

Historical Control Data on Neoplastic Findings in C Wist from 2-Year Bioassays (Planned Sacrifice Schedule: >103 Weeks)

b(4)

Liver and Common Bile Duct

Male	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Liver						
Numbers of rats examined	3737					
Hepatocellular nodules	6	0.16	0.18	0.75	0.00	4.00
Hepatocellular adenoma	79	2.11	2.10	2.00	0.00	8.00
Cholangioma	1	0.03	0.03	0.18	0.00	1.27
Hemangioma	4	0.11	0.08	0.57	0.00	4.00
Hepatocellular carcinoma	18	0.48	0.49	0.80	0.00	2.80
Cholangiocell. carcinoma	1	0.03	0.02	0.14	0.00	1.00
Hemangiosarcoma	1	0.03	0.02	0.14	0.00	1.00
Metastatic carcinoma	3	0.08	0.11	0.60	0.00	4.00
Metastatic sarcoma	2	0.05	0.07	0.34	0.00	2.00
Lymphoma infiltrate	4	0.11	0.10	0.59	0.00	2.00
Common Bile Duct						
Numbers of rats examined	19					

Female	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Liver						
Numbers of rats examined	3686					
Hepatocellular nodules	13	0.35	0.28	1.71	0.00	12.00
Hepatocellular adenoma	101	2.74	2.93	2.98	0.00	10.20
Cholangioma	7	0.19	0.22	0.70	0.00	4.00
Hemangioma	3	0.08	0.06	0.32	0.00	2.04
Hepatocellular carcinoma	16	0.43	0.45	0.69	0.00	2.00
Cholangiocell. carcinoma	0	0.00	0.00	0.00	0.00	0.00
Hemangiosarcoma	0	0.00	0.00	0.00	0.00	0.00
Metastatic carcinoma	9	0.24	0.27	0.65	0.00	2.00
Metastatic sarcoma	0	0.00	0.00	0.00	0.00	0.00
Lymphoma infiltrate	0	0.00	0.00	0.00	0.00	0.00
Common Bile Duct						
Numbers of rats examined	5					

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Historical Control Data on Neoplastic Findings in Wistar-Kyoto (WKY) Rats from 2-Year Bioassays (Planned Sacrifice Schedule: >103 Weeks)

b(4)

Thymus

Male Species	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Thymus						
Numbers of rats examined	3376					
Thymoma (b)	23	0.68	0.59	1.44	0.00	8.25
Thymoma (m)	18	0.53	0.57	1.00	0.00	3.13
Cystic sarcoma	2	0.06	0.07	0.32	0.00	1.59
Thymic lymphoma	3	0.09	0.06	0.31	0.00	2.02
Thymoma: lymphocytic benign	5	0.15	0.12	0.67	0.00	4.44
Thymoma: lymphocytic malignant	1	0.03	0.01	0.07	0.00	0.51
Thymoma: epith. malignant	1	0.03	0.01	0.07	0.00	0.51
Lymphangioma	0	0.00	0.00	0.00	0.00	0.00
Metastatic sarcoma	1	0.03	0.05	0.37	0.00	2.56
Metastatic carcinoma	0	0.00	0.00	0.00	0.00	0.00
Lymphoma infiltrate	6	0.18	0.13	0.50	0.00	2.08

Female Species	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Thymus						
Numbers of rats examined	3475					
Thymoma (b)	88	2.53	2.62	4.91	0.00	17.02
Thymoma (m)	23	0.66	0.68	1.80	0.00	11.11
Cystic sarcoma	0	0.00	0.00	0.00	0.00	0.00
Thymic lymphoma	10	0.29	0.29	1.32	0.00	8.51
Thymoma: lymphocytic benign	11	0.32	0.28	1.53	0.00	10.20
Thymoma: lymphocytic malignant	2	0.06	0.05	0.30	0.00	2.04
Thymoma: epith. malignant	0	0.00	0.00	0.00	0.00	0.00
Lymphangioma	1	0.03	0.02	0.15	0.00	1.03
Metastatic sarcoma	0	0.00	0.00	0.00	0.00	0.00
Metastatic carcinoma	3	0.09	0.10	0.41	0.00	2.00
Lymphoma infiltrate	1	0.03	0.03	0.21	0.00	1.47

Toxicokinetics:

