6
150 µg/ml	5.5	9.4	61.4	77	494	110.0	67.6	31.2
225 µg/ml	1.2*	4.1	14.7	39	360	80.1	11.8	21.7
225 µg/ml	0.7*	1.9	6.7	33	228	50.8	3.4	29.0
300 µg/ml	0.2*	0.3	1.1			NOT CLONED		
300 µg/ml	0.1*	0.0	0.0			NOT CLONED		
450 µg/ml	0.0*	0.0	0.0			NOT CLONED		
450 µg/ml	0.0*	0.1	0.0			NOT CLONED		
600 µg/ml	0.0*	0.2	0.0			NOT CLONED		
600 µg/ml	0.0*	0.0	0.0			NOT CLONED		
750 µg/ml	0.0*	0.0	0.0			NOT CLONED		
750 µg/ml	0.0*	0.0	0.0			NOT CLONED		

For evaluation of Table see page 15

It was concluded that F2207 was not mutagenic in the mouse lymphoma assay.

Study title: Clastogenic evaluation of F2207 lot H7068 in the in vivo mouse micronucleus assay

Key findings: F2207 is negative in mouse micronucleus assay in vivo.

Study no.: T017

Volume #M4, and page #: 1

Conducting laboratory and location: [redacted]

Date of study initiation: Nov 11, 1985

GLP compliance: Yes

QA reports: yes (x) no ()

Drug: F2207, lot #H7068, and % purity: The certificate of analysis was not attached in the report; however, it was reported in study # T018 as 100.6% pure.

b(4)

Methods

Strains/species/cell line: Male and female Swiss bred mice

Doses used in definitive study: Milnacipran was administered by oral gavage at 19, 63 and 190 mg/kg/oral doses. Animals were sacrificed at 24, 48 and 72 hours post dose. The vehicle control group received water per oral. Both vehicle and positive control mice were sacrificed at 24 hours after dosing. Animals were killed by carbon dioxide inhalation. Bone marrow from the tibia was collected into a centrifuge tube. Cells were separated and spread on slides. Cells were fixed with methanol and stained with Grünwald solution followed by Giemsa stain. Slides were scored for % micronucleated polychromatic (PCE). PCE:NCE ratios were determined. The study design is shown below from the Sponsor's table.

Treatment	Number of Animals At Each Kill Time			Total Animals
	24 Hr	48 Hr	72 Hr	
High level	10	10	10	30
Intermediate level	10	10	10	30
Low level	10	10	10	30
Positive control	10	-	-	10
Negative control	10	-	-	10

The route of administration was oral gavage.

Basis of dose selection: LD₅₀ data from the previous study

Negative controls: Water was used as a negative control

Positive controls: Cyclophosphamide was used as a positive control at 100 mg/kg/ip.

Incubation and sampling times: See above

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Significant increase in micronucleated cells in a dose dependent manner.

Study outcome:

Data show mortality in two males at 190 mg/kg after 24 and 72 hours of the treatment. No statistically significant increase in the % micronucleated PCE was noted except in male mice at 63 mg/kg after 48 hours. The PCE:NCE ratio did not show treatment related change. The positive control showed a significant increase in the % micronucleated PCE.

Data are shown from the Sponsor's table below.

TABLE 3

MICRONUCLEUS SUMMARY DATA TABLE

SPONSOR: P.F. Medicament TEST ARTICLE: F 2207 PROJECT NO.: 249.215.007
 SPECIES: Mouse Swiss Randoma SEX: 5 Males and 5 Females/dose level ASSAY NO.: E-9442

TREATMENT	DOSE	ROUTE OF ADMINISTRATION	NUMBER OF PCEs SCORED PER ANIMAL ^a	PERCENT MICRONUCLEATED CELLS: MEAN ± S.E. ^b			PCE/RBC RATIO ^c	
				MALES	FEMALES	TOTAL	MALES	FEMAL
Negative Control H ₂ O	NA	P.O.	1000	0.22±0.080	0.38±0.037	0.30±0.049	0.6	0.9
Positive Control 100 mg/kg CP		I.P.	1000	2.02±0.211**	2.60±0.526**	2.31±0.284**	0.6	0.7
Test Article								
24-hour kill	19 mg/kg	P.O.	1000	0.36±0.093	0.18±0.080	0.27±0.065	0.6	0.7
	63 mg/kg	P.O.	1000	0.46±0.144	0.36±0.121	0.41±0.090	0.7	0.6
	190 mg/kg	P.O.	1000	0.25±0.087	0.24±0.060	0.24±0.047	0.6	0.7
48-hour kill	19 mg/kg	P.O.	1000	0.20±0.071 ^d	0.26±0.024	0.23±0.037	0.5	0.8
	63 mg/kg	P.O.	1000	0.64±0.117* ^d	0.44±0.181 ^d	0.54±0.107	0.4	0.7
	190 mg/kg	P.O.	1000	0.26±0.147 ^d	0.24±0.087	0.25±0.081	0.4	0.6
72-hour kill	19 mg/kg	P.O.	1000	0.28±0.080	0.18±0.058	0.23±0.050 ^d	0.5	0.6
	63 mg/kg	P.O.	1000	0.44±0.169	0.24±0.060	0.34±0.091	0.6	0.6
	190 mg/kg	P.O.	1000	0.30±0.058	0.17±0.020	0.20±0.041	0.5	1.1

^aOnly Polychromatic Erythrocytes (PCEs) scored.
^bData analyzed by one-tailed t-test. Each animal constituted a data point.
 *Significant increase at ps0.05.
 **Significant increase at ps0.01.
^cRatio of PCEs to mature erythrocytes (RBCs).
^dIn one or two animals only 500 PCEs were scored.

Individual data from 48 hour sample are shown below.

TABLE 2 b

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: P.F. Medicament TEST ARTICLE: F 2207 PROJECT NO.: 249.215.007
 ASSAY NO.: E-9442

TREATMENT	ANIMAL NO.	MALES			FEMALES			PCE/RBC RATIO	
		NO. OF MN PCEs ^a	% CELLS WITH MN PCEs	PCE/RBC RATIO	ANIMAL NO.	NO. OF MN PCEs ^a	% CELLS WITH MN PCEs		
Test Article									
48 hour kill	Low	748	4	0.4	0.5	800	3	0.3	1.2
		755 ^b	0	0.0	0.7	809	2	0.2	1.0
		759	3	0.3	0.4	817	2	0.2	0.8
		765	1	0.1	0.6	834	3	0.3	0.4
	Medium	771 ^b	1	0.2	0.2	840	3	0.3	0.7
		747	4	0.4	0.4	799	1	0.1	1.0
		757	10	1.0	0.4	806	5	0.5	0.8
		766	6	0.6	0.7	818	0	0.0	0.6
	High	773	8	0.8	0.4	828 ^b	5	1.0	0.5
		778 ^b	2	0.4	0.2	833	6	0.6	0.8
		741	2	0.2	0.5	819	1	0.1	0.5
		745	3	0.3	0.5	826	3	0.3	0.6
767	0	0.0	0.4	837	3	0.3	0.4		
776	8	0.8	0.5	843	0	0.0	0.7		
787 ^b	0	0.0	0.2	845	5	0.5	0.7		

^a1000 PCEs were scored for micronuclei (MN) unless otherwise noted.
^b500 PCE's were scored.

Animal # 757 showed higher PCE than other animals at 48 hour end point. However, it was an abnormal response of the animal in the absence of treatment related changes in other treated animals.

It was concluded that milnacipran was negative in the mouse micronucleus test.

Summary and conclusions of genotoxicity studies:

Milnacipran is not mutagenic in the Ames, and chromosome aberration in human lymphocytes and mouse lymphoma in the absence and presence of S-9 liver homogenates. It was also not mutagenic in the mouse micronucleus test.

Assay	Study #	Result	Batch #	Purity
Ames	T010	Negative	CA6/174	Not provided
Chromosome aberration in human lymphocytes	T016	Negative	H7068	100.6%
Forward mutation, mouse lymphoma L5178Y TK +/-	T018	Negative	H7068	100.6%
Mouse micronucleus	T017	Negative	H7068	100.6%

Based on above table the certificate of analysis for the batch # CA6/174 is not provided to reference the purity of the drug substance. The deficiency was communicated to the Sponsor dated May 17, 2008.

Labeling recommendations:

Sponsor's label:

Mutagenesis

Milnacipran was not mutagenic in the in vitro bacterial reverse mutation assay (Ames test) or in the L5178Y TK +/- mouse lymphoma forward mutation assay. Milnacipran was also not clastogenic in an in vitro chromosomal aberration test in human lymphocytes or in the in vivo mouse micronucleus assay.

Reviewer's recommendation:

The reviewer agreed on the proposed label for mutagenicity studies. However, the certificate of analysis for Ames assay had incomplete data for the purity of the drug substance. Therefore, it is recommended that the study should be repeated as a Phase 4 study.

2.6.6.5 Carcinogenicity

Study title: 104-week oral dietary administration carcinogenicity study in the rat

Key study findings: Male rats showed increased incidences of thyroid C-cell adenoma and carcinoma at 50 mg/kg, the incidences of these findings were within historical control data. It is concluded that dietary administration of milnacipran up to 50 mg/kg for 104 weeks did not increase tumor incidences.

Study number: 6217-314/58 (T059/060) and F2207-0314/101-2004

Volume # 1 and page #: A1

Conducting laboratory and location: [REDACTED]

Date of study initiation: March 6, 1987

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Milnacipran hydrochloride, F2207, [REDACTED] 001, Lot # 5, 100% pure

CAC concurrence: No. The study was initiated prior to the implementation of SPA for carcinogenicity.

Study Type (2 yr bioassay, alternative model etc.): Two-year bioassay

Species/strain: [REDACTED] CD (SD) BR strain rats

Number/sex/group; age at start of study: 50/sex/gr. Age of rats was about 6 weeks at the beginning of the treatment.

The study design is shown below.

Group, Treatment	Dose, mg/kg/day	Male	Female	PK, Male	PK, Female	Laboratory investigation, Male	Laboratory investigation, Female
1, Control 1	-	50	50	-	-	5	5
2, Low	5	50	50	25	25	5	5
3, Mid	15	50	50	25	25	5	5
4, High	50	50	50	25	25	5	5
5, Control 2	-	50	50	-	-	-	-

Allotment of animals is shown in the table below.

Group, Treatment	Dose, mg/kg/day	CA, male	CA, female	PK, male	PK, female	Lab, male	Lab, female
1, Control 1	-	1-50	251-300			651-655	671-675
2, Low	5	51-100	301-350	501-525	576-600	656-660	676-680
3, Mid	15	101-150	351-400	526-550	601-625	661-665	681-685
4, High	50	151-200	401-450	551-575	626-650	666-670	686-690
5, Control 2	-	201-250	451-500				

CA= carcinogenicity phase

Animal housing: Animals were housed in individual cages at 19-25°C and 40-70% humidity.

Formulation/vehicle: The drug substance was mixed with the powdered diet. The test diet was prepared weekly on the basis of the most recent body weight. The food consumption was determined for each group/sex each week.

Drug stability/homogeneity: The Sponsor stated that stability and homogeneity of the test substance were determined in the three-month dose finding study. Stability and homogeneity of diet samples at 369.4 µg/g and 2942 µg/g corresponding to 30 and 200 mg/kg doses, respectively. Data showed 93-95% compliance to nominal concentrations. The drug substance in the diet mixture was stable for at least two weeks at room temperature. Furthermore, 100 g of diet-food samples from each group and sex were taken in week 1 and every 3 months for the determination of milnacipran levels in the diet. In general, the drug concentrations in the diet were in compliance with the nominal concentrations except for few incidences.

Methods:

Doses: Doses used in the study were 5, 15 and 50 mg/kg/day.

Basis of dose selection:

Two 3-month dose range-finding studies (#T023, and #T031) were conducted for the selection of doses in the carcinogenicity study. These studies were reviewed under IND 63,736 dated July 12, 2004 and presented in the CAC-EC briefing on June 15, 2004. Since a 52-week chronic toxicity study is reviewed for the NDA, it was felt that a further written review for study # T023 would not be necessary. However, some of the critical findings to support appropriate dose selection for the carcinogenicity study is presented below. Study #T031 was conducted in CD(SD)BR male rats at 60 and 200 mg/kg in diet. Study #T023 was conducted in male and female CD(SD) BR rats at 30, 60, 100, 150 and 200 mg/kg/day doses given in the diet. The actual dose was close to the nominal dose during the study. No premature deaths were reported in the study. The body weight (g) data are shown in the table below.

b(4)

Male						
Week	Control	30 mg/kg	60 mg/kg	100 mg/kg	150 mg/kg	200 mg/kg
0	160	163	160	152	161	165
13	511	472	477	407	407	379
Gain (g)	350	308*	317*	255*	247*	214*
Gain, %control	-	88%	90%	73%	70%	61%
BW, %control	-	92%	93%	79%	79%	74%
Female						
0	128	126	126	129	127	128
13	240	226	237	217	204	200
Gain (g)	112	100	111	88*	76*	71*

Gain, %control	-	90%	99%	78%	68%	64%
BW, %control	-	94%	98%	90%	85%	83%

The total food consumption (g/wk) is shown in the table below.

Group	1	2	3	4	5	6
Dose, mg/kg	Control	30	60	100	150	200
Male	2372	2257 (↓5%)	2244 (↓5%)	1961* (↓17%)	1900* (↓20%)	1814* (↓24%)
Female	1513	1488 (↓2%)	1503	1389 (8%)	1327* (↓12%)	1331* (↓12%)

*statistically significant (P<0.05)

The data suggest that the body weight and food consumption in male and female rats were affected at 100 mg/kg and higher doses. The actual dose delivered via the diet was close to the targeted dose. A decrease in the food consumption, body weight and target drug intake would indicate a palatability of the drug-diet mixtures. Since the target drug intake was achieved in the study, the decrease in food consumption does not appear to be due to palatability of the drug-diet mixtures.

Histopathology of the liver from each animal was conducted. In addition, histopathology of all gross organs was also conducted. Histological data revealed hypertrophy and vacuolation in the liver of all treated male rats. Based on a 4-point severity scale, male rats showed minimal to severe hypertrophy and vacuolation of the liver at 100 mg/kg and higher doses. No other histopathological change in the male rat was noted. The slight hepatocellular hypertrophy was observed in female rats at 100, 150 and 200 mg/kg dose.

Above study was repeated at 60 and 200 mg/kg in male rats. Hepatocellular fat deposits were evident in animals treated with either 60 or 120 mg/kg. Centrilobular hypertrophy was evident only at the 200 mg/kg group. The Sponsor's selection of 50 mg/kg as the high dose for the 104-week study was based on the presence of liver hypertrophy and a decrease in the body weight at 100 mg/kg in the three-month study.

Restriction paradigm for dietary restriction studies: No restriction

Route of administration: Oral with diet

Frequency of drug administration: Ad lib

Dual controls employed: Yes

Interim sacrifices: No

Satellite PK or special study group(s): Satellite groups were allotted for TK and clinical pathology.

Deviations from original study protocol: None

Statistical methods: Survival probability function was determined by the Kaplan-Meier technique. Hematology and clinical chemistry data were analyzed by parametric methods. Statistical analysis of the data was performed according to

Peto if the total number of incidences from all stratified tables was greater than five. Otherwise, the interval-based exact permutation test was used.

Observations and times:

Clinical signs: Animals were examined twice daily to detect mortality or moribund conditions. Clinical signs were examined once a day. Each animal had a detailed clinical examination at weekly.

Body weights: Body weights were recorded at predose, on the first day of treatment, and once a week up to week 16. Thereafter, body weights were recorded once every 4 weeks.

Food consumption: Food consumption was recorded once a week up to week 16 and at 4 week intervals for rest of the study.

Hematology: Blood samples were obtained during weeks 4, 26, 52 and 104 from satellite animals. However, due to mortality in the satellite groups, blood samples were also obtained from the main study group in week 104. Samples were collected from the orbital sinus under light ether anesthesia. Animals were fasted overnight before blood collection. Red cell parameters, WBC and platelet counts were recorded.

Clinical chemistry: Plasma samples were taken for standard clinical chemistry parameters. Plasma electrolyte levels were not determined.

Organ weights: Animals were sacrificed by IP injections of sodium pentobarbital. External surface, thoracic, abdominal and cranial cavities were examined. Organ weights for the following organs were recorded: adrenals, kidneys, ovaries, testes, uterus, heart, liver, spleen and thyroid.

Histopathology: Protocol specified tissues were fixed in 10% formalin with the exception of eyes, which were fixed in Davidson's fluid. **Histopathology was conducted** on all tissues from control groups, high dose group, gross lesions and all tissues from animals that died or were killed in moribund condition. Liver samples from groups 2 and 3 were also processed for histological examinations. Slides were stained with hematoxylin and eosin.