- Greater than dose-related increase in systemic exposure to test article in F at 10 to 125 mg/kg/day
- Linear dose-related increase in exposure (AUC₀₋₂₄) to the parent drug in the M
- Dose-proportional increase in systemic glucuronide metabolite exposure, without gender differences
- Exposure to parent drug, CG5503 but not to the glucuronide metabolite was higher in the F than in the M
- High intra-group variability in the TK parameters (note large Standard Deviations observed) may be a result of variable feeding due to the housing arrangement using 5 rats/cage, with possible inconsistent access to food within the caged groups
- The results of the TK evaluation on CG5503 and CG5503 glucuronide are presented in the following tables (provided from the original NDA submission):

Summary on exposure to CG5503 base in Wistar rats after daily oral doses of CG5503 in the diet for at least 104 weeks
(mean values $AUC_{(0-24h)} \pm S.D.$ of week 4, week 13 and week 26)

Dose	[mg/kg/day]	Males				Females			
		10	50	125	250	10	50	125	250
Week 4									
AUC	[ng·h/ml]	21.9 ±21.1	131 ±66	284 ±169	560 ±253	12.5 ±6.2	136 ±96	384 ±159	1079 ±738
Week 13									
AUC	[ng·h/ml]	18.0 ±4.6	118 ±30	331 ±175	485 ±234	18.8 ±8.7	207 ±112	994 ±537	2688 ±2719
Week 26									
AUC	[ng·h/ml]	19.1 ±9.5	94 ±65	274 ±165	328 ±138	16.5 ±7.7	156 ±56	620 ±363	1349 ±1381
Weeks 4 to 26									
		19.7 ±8.7	115 ±36	296 ±125	458 ±205	15.9 ±4.6	166 ±50	666 ±351	1705 ±1495
R_{A1}	[%]	87.2	71.8	96.5	58.6	132	115	161	125

R_{A1} = ratio of AUC from week 26 to week 4

Summary on exposure to CG5503 glucuronide in Wistar rats after daily oral doses of CG5503 in the diet for at least 104 weeks
(mean values C_{max} and $AUC_{(0-24h)} \pm S.D.$ of week 4, week 13 and week 26)

Dose	[mg/kg/day]	Males				Females			
		10	50	125	250	10	50	125	250
Week 4									
AUC	[ng·h/ml]	18630 ±4778	88881 ±22176	221058 ±49522	428530 ±167827	15750 ±4419	80647 ±20367	213118 ±8960	396595 ±181995
Week 13									
AUC	[ng·h/ml]	21977 ±1877	94668 ±20658	249068 ±66631	462639 ±98040	19312 ±6475	104501 ±24954	263560 ±51050	495182 ±158659
Week 26									
AUC	[ng·h/ml]	19534 ±6919	102417 ±29299	277884 ±111166	475181 ±181040	16881 ±4422	98980 ±32854	285241 ±95222	436231 ±150146
Weeks 4 to 26									
AUC	[ng·h/ml]	20047 ±4563	95322 ±17707	249337 ±71028	455450 ±111894	17315 ±3904	94709 ±18638	253973 ±64122	442669 ±132524
R_{A2}	[%]	105	115	126	111	107	123	134	110

R_{A2} = ratio of AUC from week 26 to week 4

3.4.6. Reproductive and developmental toxicology

Summary: The potential for reproductive and developmental toxicity by tapentadol was evaluated in rats and rabbits. Dose selection for the main studies was based on the results of preliminary range-finding toxicity studies, conducted in pregnant or non-pregnant animals. The range-finding studies included assays on tapentadol given by oral gavage, intravenous injection, and subcutaneous injection. Pivotal reproductive toxicology studies were conducted by the intravenous and subcutaneous routes to maximize systemic exposure to the parent drug, because oral tapentadol undergoes rapid and nearly complete transformation, predominantly to the O-glucuronide metabolite. An additional preliminary study in maternal and juvenile rats given tapentadol by oral gavage was conducted for dose selection in the pre- and post-natal development study in maternal and juvenile rats. Dose range-finding studies were conducted in non-pregnant rabbits administered tapentadol by intravenous and subcutaneous injections.

The results of the oral dose range-finding study in pregnant Sprague Dawley rats (Study TP2767) showed maternal hypersalivation at both doses administered (300 and 400 mg/kg/day), and reduced maternal body weight gains of -24% at 300 and -23% at 400 mg/kg/day throughout the dosing period. There were no treatment-related effects on the macroscopic examination of the dams, the numbers of implantations and dead and live fetuses. Oral dose range-finding investigation in another study conducted for dose-selection in the pre- and post-natal development and juvenile study in rats (oral doses 0, 20, 75, and 150 mg/kg b.i.d. PO, Study TP2772) showed reduced maternal body weight gains and food consumption (dose-related) and F₁ generation toxicity via transfer in milk through postnatal day (PND) 8, and by direct treatment of the pups in PND 13-26 at all doses. F₁ toxicity included HD pup deaths in the first 4 PNDs, reduced pup body weight gains in female pups during lactation (up to PND 8) and in the male and female pups dosed directly via gavage at the HD (PND 13-26). Clinical signs observed in the pups included hypoactivity at the MD and HD, and sedation and coldness at the HD before reduction of the HD from 150 to 100 mg/kg/day.