Following review of the results on June 15, 2004, the executive Carcinogenicity Assessment Committee (CAC) requested the Sponsor to provide histological data for thyroid tumors from all male rats in the carcinogenicity phase and the laboratory investigation phase including low (group 2) and mid (group 3) dose animals. The Sponsor stated that both original slides of male thyroid in control, low, mid and high dose groups, and newly prepared thyroid slides for the low and intermediate group terminal kill males were considered to be suitable for an accurate examination for both C-cell hyperplasia and C-cell neoplasia. Thyroid C-cell histopathology from all male rats was further examined by a peer review committee assigned by the Sponsor.

Toxicokinetics: Five rats/sex/group were bled in the morning in weeks 1, 4, 26, 52 and 104 for the determination of drug plasma levels. Animals were discarded after blood collection. Blood samples were also collected from animals in main study groups on week 104 for the determination of plasma drug levels. The blood samples were collected one hour following the start of light cycle. However, the Sponsor did not mention the time intervals for the collection of the sample.

Results:

Mortality: The percent of rats that survived at the end of the study in each group are shown in the table below.

	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	5 mg/kg	15 mg/kg	50 mg/kg	Control
Male	44%	46%	46%	44%	48%
Female	52%	64%	58%	53%	54%

The survival data from the statistical report are shown in the table below.

Gender	Group 1 (Control)	Group 2 (control)	Group 3 (5 mg/kg)	Group 4 (15 mg/kg)	Group 5 (50 mg/kg)
Male	30/55	26/50	29/55	31/55	30/55
Female	26/55	23/50	21/55	25/55	29/55

The above data indicate that animals allotted to clinical pathology were counted for the analysis for survival. There were no differences in the survival between groups.

Clinical signs: No treatment related clinical signs were reported in male and female rats.

Body weights: Average body weight (g) data are shown in the table below. Percent change compared to the combined control is presented in the table.

	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	5 mg/kg	15 mg/kg	50 mg/kg	Control
Male					
Week 0	195	197	196	200	199
104 week	647	636	653	585	664
BW, % Control		97%	99.6%	89%	
Wt gain	457	440	456	384*	468
% Wt gain		95%	98.6%	83%	
Female					
Week 0	149	152	151	148	149
Week 104	470	455	415	374	468
BW, % Control		97%	88%	79.7%	
Wt gain	322	304	262*	224*	319
%Wt gain		95%	81.8%	70%	

Male rats at 50 mg/kg showed 11 and 17% reduction in the body weight and weight gain, respectively, compared to combined control animals. Female rats showed average 20 and 30% reduction in the body weight and weight gain, respectively, compared to the

combined control at 50 mg/kg. Female rats also showed average 12% reduction in the body weight compared to the combined control at 15 mg/kg.

Food consumption: Average food consumption (g/animal/week) for week 1 and 104 is shown in the table below. Data suggested that food consumption was reduced in male rats treated with 50 mg/kg at several weeks during the treatment period. Female rats also showed a similar reduction in the food consumption following dosing at 15 and 50 mg/kg. These data suggest the possibility that the reduction in weight gain in male and female rats may be due to a reduction in food intake during the treatment period.

	Control	5 mg/kg	15 mg/kg	50 mg/kg	Control
Male					
Week 1	168	164	163	156	168
Week 104	160	157	158	144	155
Female					
Week 1	120	117	117	108	122
Week 104	136	123	118	119	133

Calculated milnacipran consumption was acceptable, although some variations were observed.

Hematology: Hematology data did not show any treatment related changes.

Clinical chemistry: There were no treatment related changes of toxicological significance in the study. Plasma levels of thyroid hormones were not determined.

Organ weights: Organ weight data showed a slight increase in the mean thyroid weight in both males and females when data were normalized to percent body weight. There were changes in heart and kidney weights at the high dose that were statistically significant but biologically insignificant. The weight of thyroid as % body weight is shown in the table below.

	Group 1 Control	Group 2 5 mg/kg	Group 3 15 mg/kg	Group 4 50 mg/kg	Group 5 Control
Male					
Thyroid, left	0.0025	0.0035*	0.0025	0.0041*	0.0027
Thyroid, right	0.0030	0.0035*	0.0027	0.0034*	0.0024
Female					
Thyroid, left	0.0030	0.0032	0.0036*	0.0039*	0.0031
Thyroid, right	0.0026	0.0032	0.0034*	0.0039*	0.0031

Gross pathology: Necropsy findings showed a lower incidence of enlarged pituitary in drug treated animals compared to controls. However, changes in the pituitary were neither statistically significant nor were of a magnitude that would support biological significance.

Histopathology:

Non-neoplastic:

Some of the non-neoplastic findings are shown in the table.

Group	Males					Females				
	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5
Dose (mg/kg)	Cont	5	15	50	Cont	Cont	5	15	50	Cont
Keratitis	1	0	2	4	1	0	0	2	5	1
Liver, Eosinophilic focus	5	8	16	16	7	1	5	9	10	3
Liver, centrilobular vacuolation	0	0	3	13	0	0	1	1	0	0
Urinary bladder, cystitis	1	0	3	4	1	0	1	0	0	0

Keratitis and the presence of focal eosinophilia in the liver were observed in male and female rats at 15 and 50 mg/kg. Keratitis could be related to dryness and scratching of eyes. The issue needs to be addressed to the medical reviewer and mention as the non-clinical finding in the package insert upon chronic uses of the drug. The cause of liver eosinophilic focus and vacuolation is not known. However, doses higher than 30 mg/kg showed vacuolation in the liver in several studies in rats. Therefore, based on the non-clinical data yearly check up for liver transaminase activity should be recommended in the label for clinical uses.

Thyroid C-cell hyperplasia in male and female rats is shown in the table below.

	Males					Females				
	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5
Dose (mg/kg)	Cont	5	15	50	Cont	Cont	5	15	50	Cont
Number examined	50	30	28	51	49	55	55	55	55	50
Thyroid C-cell hyperplasia	22	8	7	18	19	30	10	13	24	34

Histopathology of all animals for thyroid C-cell hyperplasia in male rats based on consensus diagnosis was resubmitted on Aug 24, 2005 (SN # 113, IND 63,736) and study # F2207-0314/101-2004 for NDA 22-256 as shown in the table below.

	Gr 1 control	Gr 2 5 mg/kg	Gr 3 15 mg/kg	Gr 4 50 mg/kg	Gr 5 control
# examined	50	53	47	51	49
Incidence	23	26	18	18	20
Grade 1	13	10	6	11	12
Grade 2	9	16	8	7	8
Grade 3	1	0	4	0	0

Thyroid C cell hyperplasia was noted in high incidences in the control and treated animals.

Neoplastic: Neoplastic findings from the statistical database were provided by the statistical reviewer before updating the histopathology data with respect to thyroid. Data are shown below. Group 0=control 1, group 1= control 2, group 2= 5 mg/kg, group 3= 15 mg/kg, group 4= 50 mg/kg.

**APPEARS THIS WAY
ON ORIGINAL**

SEX	ORGANNAM	TUMORNAM	0	1	2	3	4
Male	adrenal	adenoma-b	1
		pheochromocytoma-b	10	15	8	2	5
	bone	pheochromocytoma-m	2	1	3	1	1
		osteoma-b	1
	brain	adenoma-b	.	.	.	1	.
		glioma-m	.	1	.	.	1
		menigioma-b	.	.	1	.	.
	caecum	papilloma-b	.	.	.	1	.
		adenoma-b	.	.	.	1	.
	connective tiss	fibroma-b	.	.	.	1	.
		lipoma-b	.	.	.	1	.
		liposarcoma-m	.	.	1	.	.
	ear	sarcoma-m	1
		papilloma-b	.	.	1	.	1
	eyes	melanoma-b	1	.	.	.	1
	foot/leg	papilloma-b	1
	hem/lymph/retic	histiocytic sarcoma-	1	.	2	1	3
		leukemia granulocyti	.	1	1	.	2
	kidneys	lipomatous tumor-b	.	2	.	.	.
		sarcoma-m	1
	liver	adenoma-b	1	.	2	.	.
	mammary	carcinoma-m	.	.	1	.	.
	nerve	schwannoma-m	.	.	.	1	.
	pancreas	islet cell adenoma-b	1	4	1	2	3
		islet cell carcinoma	1
	pituitary	adenoma-b	28	32	21	18	28
		carcinoma-m	.	1	.	.	1
	skin subcutis	basal cell tumor-b	.	1	.	1	.
		fibroma-b	1	3	8	4	3
		fibroma-b, dermal	3	6	3	2	2
		fibrosarcoma-m	2	.	1	1	.
		lipoma-b	3	4	2	.	1
		liposarcoma-m	.	1	.	.	.
		papilloma-b	9	7	9	6	4
		sarcoma-m	.	1	.	1	.
	stomach	squamous carcinoma-m	.	.	.	1	.
	systemic tumor	hemangioma-b	1	.	2	.	.
		hemangiosarcoma-m	.	.	1	.	.
	tail	papilloma-b	.	1	.	.	.
	testes	Leydig cell tumor-b	5	2	6	3	6
	thyroids	c-cell adenoma-b	4	2	3	1	11
		c-cell carcinoma-m	1	.	1	.	.
		follicular adenoma-b	5	4	.	.	3
		follicular carcinoma	.	.	.	1	.

APPEARS THIS WAY
ON ORIGINAL

SEX	ORGANNAM	TUMORNAM	DOSEGRP					
			0	1	2	3	4	
Female	abdominal cavit	lipoma-b	1
		adrenal	1
	adrenal	adenoma-b	1
		carcinoma-m	1
		pheochromocytoma-b	2	2
	brain	adenoma-b	.	.	1	.	.	.
		carcinoma-m	1	.
		glioma-m	.	1	.	.	.	2
	caecum	menigioma-b	.	.	.	1	.	.
		adenoma-b	1
	colon	lipoma-b	.	.	1	.	.	.
		papilloma-b	.	.	1	.	.	.
	duodenum	adenoma-b	1
		fibroadenoma-b	.	.	2	.	.	.
	esophagus	c-cell adenoma-b	.	.	1	.	.	.
		carcinoma-m	.	.	1	.	.	.
	ear	papilloma-b	1
	eyes	adenoma-b	1
		follicular adenoma-b	1
	heart	adenoma-b	1
	hem/lymph/retic	histiocytic sarcoma-	.	.	1	.	.	.
		leukemia granulocyti	.	1
	ileum	adenoma-b	1
	jejunum	fibroadenoma-b	1
	kidneys	carcinoma-m	1
		lipomatous tumor-b	1	.	.	1	.	.
	liver	sarcoma-m	.	1
		adenoma-b	.	1
	mammary	carcinoma-m	3	2	4	3	4	.
		fibroadenoma-b	24	18	14	14	9	.
	ovaries	granulosa theca tumo	.	.	.	1	.	.
	pancreas	islet cell adenoma-b	.	.	.	2	.	.
		islet cell carcinoma	1	2	.	.	.	1
	parathyroid	adenoma-b	2
	pituitary	adenoma-b	43	38	27	29	36	.
		carcinoma-m	4	.	1	2	2	.
	skin subcutis	fibroma-b	2	3	2	.	1	.
		fibroma-b, dermal	.	.	1	.	.	.
		lipoma-b	2	1	1	.	.	.
		liposarcoma-m	.	.	1	.	.	.
	stomach	papilloma-b	.	.	.	1	.	.
	systemic tumor	hemangiosarcoma-m	1
		papilloma-b	1
	tail	adenoma-b
		carcinoma-m
	thyroids	c-cell adenoma-b	12	10	2	2	4	.
		c-cell carcinoma-m	.	1	.	.	.	1
	thyroids	follicular adenoma-b	1	1	1	1	.	.
		follicular carcinoma	1
	uterus	adenomatous polyp-b	.	.	1	.	.	1
carcinoma-m		1	
vagina	stromal polyp-b	6	3	4	3	2	.	
	stromal sarcoma-m	.	.	.	1	1	.	
	polyp-b	.	.	1	.	.	.	

Absence of any histological incidence was marked by a dot in the above table.

Histopathology data showed an increased incidence of thyroid C-cell adenoma in male rats. Otherwise, there was no treatment-related increase in tumor incidences. Incidences were 4/50, 2/49, 3/30, 1/28 and 11/51 (21.6%) for control 1, control 2, low, mid and high doses, respectively. Incidence as high as 14.2% was reported in the literature in male rats of the strain used in the study. The Sponsor provided the incidence of 4-24% in male and female rats based on historical control data collected between 1987 to 1989 from

The historical control data for male rats showed 11-24% during 1987 to 1988. In the present study high incidences of the common tumor were observed in male rats only. The Sponsor stated that the occurrence of this common tumor in the control male groups was smaller than that in the high dose group and that resulted statistically significant outcome in the treated group. Female rats did not show any increased trend in tumor incidence.

b(4)

The amendment # 113 (IND 63,736) and study # F2207-0314/101-2004 for NDA 22-256 provided data for thyroid C-cell hyperplasia and neoplasia from all male rats as shown in the table below.

Lesion	# examined	Control 1	Control 2	5 mg/kg	15 mg/kg	50 mg/kg
C-cell hyperplasia		23 Gr 1 = 13, Gr 2 = 9, Gr 3 = 1	20 Gr 1 = 12, Gr 2 = 8	26 Gr 1 = 10, Gr 2 = 16,	18 Gr 1 = 6, Gr 2 = 8, Gr 3 = 4	18 Gr 1 = 11, Gr 2 = 7
C-cell adenoma		3	2	6	6	11
C-cell adenoma (%)		6%	4.08%	11.3%	12.76%	21.56%
C-cell carcinoma		1	0	1	0	0
C-cell carcinoma (%)		2%	0	1.88%	0	0
C-cell adenoma or carcinoma		4	2	6	6	11
C-cell adenoma or carcinoma (%)		8%	4%	11%	13%	22%

Gr 1 = minimal, Gr 2 = slight, Gr 3 = moderate

Study Number 0314/101
Final Report

b(4)

Appendix 1
Summary of histopathology findings:

	Dose Group	Control 1			Low			Intermediate			High			Control 2
		CP	LI	T	CP	LI	T	CP	LI	T	CP	LI	T	CP
	AUTOLYSED	50	4 ⁽¹⁾	54	50	3 ⁽²⁾	53	50	5	55	50	4 ⁽³⁾	54	50
	EXAMINED	4	0		0	0		6	2		2	1		1
THYROID	TOTAL			50			53			47			51	49
C-cell hyperplasia	OVERALL INCIDENCE			23			26			18			18	20
	Grade :-	26	1	27	26	1	27	29	0	29	33	0	33	29
	Grade 1	10	3	13	8	2	10	5	1	6	10	1	11	12
	Grade 2	9	0	9	16	0	16	7	1	8	7	0	7	8
	Grade 3	1	0	1	0	0	0	4	0	4	0	0	0	0
	C-cell adenoma	3	0	3	6	0	6	5	1	6	9	2	11	2
	C-cell carcinoma	1	0	1	1	0	1	0	0	0	0	0	0	0
	Adenoma /carcinoma-tumour bearing	4	0	4	6	0	6	5	1	6	9	2	11*	2
	%incidence			8			11			13			22	4

Key:

- CP= Findings from animals assigned to the Carcinogenicity Phase
- LI= Findings from animals assigned to the Lab. Investigations Phase
- T= Findings from total animals (CP+LI)
- Grade - : finding not present
- Grade 1 : minimal
- Grade 2 : slight
- Grade 3 : moderate
- * P = 0.003

(1) 4 animals in satellite group reported instead of 5. Slide from animal 655 not available
 (2) 3 animals in satellite group reported instead of 5. Slide from animals 658 & 659 not available
 (3) 4 animals in satellite group reported instead of 5. Slides from animal 670 not available

The Sponsor's assembled peer review committee also examined the data. The peer review committee consisted of an external pathologist chosen by the Sponsor and a pathologist representing the Sponsor. The peer review committee consisted of [redacted] and R. Grosse, M.D., Institute de Recherche Pierre Fabre, France, carried out the peer review in accordance to NTP, Society of Toxicology and IARC guidelines. The consensus for three male rats in the control and low dose group differed from the original diagnosis as shown below.

b(4)

1. Group 1 (control), male 28: Original diagnosis was thyroid C-cell adenoma
Consensus diagnosis: Moderate Thyroid C-cell hyperplasia
2. Group 2 (5 mg/kg), male 69: Original diagnosis was thyroid C-cell carcinoma
Consensus diagnosis: C-cell carcinoma, C-cell adenoma, minimal C-cell hyperplasia

3. Group 2 (5 mg/kg), male 81: Original diagnosis was thyroid adenoma
Consensus diagnosis: C-cell adenoma and minimal C-cell hyperplasia

Statistical significance in C-cell adenoma and carcinoma was observed only at 50 mg/kg with a p value of 0.003 between control and the high dose group (Sponsor's analysis). The low and mid doses did not show statistical significance when compared to the control. The Sponsor stated that a dose response trend was observed for C-cell adenoma and carcinoma (p=0.001). The level of statistical significance (p value) for the common tumor was 0.01 for pair wise comparison and 0.005 for the trend test. Overall, the resubmitted data base for thyroid C-cell adenoma and carcinoma in the male rats showed higher incidence in the high dose group. A statistical review of the data was requested to the Division of Biometrics VI. The statistical review dated Jan 27, 2006 showed that the incidence of adenoma in the thyroid C cell, combined incidences of adenoma and carcinoma of thyroid C cell were significantly higher in the high dose group compared to the combined control groups in male rats.