The results of the intravenous dose range-finding study in rats (Study TP2060) showed decreased maternal body weights at the high dose (15 mg/kg/day), and maternal deaths at the 7 and 15 mg/kg/day doses (2/20 rats in each group), with tonic or clonic convulsions, flaccid position, and hemorrhagic snout in a high dose dam. Reduced numbers of fetuses, implantation sites and increased late resorptions were attributed to maternal toxicity, in agreement with the Sponsor. No evidence of teratogenicity by tapentadol was found in the fetal examinations in this range-finding study.

Subcutaneous tapentadol injection in pregnant rats for 2 weeks (Study TP2465) produced injection site discoloration and erythemas at the MD of 30 mg/kg/day given in divided doses (b.i.d.) and HD of 50 mg/kg/day (divided, b.i.d.), and weeping eroded hemorrhagic lesions, eschar formation, and injection site indurations at the HD in most of the dams. Food consumption was reduced in a dose-related manner at the MD and HD, and reduced motility was observed at the HD.

The results of the dose range-finding study in non-pregnant Himalayan rabbits administered tapentadol by intravenous injection at doses of 0, 3, 7, and 15 mg/kg/day for 2 weeks (Study TP2061) showed reduced dose-related increases in severity and duration of motility and flaccid position at all dose levels. Opisthotonus, increased respiratory rate, and reduced food consumption and body weights were observed at the MD and HD, and reduced respiratory volume and exophthalmus was found at the HD. Dose range-finding evaluation was also performed in non-pregnant rabbits by the subcutaneous route, at doses of 0, 10, 30 and 50 mg/kg/day (divided, b.i.d.) for 14 consecutive days (Study TP2464). Local toxicity was observed, similar to that in the subcutaneous toxicity study in the rats, at the high dose and included red or brown discoloration at the injection sites with subcutaneous lesions and skin thickening. Miosis and dose-related reduced food consumption was observed at all doses, and body weights were reduced (-18%) at the HD. The clinical signs also included reduced motility and abdominal position at the highest dose administered. The necroscopic examination in that study revealed pale liver and urinary bladder gritty substance at the high dose.

In the main study on potential treatment-related adverse effects on fertility and early embryonic development to implantation in male (M) and female (F) Wistar rats, tapentadol was negative for adverse effects on mating and fertility at intravenous (IV) doses of up to the maximum tolerated (MTD based on toxicity, Study TP2445). The maternal (F₀) rats were administered consecutive daily IV doses of 0 (saline vehicle control), 3, 6, and 12 mg/kg/day, from 28 days before mating through 9 days after mating in the M, and from 14 days before mating through Day 6 post-coitum in the F. The key study findings in the pivotal fertility study were maternal and early embryonic (F₁ generation) toxicity. Maternal toxicity was indicated by slight body weight loss and decreased food consumption at 6 and 12 mg/kg/day IV tapentadol. The mean numbers of corpora lutea were reduced by 4% and 6%, and there were decreased mean numbers of implantations by 8% and 11% at those doses, respectively. Additionally, there were dose related increases in mean percent pre-implantation and post-implantation losses, and dose-related decreased mean numbers of live conceptuses at all doses, compared to controls. No adverse treatment-related effects on mating performance and in the necroscopic examination of the reproductive organs were observed in the M rats. The embryonic developmental abnormalities (pre-implantation and post-implantation losses) are considered to be secondary to maternal toxicity, in agreement with the Sponsor. The NOAEL value for F₀ toxicity was 3 mg/kg/day IV, for adverse effects on fertility in the F₀ M and F rats was 12 mg/kg/day IV and for adverse effects on early embryonic development (F₁ generation) was 3 mg/kg/day IV. Toxicokinetics analyses were not conducted in this study; estimated systemic exposures based on the toxicokinetics analyses in a 4-week intravenous study in rats (PT1966) at the highest IV dose of 15 mg/kg/day represented approximately 0.41 times in the M and 0.35 times in the F rats, the MRHD, on an AUC basis.

Embryo-fetal development toxicity (Segment II studies) was studied in rats and rabbits administered tapentadol by intravenous and subcutaneous administration. Tapentadol was negative for teratogenicity in rats at up to maternally toxic doses, but found to

increase the incidence and severity of fetal variations and malformations in the rabbit studies.