The historical control data for thyroid C-cell neoplasm in the same strain of rats and the laboratory (see the table below) is higher than that observed, it is concluded that the treatment with milnacipran did not show neoplastic changes in the male and female rats in the 2-year carcinogenicity study.

Historical control data for thyroid C-cell neoplasia are shown below.

Study #	%Male	% Female	Study month/year
A	24	12	11/88
B	20	11	9/88
C	17	15	6/88
D	16	2	2/88
E	18	16	1/88
F	12	8	6/87
G	17	4	6/87
H	11	10	4/87

Toxicokinetics: Based on the data of plasma milnacipran levels, there was evidence of absorption of the drug when given in the diet. The limit of detection was 2 ng/mL. However, the level was often non-detectable at 5 mg/kg dose in male rats. Female rats showed higher levels than male rats and the level increased dose proportionately. Data were not collected at multiple time points and plasma exposure to the drug is unknown.

The plasma level from male and female rats is shown from the Sponsor's table below.

TABLE 1 - PLASMA LEVELS OF UNCHANGED P 2207 AS A FUNCTION OF TIME IN MALE RATS TREATED FOR 104 WEEKS

Group Dose	Week 1		Week 4		Week 26		Week 52		Week 104	
	Animal number	Concentration (ng.ml ⁻¹)	Animal number	Concentration (ng.ml ⁻¹)	Animal number	Concentration (ng.ml ⁻¹)	Animal number	Concentration (ng.ml ⁻¹)	Animal number	Concentration (ng.ml ⁻¹)
(2) 5 mg/kg/d	501	72	506	9*	511	N.D.	516	N.D.	97	27
	502	N.D.	507	N.D.	512	16*	517	N.D.	521	N.D.
	503	N.D.	508	14*	513	26*	518	N.D.	523	N.D.
	504	N.D.	509	12*	514	18*	519	N.D.	524	N.D.
	505	N.D.	510	16*	515	N.D.	520	N.D.	525	62
	N.D. (4) 72 (1)			N.D. (1) 13 (4)		N.D. (2) 20 (3)		N.D.		N.D. (3) 44.5 (2)
(3) 15 mg/kg/d	526	33	531	62	536	119	541	108	146	N.D.
	527	29*	532	48	537	92	542	81	148	30
	528	38	533	68	538	123	543	51	149	305
	529	N.D.	534	69	539	80	544	64	546	97
	530	42	535	75	540	132	545	9*	547	120
	N.D. (1) 36 (4)			64		109		63	549	128
(4) 50 mg/kg/d	551	326	556	428	561	785	566	553	186	100
	552	158	557	278	562	515	567	459	188	562
	553	203	558	489	563	620	568	559	189	404
	554	155	559	557	564	568	569	730	199	511
	555	239	560	380	565	597	570	505	200	1068
	216		426		617		561		571	976
									574	1048
									667	

N.D. : not detected (< 2 ng)

* : extrapolated values

APPEARS THIS WAY
ON ORIGINAL

TABLE 2 - PLASMA LEVELS OF UNCHANGED P 2207 AS A FUNCTION OF TIME IN FEMALE RATS TREATED FOR 104 WEEKS

Group Dose	Week 1		Week 4		Week 26		Week 52		Week 104	
	Animal number	Concentration (ng.ml ⁻¹)								
5 mg/kg/d	576	N.D.	581	N.D.	586	50	591	N.D.	347	N.D.
	577	N.D.	582	17*	587	N.D.	592	N.D.	348	38
	578	120	583	20*	588	64	593	32	350	30
	579	N.D.	584	15*	589	N.D.	594	17	599	38
	580	N.D.	585	12*	590	N.D.	595	N.D.	600	25
		N.D. (4) 120 (1)		N.D. (1) 16 (4)		N.D. (3) 57 (2)		N.D. (3) 25 (2)		N.D. (1) 33 (4)
15 mg/kg/d	601	43	606	106	611	161	616	146	621	204
	602	73	607	152	612	231	617	88	622	91
	603	48	608	133	613	118	618	96	623	124
	604	52	609	75	614	820	619	100		
	605	42	610	155	615	313	620	155	398	162
								400		125
50 mg/kg/d		57		124		329		117		141
	626	187	631	514	636	756	641	460		
	627	280	632	496	637	510	642	720	446	834
	628	171	633	447	638	299	643	755	447	1125
	629	202	634	505	639	409	644	557	448	1142
								449		963
								648		73
		234		486		567		644		827

N.D. : not detected (< 2 ng)

* : extrapolated values

Above data suggest that there was no difference in the plasma levels of milnacipran between male and female rats and the concentration increased with the dose. The average concentrations were 667 and 827 ng/mL at 50 mg/kg in male and female rats, respectively.

Summary of individual study findings:

The 104-week dietary carcinogenicity study in rats did not show excess mortality at the high dose compared to the control. However, there was a reduction in the body weight and weight gain at the high dose. The ECAC recommendation for the rat carcinogenicity study on June 15, 2004 is shown below.

“The Committee concluded that the MTD was achieved, based on the reduced relative body weight of >10 % observed in both male and female rats at the high dose, 50 mg/kg, in the 2-year studies. However, histopathologic evaluation of the thyroid for all male rats in the low and mid doses is needed for the statistical evaluation. The evaluation of the new data should employ trend analysis and pairwise comparison with controls individually and combined.”

The Sponsor submitted updated thyroid C-cell histopathology data for male rats at low and mid doses according to the recommendation of ECAC on June 15, 2005. The

updated data showed statistically significant increase in incidences of thyroid C-cell neoplasm (adenoma and carcinoma combined) at 50 mg/kg compared to the control. However, incidences were within the historical range (4-24%) in the same strain of male and female rats from [redacted] collected during 1987-1989. The historical control data for same strain male rats for thyroid C-cell neoplasm was between 11 to 24%. The statistical analysis of combined adenoma and carcinoma of thyroid C cell in the male rat showed statistically significant trend. Pairwise comparison with the combined control also showed statistically significant increase in the thyroid C-cell tumor at 50 mg/kg (see statistical review dated Jan 26, 2006). On the basis of high incidences of thyroid C-cell hyperplasia, adenoma and historical control data, the reviewer concluded that milnacipran is not carcinogenic in the rat. However, carcinogenicity assessment committee executive committee (CAC-EC) recommended that the finding of thyroid C-cell adenoma in male rats should be included in the package insert. b(4)

Adequacy of the carcinogenicity study and appropriateness of the test model:

The study is acceptable based on the body weight parameter. A reduction in the weight gain is consistent with the known pharmacological action of this class of drugs, and the high dose achieved the pharmacological effect during the treatment. Average plasma concentrations of the drug in male and female rats was 39, 139 and 747 ng/mL at 5, 15 and 50 mg/kg, respectively.

Evaluation of tumor findings: Thyroid C-cell adenoma is a common tumor and prevalent in untreated rats. Considering a higher tumor incidence of this neoplasm only in the male rats and within the historical data range, the reviewer considered that the incidence is not treatment related.

Carcinogenicity summary: The 104-week carcinogenicity study was conducted in [redacted] rats. The drug substance was delivered through diet. Data suggested that milnacipran was stable in the diet during the feeding period. There was a proof of absorption and the calculated doses were in compliance with the target doses. b(4)

The rat study was conducted at doses 5, 15 and 50 mg/kg. There was a reduction in the body weight and food consumption at 50 mg/kg. The mortality was not affected. There was no treatment related tumor incidences except thyroid C-cell adenoma in male rats. Thyroid C-cell tumor is a common tumor. The incidence might not be treatment-related even it reached statistical significance. In addition, it was within the historical data range (4-24% for male and female rats provided by the Sponsor).

The Sponsor provided following kinetic data in humans at 100 mg bid dose that is considered to be maximum recommended human dose (MRHD), these data could be factored for animal to human dose comparison:

Cmax: 539 ng/mL, AUC 6650 ng.h/mL at the steady state

Carcinogenicity conclusions: It is concluded that MTD was achieved in the rat study. The reviewer recommended that milnacipran did not show carcinogenic potential up to 50 mg/kg based on the updated histopathology data and data from the historical control. However, CAC-EC recommended that the finding of thyroid C-cell adenoma needs to be addressed in the package insert.

Recommendations for further analysis: Nil

Labeling Recommendations:

The Sponsor's annotated label is shown below.

b(4)

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: A fertility study in rats treated orally with TN-912

Key study findings: Data suggest that the mating performance was delayed at 20 and 80 mg/kg. The fertility of rats was reduced at 80 mg/kg. The no effect dose was 5 mg/kg in Wistar rats.

Study no.: T092

Volume #M4, and page #: 1

Conducting laboratory and location: []

b(4)

Date of study initiation: June 27, 1989

GLP compliance: Yes

QA reports: yes (x) no ()

Drug: TN-912, lot # C 003, and % purity: Above 99.9%, Lot certificate was not provided on June 9, 2008.

b(4)

Methods

Doses: 5, 20 and 80 mg/kg/oral, the dose level was determined from previously conducted 4-week repeat dose study at 10, 35 and 120 mg/kg. The body weight was reduced at 35 mg/kg and above doses. Another reproductive safety study at 5, 15 and 60 mg/kg also showed a decrease in the body weight gain at 60 mg/kg.

Species/strain: Wistar SPF rats, male weighed 138-153 g and female weighed 208-244 g at start of administration of the drug. At the beginning of the treatment, age of animals was 6 and 13 weeks for male and female, respectively,

Number/sex/group: The study design is shown below.

Test Groups	Dose Levels* (mg/kg)	Concentration of Test Solutions (%)	Males		Females	
			No. of Animals	Animal No.	No. of Animals	Animal No.
Control	0	0	24	1001 - 1024	24	1101 - 1124
Low	5	0.1	24	2001 - 2024	24	2101 - 2124
Intermediate	20	0.4	24	3001 - 3024	24	3101 - 3124
Highest	80	1.6	24	4001 - 4024	24	4101 - 4124

*: Dose levels shown as bulk levels, purity conversion was not done.

Route, formulation, volume, and infusion rate: The test article was dissolved in water for injection. The drug substance solution was administered by oral gavage to male rats for 9 weeks before mating and during mating. Female rats were treated for 2 weeks before mating, during mating and up to gestation day 7. The dose volume was 0.5 mL/kg. Control animals were treated with water for injection.

Satellite groups used for toxicokinetics: No satellite group was allotted for TK.

Study design: Animals were observed 3 times daily during the treatment and twice daily during other periods for mortality and clinical signs. The body weight was recorded twice a week during pre-mating and mating periods. The body weight of female rats was recorded on gestation days 0, 4, 7, 11, 14, 17 and 20. The food consumption was recorded at the time of body weight recording. The food consumption of female rats was also recorded on gestation days 1, 4, 7, 11, 14, 17 and 20.

Parameters and endpoints evaluated: Estrus cycle of female rats were monitored during the pre-mating period. Male and female rats after 9 and 2 weeks of dosing were allowed to mate from the same treatment group on a one to one basis. Presence of vaginal plug was considered as gestation day 0. Animals were allowed to mate for a maximum period of 2 weeks. Unmated male rats were allowed to mate with an untreated female with normal estrus cycle and the unmated female rat was paired with a proven male for another one week. Following parameters were determined:

Copulation index = # copulated animals / # housed together x 100

Insemination index = #male inseminated/# male copulated x 100

Fertility index = # Pregnant rats/ # copulated females x 100

Male rats:

Male rats were sacrificed on the day of copulation by ether anesthesia. Testes, epididymis, seminal vesicles, and prostate were weighed and fixed in 10% formalin. Reproductive tissues from male rats where copulation was confirmed but females were not pregnant were stained with hematoxylin and eosin for histopathological examinations.

Female rats:

Pregnant rats were sacrificed on day 20 and following parameters were determined: number of corpora lutea, number of implantations, number of live fetuses, number of resorption and dead fetuses.

Ovaries and uteri from non pregnant animals were examined histologically following hematoxylin and eosin staining.

Live fetuses were examined for external abnormalities. Half of total fetuses from each litter were fixed in Bouin's fixative to examine visceral abnormalities according to Wilson's methods. The other half were treated with 70% alcohol, stained with Alizarin red to examine the skeletal abnormalities according to Dawson's methods.

Results

Mortality: No treatment related mortality was reported.

Clinical signs: Salivation was noted at 80 mg/kg in male rats during premating period. Female rats also showed salivation during premating and gestation period at 80 mg/kg.

Body weight:

Male:

The average body weight (g) for male rats before mating is shown below.

Day	Control	5 mg/kg	20 mg/kg	80 mg/kg
0	144	144	144	144
63	374	371	363	348*
Weight gain	230	227	219	204

*statistically significant

At the end of pre-mating period, the body weight gain of male rats was reduced by about 12% at 80 mg/kg.

Female:

The body weight of female rats did not change substantially due to the treatment during the pre-mating period. However, a slight reduction the body weight was noted at 80 mg/kg during the gestation period as shown in the Sponsor's table below.

Table 9 Fertility study in rats treated orally with TN-912
Body weights of F0 dams during the gestation period

Dose mg/kg		-- Administration --						
		0	4	7	11	14	17	20a)
0	No.	24	24	24	24	24	24	24
	Mean	233.4	243.2	250.8	265.3	277.0	298.7	334.8
	S.D.	10.1	9.8	10.9	11.7	11.4	12.3	12.8
5	No.	23	23	23	23	23	23	23
	Mean	232.0	240.2	247.0	260.7	274.0	294.7	331.2
	S.D.	11.3	11.2	11.5	12.0	12.3	15.7	19.8
20	No.	24	24	24	24	24	24	24
	Mean	232.0	241.4	247.3	261.3	274.5	297.1	332.0
	S.D.	12.6	11.7	12.2	13.1	14.7	16.9	21.2
80	No.	21	21	21	21	21	21	21
	Mean	228.4	233.2**	238.7**	254.0**	269.3*	291.5	327.7
	S.D.	11.5	11.7	11.9	12.8	12.9	14.8	17.7

No.: No. of dams
a) : Day of gestation
* : p<0.05 ; ** : p<0.01 (Significant difference from control)
Unit : g

Food consumption: The average food consumption of male rats at the end of pre-mating period was not affected by the treatment.

Female rats showed a slight reduction in the food consumption at 20 and 80 mg/kg before mating. A slight reduction in the food consumption was noted at 80 mg/kg from gestation days 1-14 at 80 mg/kg. However, the food consumption at 80 mg/kg at the end of gestation period was comparable to the control.

Toxicokinetics: Nil

Necropsy: There were no macroscopical changes due to the treatment in male rats at necropsy. The weight of testes was not affected by the treatment. However, the weight of epididymis was slightly reduced at 5, 20 and 80 mg/kg as shown from the Sponsor's table below.

Table 5 Fertility study in rats treated orally with TN-912
Testis and epididymis weights of F0 male rats

Dose mg/kg	No. of animals		----Testis----		--Epididymis--	
			(R) g	(L) g	(R) mg	(L) mg
0	24	Mean	1.52	1.57	464.9	465.2
		S.D.	0.06	0.06	26.7	26.1
5	24	Mean	1.50	1.53	439.3**	436.7**
		S.D.	0.07	0.07	29.6	27.0
20	24	Mean	1.51	1.51	448.0*	435.7*
		S.D.	0.09	0.18	30.8	57.7
80	24	Mean	1.55	1.61	446.7*	450.8
		S.D.	0.09	0.09	34.6	31.3

* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

One rat pair at 5 mg/kg and 3 rat pairs at 80 mg/kg showed lack of pregnancy after copulation. However, histopathology data of reproductive organs did not show treatment related changes.

Estrus cycle was not affected by the treatment. The average estrus cycle was between 3.4 to 3.6 days. No macroscopic changes due to the treatment were observed in dams at necropsy. However, the weight of the ovary at 80 mg/kg was reduced as shown from the Sponsor's table below.

Table 14 Fertility study in rats treated orally with TN-912
Ovary weights of F0 dams at cesarean section

Dose mg/kg	No. of dams		----Ovary----	
			(R) mg	(L) mg
0	24	Mean	55.4	52.4
		S.D.	13.6	10.1
5	23	Mean	50.3	50.2
		S.D.	12.7	11.8
20	24	Mean	49.0	50.8
		S.D.	9.8	8.1
80	21	Mean	47.9*	53.1
		S.D.	9.9	9.7

* : p<0.05 (Significant difference from control)

A slight reduction in the copulation index was noted at 20 mg/kg after first mating. Insemination index, copulation index and fertility index was reduced at 80 mg/kg after first mating indicating that the treatment had an delayed effect on the mating performance in male and female rats at 20 and 80 mg/kg as shown from the Sponsor's table below.