Pregnant Sprague Dawley rats were given tapentadol by intravenous injection at doses of 0, 3 (LD), 7 (MD), and 15 (HD) mg/kg/day on gestation days 6-17 (inclusive, Study TP2060). There were 2 maternal deaths each in the MD and HD groups, within 2-45 minutes of the first injection, preceded by convulsions, exophthalmus, flaccid position and hemorrhagic snout. There were reduced numbers of fetuses, decreased implantation sites and increased late resorptions that correlated with decreased maternal body weights and severe maternal clinical signs. There were no treatment-related effects on sex distribution, placenta weight, fetal weight, fetal deaths, incidence of runts, and no external, skeletal and soft tissue malformations, variations and retardations. Subsequent investigation of embryotoxicity by tapentadol in using the subcutaneous route (Study TP2510) at doses of 0, 5 (LD), 10 (MD), and 20 (HD) mg/kg b.i.d. (10, 20, and 40 mg/kg/day) on Gestation days 6-17 (inclusive) showed maternal toxicity at all dose levels, with dose-related increases in severity and duration of reduced body weight gain, abdominal position lasting 1-2 hours, and at the MD and HD local toxicity (eschar formation and hemorrhagic foci). No treatment-related malformations or variations were observed in this study. However, tapentadol was embryotoxic at the HD (representing systemic exposure of approximately 3 times the clinical systemic exposure at the MRHD, on an AUC basis). There were observations indicative of treatment-related developmental delay (skeletal retardation), with increased incidence of incomplete or missing ossification of the sternebra and caudal vertebral bodies when compared to control fetuses. A relationship of the observed embryotoxicity to maternal toxicity, particularly to treatment-related reduced food consumption and body weight gain is likely. The NOAEL for embryotoxicity was 20 mg/kg/day (representing systemic exposure of approximately 1.5 times the clinical systemic exposure at the MRHD, on an AUC basis). Exposure to the O-glucuronide metabolite at the highest dose tested was approximately equivalent to clinical exposure to the metabolite at the MRHD, on an AUC basis.

An early embryo-fetal toxicity study in Himalayan rabbits (Study TP2061) evaluated intravenous doses of 0, 1 (LD), 3 (MD), and 9 (HD) mg/kg/day from Gestation Days 6-20, inclusive (Study TP2062). Maternal toxicity, noted at the HD, included flaccid position, increased respiratory rate, opisthotonus and tremor. There was one abortion at the HD on Gestation Day 26 (within range of historical background incidence). This study was negative for external, visceral and skeletal malformations, variations and retardations by IV tapentadol administration, although there was a treatment-related decrease in number of live fetuses and increased post-implantation loss at the HD, due to the deaths of 10 fetuses in 2 of the 16 litters evaluated. Toxicokinetic evaluation results were not provided.

A second embryo-fetal development study on tapentadol was conducted in Himalayan rabbits, dosed twice daily by the subcutaneous route at doses of 0, 4 (LD), 10 (MD), and 24 (HD) mg/kg/day (Study TP2511). There were dose-related increases in incidence and severity of maternal toxicity, indicated by miosis, abdominal position and reduced body

weight gain and food consumption at all doses administered. Statistically significant reductions in fetal body weights were found at the MD (-10%) and HD (-13%). The fetal examinations also revealed dose-related decreases in fetal viability, with increased post-Caesarian deaths during the 24-hour incubator stay at the MD (9 fetal deaths) and HD (7 fetal deaths), when compared to the control and LD groups (3 deaths each). There were 4 runts at the HD (compared to 2 in the control group), all of which died during the 24 hour post-Caesarian incubator stay. Increased incidence of multiple internal malformations, with gastroschisis or thoracogastroschisis, prolapsed organs, amelia, and phocomelia were observed at the MD (+0.9% incidence compared to controls) and HD (+1.7% incidence compared to controls). Encephalocele was seen in 1 HD runt, spina bifida in 1 HD runt, kyphosis in 1 HD runt, ablepharia in 3 HD fetuses from 1 litter, and cleft palate in 1 MD fetus (0.9%), 3 fetuses from 1 HD litter and 1 additional fetus from another HD litter (3.4%). Skeletal variations (accessory 13th rib, shortened ribs, caudal vertebral bodies misaligned or fused, unossified parietal area of the skull and sternum fused or misaligned), and skeletal retardations (incomplete ossification in the frontal, parietal, interparietal, and supraoccipital skull, unossified hyoid, incomplete or unossified small sternum, and reduced, unossified or dumbbell-shaped vertebral bodies) were found with statistically significant increases at the HD compared to control incidence. The NOAEL for teratogenicity in this study was 4 mg/kg/d SC, representing exposures in the dams that were approximately equivalent to the clinical systemic exposure at the MRHD, on an AUC basis. The embryo-fetal toxicity was observed in the litters from dams showing severe clinical signs, body weight loss, and reduced body weight gain and food consumption, although not all dams showing toxicity had adversely affected fetuses. A possible relationship of the observed fetal toxicity to maternal toxicity, along with the incidence of these findings within the upper limit of the historical range of background incidence for the performing laboratory suggest that the findings in this study may not be entirely related to direct effect of tapentadol on embryo-fetal development in rabbits, although a direct treatment-related teratogenic effect cannot be ruled out, either.