Table 15 Fertility study in rats treated orally with TN-912
Mating and fertility of F0 animals

	Dose mg/kg	No. of males	Days until copulation Mean±S.D.	Male		No. of females	Days until copulation Mean±S.D.	Female	
				Copulation index (%) a)	Insemination index (%) b)			Copulation index (%) c)	Fertility index (%) d)
1st mating	0	24	2.3±1.4	24/24(100.0)	24/24(100.0)	24	2.3±1.4	24/24(100.0)	24/24(100.0)
	5	24	2.7±1.2	24/24(100.0)	23/24(95.8)	24	2.7±1.2	24/24(100.0)	23/24(95.8)
	20	24	2.0±2.6	23/24(95.8)	23/23(100.0)	24	2.9±2.6	23/24(95.8)	23/23(100.0)
	80	24	2.8±1.4	22/24(91.7)	19/22(86.4)	24	2.3±1.4	22/24(91.7)	19/22(86.4)
2nd mating	0	0				0			
	5	0				0			
	20	1	4.0	1/ 1(100.0)	1/ 1(100.0)	1	1.0	1/ 1(100.0)	1/ 1(100.0)
	80	2	5.0±1.4	2/ 2(100.0)	2/ 2(100.0)	2	3.5±0.0	2/ 2(100.0)	2/ 2(100.0)
Total	0	24		24/24(100.0)	24/24(100.0)	24		24/24(100.0)	24/24(100.0)
	5	24		24/24(100.0)	23/24(95.8)	24		24/24(100.0)	23/24(95.8)
	20	24		24/24(100.0)	24/24(100.0)	24		24/24(100.0)	24/24(100.0)
	80	24		24/24(100.0)	21/24(87.5)	24		24/24(100.0)	21/24(87.5)

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 caesarean data did not show a treatment related trend on the fetuses although an increased post-implantation loss was noted at 5 mg/kg. Data are shown in the Sponsor's table below.

APPEARS THIS WAY
ON ORIGINAL

Table 16 Fertility study in rats treated orally with TN-912
 Cesarean section data on F0 dams

Dose mg/kg	No. of dams	No. of corpora lutea	No. of implan- tations	Pre- implan- tation loss % a)	No. of resorbed or dead fetuses				Live fetuses				Placental weight(g)	No. of f) live fetuses with ext. mal. (x)h)		
					Total (x)b)	Early c)	Late d)	No. of live fetuses			Sex ratio (M/F)e)	Fetal body weight(g)				
								Total	Male	Female		Male			Female	
0	24	Total	376	356	5.3	23(6.5)	22	1	333	182	151	1.21	3.14	2.93	0.42	0(0.0)
		Mean	15.7	14.8					13.9	7.6	6.3		0.19	0.20	0.04	
		S.D.	1.1	1.9					2.1	2.1	2.0					
5	23	Total	360	343	4.7	33(9.6)	32	1	310	167	143	1.17	3.24	2.98	0.40	0(0.0)
		Mean	15.6	14.9					13.5	7.3	5.2		0.36	0.31	0.03	
		S.D.	1.5	2.0					1.8	2.1	2.3					
20	24	Total	375	343	8.5	24(7.0)	20	4	319	155	164	0.95	3.23	3.02	0.41	1(0.33)
		Mean	15.6	14.3					13.3	6.5	6.8		0.19	0.21	0.03	
		S.D.	1.3	2.4					2.7	2.4	2.2					
80	21	Total	315	302	4.1	23(7.6)	23	0	279	139	140	0.99	3.16	2.88	0.42	0(0.0)
		Mean	15.0	14.4					13.3	6.6	6.7		0.19	0.21	0.03	
		S.D.	1.2	1.7					2.3	2.0	1.7					

a): [(No. of corpora lutea - No. of implantations) / No. of corpora lutea] X 100
 b): (No. of resorbed or dead fetuses / No. of implantations) X 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) X 100
 i): External inguinal hernia

Visceral and skeletal examinations of fetuses did not show any treatment related changes.

Summary of the study:

Data suggest that the mating performance was delayed at 20 and 80 mg/kg. The fertility of rats was reduced at 80 mg/kg. The no effect dose was 5 mg/kg in Wistar rats. The study was conducted at MTD and acceptable.

Study title: F2207 oral gavage fertility study in the rat

Key study findings: Milnacipran had no effect on mating performance in rats up to 60 mg/kg (360 mg/m³). However, reduced fertility and embryocidal effect was observed in female rats at 5 mg/kg (30 mg/m²) and higher doses in Sprague Dawley rats.

Study no.: 314/28, T030

Volume # M4, and page #: 1

Conducting laboratory and location:

b(4)

Date of study initiation: March 18, 1986

GLP compliance: Yes

QA reports: yes (x) no ()

Drug: Milnacipran, lot # D9032, and % purity: 101%

Methods

Doses: The study design is shown below from the Sponsor's table on page 5 of the report.

Group number	Group description	Dose level mg/kg/day	Concentration mg/ml	Dose volume ml/kg/day
1	Control	0	0.0	10
2	Low	5	0.5	10
3	Intermediate	15	1.5	10
4	High	60	6.0	10

Species/strain: C57BL/6J (SD) Br strain of rats, the body weight of male ranged from 205 to 250 g, female ranged from 175 to 215 g.

Number/sex/group: 30/group for male, Female (caesarean group): 15, 14, 15, 16; littering group: 15, 16, 15 and 14, for control, 5, 15 and 60 mg/kg, respectively. From clinical findings summary on Table 1, number of female rats in caesarean section group appeared to be 15, 15, 15 and 16 at control, 5, 15 and 60 mg/kg, respectively.

Route, formulation, volume, and infusion rate: The test article was dissolved in distilled water daily. The solution of test article (milnacipran) or water was administered orally at 10 mL/kg/day. Male and female animals were dosed daily before the mating period for 70 and 14 days, respectively. The dosing was continued for another 14 days during the mating for male rats. Female rats were treated during the mating and gestation period (day 20 of pregnancy) for caesarean groups. Female in the littering group were treated up to post-partum day 21.

Satellite groups used for toxicokinetics: The Sponsor stated that proof of absorption was not obtained in the study and no satellite group was allotted.

Study design: Treated males were cohabited with treated female rats at 1:1 basis for a maximum of 14 days. Mating performance was checked by vaginal smears daily for the presence of sperm cells. Gestation day 0 correspond to day of mating as confirmed by the presence of sperm cells in the vaginal smears. Vaginal smears were not obtained at the end of 14 day cohabitation.

Parameters and endpoints evaluated:

Caesarean Group:

Designated females were sacrificed by caesarean section on day 20. Animals were sacrificed by carbon dioxide inhalation. Any macroscopic changes on the external and internal organs were recorded. Uteri and ovaries were dissected to determine following:

1. Number of corpora lutea and implantation by treating uteri with ammonium sulfate
2. Live fetuses
3. Early resorption
4. Late resorption

b(4)

5. Dead fetuses
6. Fetal weight and sex

Fetal examinations:

1. external malformation
2. Skeletal abnormality, variations or malformations following digestion of tissues with 95% ethanol. Animals were stained with Alizarin and skeletal abnormality was examined under a microscope. About half of the litter was examined by this method.
3. Visceral examination for variations and any abnormality was performed for other half of the litter following fixation with Bouin's fixative. The method is referred as Wilson's method.

Calculations:

% insemination: $\frac{\text{\# inseminated animals}}{\text{\# paired animals}} \times 100$

% Fecundity: $\frac{\text{\# pregnant animals}}{\text{\#inseminated animals}} \times 100$

% Fertility: $\frac{\text{\# pregnant}}{\text{\# paired}} \times 100$

% Gestation: $\frac{\text{\# live pups}}{\text{\# Pregnants}} \times 100$

Littering group:

Designated animals were allowed to deliver, nurse F₁ pups to weaning. The gestation period was noted to determine if the treatment had any effect on gestation. The numbers of live and dead pups were determined. The sex of the pups was determined during the weaning period. The weights of pups were recorded at several time points up to weaning day 21. Dead pups were examined for external and visceral abnormalities. Skeletal abnormalities were recorded by Alizarin staining method. Physical development of F₁ pups were examined by recording following parameters:

1. Pinna folding
2. Hair growth
3. Tooth eruption
4. Eye opening

Calculations:

% Live birth: $\frac{\text{\# pups alive on day 1}}{\text{\# pups born}} \times 100$

% Viability: $\frac{\text{\# pups alive on day 4}}{\text{\# Live pups delivered}} \times 100$

% Weaning: $\frac{\text{\# pups alive on day 21}}{\text{\# pups alive on day 4}} \times 100$

Results

Mortality: Male #296, #304, #307 and 320 at 60 mg/kg died and the Sponsor stated that the cause of death was dosing errors.