Pre- and post-natal developmental toxicity by tapentadol was investigated in Sprague-Dawley rats at maternal (F₀ generation) oral gavage doses of 0, 10 (LD), 25 (LMD), 75 (HMD), and 150 (HD) mg/kg b.i.d. (20, 50, 150, and 300 mg/kg/day) administered from Gestation Day (GD) 6 through Post-Partum Day (PPD) 21, inclusive. Maternal toxicity was indicated by deaths (4 HD dams found dead and the remaining maternal deaths by sacrifice *in extremis*) in 6 HD, 1 HMD, 1 LMD, and 2 LD dams, and clinical signs of ptialism (HD), piloerection and round back (HD, HMD), reduced body weight and body weight gain (BWG, -22% at the HMD and -24% at the HD), and reduced food consumption at the HMD and HD throughout the dosing period. There were no treatment-related effects on pregnancy and parturition. There was a statistically significant treatment-related reduction in the viability index indicating increased pup mortality, with complete litter deaths in 1 HMD litter and 2 HD litters, and increased numbers of pup deaths at the HMD (16 pups) and HD (18 pups) compared to 2 control pup deaths. Statistically significant reduction in pup body weights and body weight gains were also observed at the HD from PPD1 throughout lactation. Unossified 6th centrum of the cervical vertebrae was noted in 50% of the HD pups that were found dead during PPD

1-4, and there were non-ossified or incomplete ossification in other bones in several pups, probably due to lower body weights in these pups.

Pup (F₁ generation) post-weaning physical development measurements showed reduced body weights and body weight gains in the F₁ HD M from PPD22 to the end of the study (pup ages 10-11 weeks), and in the MD (PPD22-37) and HD (PPD22-ages 10-11 weeks) F compared to controls. There were slight, but not significant increases in horizontal movements and rearing at the MD and HD in the M (+7% and +10%, respectively) and F at all dose levels (+2%-+20%). The results of the T-maze test on the F₁ generation showed slight, but not significant increases in test time in the learning phase in M at the HMD (+63%) and HD (+52%), and in the memory phase at the HD (+12%), compared to controls. No treatment-related effects on F₁ generation sexual development, auditory function and pupil constriction were found. Specifically, there were no treatment-related effects on F mating, mean numbers of days to mate, fertility data pregnancy status, hysterectomy data (e.g., corpora lutea, implantations, concepti, etc). There was a failure to mate in 2, 1, and 1 F₁ M at the LD, MD, and HD, respectively, that is unlikely related to treatment due to absence of dose-effect. Toxicokinetic evaluation showed dose-related linear increases in exposure to parent drug and the glucuronide metabolite in the F₁ pregnant dams, with increased exposure to the parent drug with repeated dosing at all but the HD, and to the glucuronide at all doses, suggesting accumulation. The NOAEL for F₀ maternal toxicity was 50 mg/kg/day PO, based on reduced BWG and food consumption (representing systemic exposure of approximately 1.5 times the clinical exposure at the proposed MRHD on an AUC basis). The NOAEL for treatment-related abnormalities in pup development was 20 mg/kg/day PO, due to pup deaths from PPD 1-4 (exposure of approximately 0.3 times the clinical exposure at the MRHD, on an AUC basis). The NOAEL for the F₁ generation was 300 mg/kg/day (systemic exposure of approximately 10 times the clinical exposure to the parent drug at the MRHD, on an AUC basis); although body weights were reduced, there were no effects on mating, fertility, and neurobehavioral parameters. Maternal systemic exposure to the main O-glucuronide metabolite at the NOAEL represented approximately 35 times the clinical exposure to the metabolite at the MRHD, on an AUC basis.

In conclusion, the results of the studies on reproductive toxicity in rats and rabbits suggested potential adverse effects by tapentadol on the developing fetus and newborn, although these effects may be related in part to drug-related poor maternal condition, such as nutritional deficiencies, due to decreased appetite and food consumption, and resulting weight loss. No direct effects on fertility were observed in male and female rats at doses up to the maximum tolerated, based on treatment-related toxicity and representing systemic exposure that were approximately equivalent to clinical exposure at the MRHD of 700 mg/day, using AUC comparison. No clear, treatment-related teratogenic effects were observed in rats (given subcutaneous and intravenous doses) and rabbits (given intravenous doses) at doses up to the maximum tolerated based on maternal toxicity during organogenesis (approximately 3 times in rats and 10 times in rabbits, the clinical exposure at the MRHD on an AUC basis); however, tapentadol was embryotoxic in these studies. However, malformations were seen in the subcutaneous study on embryo-fetal development in the rabbits. The degree to which maternal toxicity,

known to affect embryofetal development in rodents, was responsible for these findings, vs. direct teratogenicity by tapentadol is not known. The Segment II studies revealed developmental delay in the rats, indicated by incomplete or non-ossification of the sternbrae and caudal vertebral bodies. In the rabbits, decreased viability, developmental delay (delayed ossification of fetal skeleton), and slight dose-related increased incidence of variations and malformations were observed, although predominantly in the litters of dams showing severe treatment-related toxicity. The malformations seen in the fetal rabbits were within the upper range of historical incidence. Tapentadol exposure *in utero* and during lactation (Segment III) increased rat pup mortality in the days following birth, reduced pup body weight gain and skeletal development, and suggested a slight but not significant effect on learning and memory, probably due to secondary effects of maternal toxicity and treatment-related CNS sedation, in the offspring, at maternal doses higher than those representing exposure approximately equivalent to the clinical exposure at the MRHD on an AUC basis.

Dose range-finding studies:

Dose range finding studies were conducted in pregnant rats and rabbits. The results of two embryo-fetal toxicity studies (Studies 10434 (TP 2060) and TP2062) were reviewed under the original IND submission (IND 61,345, SN000, December 1, 2000, Kathleen Haberny, Ph.D., now Kathleen Young, Ph.D., Pharmacology and Toxicology Reviewer). The previous study reviews are provided, below.

Examination of the Influence of BM-200 on the Pregnant Rat and the Fetuses by Intravenous Administration – Embryotoxicity Study: study 10434/97

Amendment #000, Vol #4, Page #1

Conducting laboratory and location: Testing facility:

Date of study initiation: May 6, 1997

GLP compliance: Signed and present

QA- Report Yes (x) No ()

Protocol reviewed by Division Yes () No (x)

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Methods: Pregnant Sprague-Dawley SPRD rats (ages 7 weeks, weights 184-231 g, n=20/dose) were used in this study. The rats were administered BN-200 (presently known as CG5503, Batch #06, purity 98% by TLC, in physiological saline) at doses of 0, 3, 7 and 15 mg/kg/day by intravenous bolus injection at 10 ml/kg over 1 minute, daily from the 6th to 17th day of pregnancy.

The maternal observations were mortality (daily), clinical signs (daily), body weights (daily), and food and water consumption (daily).

The necropsy evaluations, conducted on gestation day 20, were uterine weights, examination of maternal internal organs, numbers of alive and dead fetuses, placenta count, sex and viability of fetuses, number and size of resorptions, corpora lutea in the ovaries, implantations, locations of fetuses in the uterus, gravid uterus weight, weights of fetuses and placentae, external and internal inspection of fetuses for skeletal, internal organ, and soft tissue damages/malformations, variations, and retardations. The parameters were corpora lutea, implants, resorptions, placental weights, fetal weights, number of alive and dead fetuses per dam, per group and per sex, distribution of fetuses in uterine horns, runts, malformed fetuses, malformation rate, variations and variation rate, retardations and retardation rate, pre-implantation loss and post-implantation loss. No toxicokinetic analysis was conducted.

Results: There were 2 maternal deaths each in the mid-dose (7 mg/kg/d) and high-dose (15 mg/kg/d) groups. The mortalities occurred within 2-45 minutes of the first injection on gestation day 6. The deaths were preceded by tonic or clonic convulsions, exophthalmos, flaccid position, and in one high dose dam, hemorrhagic snout.