Female #47 at 5 mg/kg (caesarean group, pregnant animal) died on post coital day 18 due to intubation error.

Female #88 at 15 mg/kg died on post mating day 8 due to intubation error.

Female # 93 (pre mating day 12), #95 (pre mating day 8), #112 (post coital day 16), #115 (post coital day 12) died at 60 mg/kg. The Sponsor indicated that deaths were due to dosing error.

Based on above data 4 male rats in the 60 mg/kg group died during the study. For female rats, 1, 1, 4 rats died at 5, 15 and 60 mg/kg, respectively, due to dosing error.

Clinical signs: Male rats did not show any treatment related clinical signs except for minor incidences of diarrhea and retching reflex after dosing.

Body weight: The average body weight (g) data are shown below.

Male rats:

Week	Group 1	Group 2	Group 3	Group 4
	0	5	15	60 mg/kg
1	230	230	228	228
Before mating	491	481	470	453
Gain, % Control		96%	92%	86%

Above data suggest that male rats lost 14% of the body weight gain compared to the control at 60 mg/kg before mating due to the treatment.

Female rats:

Week/ Day	Group 1	Group 2	Group 3	Group 4
	0	5	15	60 mg/kg
Week 1	198	197	196	195
Before mating	226	226	225	221
Gain, % control		-	-	91%
Gestation Day 0	231	233	231	224
Gestation Day 20 (Caesarean)	361	361	361	336

section)				
Gain, % control		99%	100%	86%
Lactation, Day 1	275	272	270	269
Lactation, Day 21	319	316	309	294
Gain, % control		100%	86%	56%

Female rats showed about 9%, 14% and 44% loss of body weight gain due to the treatment at 60 mg/kg compared to the control before mating, during gestation and end of weaning, respectively.

Based on the average data, 60 mg/kg had an adverse effect on the body weight gain in male and female rats.

Food consumption: No food consumption data were provided in the report.

Toxicokinetics: No toxicokinetic data were provided in the report.

Necropsy:

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Data for mating and pregnancy are shown in the Sponsor's table 1 below.

**APPEARS THIS WAY
ON ORIGINAL**

Data on live fetuses in caesarean sections and littering animals are shown below from Sponsor's Table 1.

Table 1 (cont.) Project No. 314/28

Test Animals and Group Indices

P Generation

Parameter	Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 15 mg/kg	Group 4 - 60 mg/kg
Number of females used for caesarian section	15	14	15	16
Number of females with 100 per cent intra-uterine deaths	0	0	0	1
Number of females with live foetuses in utero at necropsy	15	12	12	8
Number of females used for rearing	15	16	15	14
Number of pregnant animals used for rearing	15	14	13	13
Number of females with live pups on day 1 p.p.	15	14	13	12
Number of females with live pups on day 21 p.p.	15	14	11	6
Implantation index in %	96.7	100.0	93.1	96.3
Fecundity index in %	96.6	90.0	92.6	84.6
Fertility index in %	95.3	90.0	86.2	81.5
Gestation index in %	100.0	100.0	100.0	92.3

Data on "number of females with live fetuses in utero at necropsy" in Sponsor's table 1 on pregnant rats that were sacrificed on gestation day 20 showed about 15, 20 and 50% of pregnant had some dead fetuses at 5, 15 and 60 mg/kg, respectively, compared to 0% in the control. The Sponsor did not provide any historical control data. However, the death in utero could have resulted from the treatment even at 5 mg/kg doses. It should be noted that fecundity index and fertility indexes were also reduced at 5 mg/kg and higher doses.

APPEARS THIS WAY
ON ORIGINAL

Detailed data on pregnancy among caesarean sectioned rats are shown below from Sponsor's table 10. The Sponsor submitted data on corpora lutea and pre-implantation loss from animals with live fetuses in utero as well as deaths in utero at necropsy. Based on the Sponsor's table below, total implantations from animals with live fetuses in utero at necropsy and total intra-uterine deaths suggested a decrease in implantations in all treated groups. However, mean data between control and treated animals were comparable.

Table 10 Group Mean Caesarian Data Project No. 314/28
Ovarian and Uterine Data
P Generation

Parameter		Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 15 mg/kg	Group 4 - 60 mg/kg
Corpora lutea	A total number	263	196	195	118
	A mean number	17.5 ± 3.3	16.3 ± 2.7	16.3 ± 2.3	14.8 ± 1.8
Implantations	A total number	213	147	171	98
	A mean number	14.2 ± 2.7	12.3 ± 5.1	14.3 ± 1.8	12.3 ± 3.3
Implantations	B total number	213	147	171	110
	B mean number	14.2 ± 2.7	12.3 ± 5.1	14.5 ± 1.8	12.2 ± 3.1
Per cent pre-implantation loss	A	17.2	26.2	12.6	17.6

A values calculated from animals with live foetuses in utero at necropsy
B values calculated from animals with live foetuses in utero at necropsy and total intra-uterine deaths

APPEARS THIS WAY
ON ORIGINAL

The Sponsor presented data in table 11 on resorptions from animals with live fetuses in utero as shown below. However, the data are not relevant because did not represent all female rats deployed to the caesarean section groups.

Table 11

Group Mean Caesarian Data

Project No. 314/28

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

P Generation

Parameter		Group 1 - 0 mg/kg	Group 2 - 5 mg/kg	Group 3 - 15 mg/kg	Group 4 - 40 mg/kg
		n = 15	n = 12	n = 12	n = 8
Implantations	total number	215	147	171	96
	mean number	14.2 ± 2.7	12.3 ± 5.1	14.3 ± 1.8	12.3 ± 3.3
Live fetuses	total number	198	131	164	89
	mean number	13.2 ± 3.2	10.9 ± 5.6	13.7 ± 1.0	11.1 ± 2.8
	% of implantations	92.6	89.8	96.3	91.8
Early resorptions	total number	13	6	7	8
	mean number	0.9 ± 1.4	0.5 ± 1.0	0.6 ± 1.0	1.0 ± 0.9
Late resorptions	total number	2	10	0	1
	mean number	0.1 ± 0.4	0.8 ± 2.9	0.0 ± 0.0	0.1 ± 0.4
Dead fetuses	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	15	16	7	9
	mean number	1.0 ± 1.4	1.3 ± 2.9	0.6 ± 1.0	1.1 ± 1.0
% Post-implantation loss		7.4	10.2	3.7	8.2

APPEARS THIS WAY
ON ORIGINAL

Sponsor's table 12 was presented for animals with live fetuses in utero at necropsy and total intra-uterine deaths as shown below. Total number of animals at 60 mg/kg shown as 9 (one rat more than that in table 11). However, none of dose groups showed dead fetuses. These table as presented here do not represent all animals deployed in the caesarean section for an analysis of post mating events in female rats.

PREPARED BY: DR. ANIL KUMAR
 43, DINESH ANAND COLONY
 P-27014, INDIA

Table 12		Group Mean Caesarian Data				Project no. 314/28
Implantation Data						
(calculated from animals with live fetuses in utero at necropsy and total intra-uterine deaths)						
P Generation						
Parameter		Group 1 - 0 mg/kg n = 15	Group 2 - 5 mg/kg n = 12	Group 3 - 15 mg/kg n = 12	Group 4 - 60 mg/kg n = 9	
Implantations	total number	213	167	171	110	
	mean number	14.2 ± 2.7	13.9 ± 5.1	14.3 ± 1.0	12.2 ± 1.1	
Early resorptions	total number	13	0	7	9	
	mean number	0.9 ± 1.4	0.5 ± 1.0	0.6 ± 1.0	1.0 ± 0.9	
Late resorptions	total number	2	10	0	12	
	mean number	0.1 ± 0.4	0.8 ± 2.9	0.0 ± 0.0	1.3 ± 1.6	
Dead fetuses	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	15	10	7	21	
	mean number	1.0 ± 1.6	1.3 ± 2.9	0.6 ± 1.0	2.3 ± 1.7	
b Post-implantation loss		7.6	10.2	1.7	10.4	

PROJECT NO. 314/28

- 47 -

b(4)

APPEARS THIS WAY
ON ORIGINAL