There were no local reactions at or near the injection sites in any group. The clinical signs in the dams given the low dose (3 mg/kg/d) were decreased activity, head bobbing, and flaccid position beginning at 5-20 minutes after dosing and lasting 5-20 minutes, on the first 2-3 dosing days. The treatment-related clinical signs in the mid-dose group were exophthalmos and flaccid position, and in one dam respiratory depression, beginning 5 minutes after dosing and lasting 20 minutes-2 hours on all dosing days. In the high-dose group, the clinical signs were tendency toward convulsions, flaccid position, and exophthalmos. Body weights were decreased slightly (-9% compared to controls) in the high-dose dams on gestation days 8-20 and body weight gain was reduced (57% increase compared to 69% increase in controls) on days 6-9 and 9-12. Food consumption was reduced in the high-dose group on gestation days 8 and 12. There were no systemic necropsy findings in the dams that survived to necropsy. Dark-red discolored lungs were observed in the necropsy examination of the animals that died. The uterus weight was decreased in the mid-dose and high-dose dams.

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The reproduction data of the dams is presented in the following table (reproduced from the original IND submission):

Parameter		Dose (mg/kg/day)			
		0	3	7	15
Corpora lutea	total	258	256	262	235
	per dam	12.9	12.8	13.1	11.8
Implantation sites	total	258	255	252**	232*
	per dam	12.9	12.8	12.6	11.6
Resorptions	total	22	19	21	11
	per dam	1.1	1.0	1.1	0.6
Early resorptions	total	6	12	7	8
	per dam	0.3	0.6	0.4	0.4
Late resorptions	total	16	7*	14	3**
	per dam	0.8	0.4	0.7	0.2
Live fetuses	total	235	233	230	221*
	per dam	11.8	11.7	11.5	11.1
Pre-implantation loss	mean %	0.0	0.7	3.9	1.1
Post-implantation loss	mean %	8.6	9.3	9.4	5.2

*Significantly different from controls at $p \leq 0.05$

**Significantly different from controls at $p \leq 0.01$

Implantation sites were decreased at the mid-dose and high-dose. Late resorptions and number of live fetuses were decreased in the high-dose animals.

The examination of the fetuses revealed no treatment-related effects on sex distribution, mean placental weights, and mean fetal weights. The external examination showed encephalocele in one control fetus, and an abdominal hematoma in one low-dose fetus. There were 5 dead fetuses (1 control, 3 at 3 mg/kg/d, and 1 at 7 mg/kg/d). There were 4 runts (2 control, 1 at 3 mg/kg/d, 1 at 15 mg/kg/d). In the skeletal examination of the fetuses, there were no malformations. Skeletal variations included accessory 14th ribs, sternum bipartite and misaligned sternbrae in similar incidence among the control and treatment groups. Skeletal retardations were also observed to a similar extent (degree and incidence) in all groups and included incomplete ossification

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of the skull and hyoid, incomplete and missing ossification or reduced size of sternbrae, incompletely ossified or dumbbell-shaped and bipartite thoracic, lumbar and caudal vertebral bodies, and unossified 5th metacarpalia and 5th metatarsalia. There were no treatment-related soft tissue malformations or variations in any group.

Key Study Findings:

- High dose (15 mg/kg/d) resulted in maternal toxicity (tremor, convulsions, flaccid position, exophthalmos, decreased body weights and decreased food consumption)
- Reduced number of fetuses correlated with decreased maternal body weights in the high-dose group
- Decreased implantation sites and late resorptions, probably related to maternal toxicity, at 15 mg/kg/d
- No treatment-related effects on sex distribution, placenta weight, fetal weight, fetal deaths and incidence of runts
- No effects of BN-200 on external, skeletal and soft tissue malformations, variations and retardations at doses ranging from 3-15 mg/kg/d IV, when administered on gestation days 6-17 in rats

Examination of the Influence of BN-200 on the Pregnant Rabbit and the Fetus by Intravenous Administration – Embryotoxicity Study: Study TP 2062

Amendment #000, Vol #3, Page #1

Conducting laboratory and location: Testing facility: 





b(4)

Date of study initiation: June 17, 1997

GLP compliance: Signed and present

QA- Report Yes (x) No ()

Protocol reviewed by Division Yes () No (x)

Methods: Pregnant Himalayan rabbits (own breed, ages 5.5-7 months, weights 2.53-3.60 kg, n=16/dose) were used in this study. The rabbits were administered BN-200 (presently known as CG5503, Batch #06, purity 98% by TLC, in physiological saline) at doses of 0, 1, 3 and 9 mg/kg/day by intravenous bolus injection in an ear vein at 3 ml/kg over 1 minute, daily from the 6th to 20th day of pregnancy.

The maternal observations were mortality, local tolerance at the injection site, clinical signs, body weights, and food and water consumption.

Necropsic examination on gestation day 29 included number of dead and alive fetuses, placental count, fetal viability (after 6-h and 24-h stay in incubator), number and size of resorptions, corpora lutea in ovaries, and implantations, and location of fetuses in uterus, fetal weights, placental weights, external inspection for fetal damage (malformations, variations, retardations), fetal dissection for location, size and condition of the internal organs, abnormalities of soft tissue, internal head structure and brain,

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fetal sex, and skeletal retardations, variations and malformations. The evaluations were corpora lutea, implants, resorptions, weight of placentae, weight of fetuses, live and dead fetuses, runts, malformed fetuses, malformation rate per group, fetuses with variations, variation rate, fetuses with retardations, retardation rate, pre-implantation loss, and post-implantation loss.

Blood was withdrawn for toxicokinetic analysis before dosing and at 15, 60 and 120 minutes on pregnancy days 6 and 20, from 3 rabbits per group.

Results: There were no maternal deaths, and no local reactions at or near the injection sites in any group. Clinical signs were observed in all dams at the high dose of 9 mg/kg/d IV on several dosing days, and included opisthotonus (spasm consisting of hyperextension of the body), flaccid position, and increased respiratory rate. Tremor was occasionally observed, and there was one abortion on gestation day 26 at the high dose. There were no treatment-related effects on body weights, body weight changes, food and water consumption, and no pathologic findings at the injection site in the necropsy examination. The systemic necropsy showed liver discoloration in one dam each in the low-dose (1 mg/kg/d) and high-dose (9 mg/kg/d) groups. In one high dose dam, there was a hemorrhage in the right uterine horn, with a thickened, malshaped placenta; all fetuses were found dead in this dam. The single abortion contained 2 normal appearing fetuses with 1 placenta; 7 dead fetuses and 8 placentae remained in the uterus. Net weight change from day 6 to necropsy was slightly reduced in the high dose dams.

The reproduction data of the dams is presented in the following table (reproduced from the original IND submission):

Parameter		Dose (mg/kg/day)			
		0	1	3	9
Corpora lutea	total	152	142	145	141
	per dam	9.5	8.9	9.1	9.4
Implantation sites	total	137	133	131	128
	per dam	8.6	8.3	8.2	8.5
Resorptions	total	18	14	10	24
	per dam	1.1	0.9	0.6	1.6
Early resorptions	total	7	3	4	10
	per dam	0.4	0.2	0.3	0.7
Late resorptions	total	11	11	6	14
	per dam	0.7	0.7	0.4	0.9
Live fetuses	total	119	119	121	94**
	per dam	7.4	7.4	7.6	7.2
Pre-implantation loss	mean %	9.7	5.4	8.5	8.8
Post-implantation loss	mean %	11.2	10.7	7.6	27.1

*Significantly different from controls at $p \leq 0.05$

**Significantly different from controls at $p \leq 0.01$

Post-implantation loss was increased, although not significantly, in the high dose group due to the significant decrease in the number of live fetuses. The decrease in the number of live fetuses was due to the deaths of 10 fetuses in 2 high dose litters, found

at laparotomy. There were no treatment-related effects on viability of the fetuses at 6 and 24 hours after laparotomy.

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The examination of the fetuses revealed no treatment-related effects on placental weights and fetal weights, sex distribution of fetuses, and no treatment-related effects on incidence of external malformations and variations. There were 3 runts, one each in the control, low dose group and mid-dose groups. There was one fetus with cleft palate (coincident with an encephalocele (herniation of brain through skull defect), and indented and elongated os nasale (nasal bone)) in the low dose group (1 mg/kg/d). There were no significant treatment-related effects on skeletal variations, but in the examination for skeletal malformations there was one high dose fetus with malformation of the thoracolumbar region in addition to the low-dose fetus that showed encephalocele with indented and elongated os nasale. The skeletal variations were accessory 13th ribs, thickened ribs, fused sternbrae and fused thoracic vertebral bodies at 1 and 3 mg/kg/d. Total retardations were significantly decreased at the mid dose (3 mg/kg/d) and high dose (9 mg/kg/d) due to a decrease in the incidence of incomplete ossification or reduction in size of the sternbrae and decreased incidence of bipartite and dumbbell-shaped thoracic vertebral bodies.

The results of the toxicokinetics analysis demonstrated dose-related increases in systemic exposure of the pregnant rabbits to CG5503 on the first (gestation day 6) and last (gestation day 20) dosing days. There were no significant differences in plasma CG5503 concentrations between gestation days 6 and 20, measured at any timepoint (0.25, 60 and 120 minutes).

Key Study Findings:

- Maternal toxicity at 9 mg/kg/d: flaccid position, increased respiratory rate, opisthotonus and tremor
- One abortion at 9 mg/kg/d on gestation day 26 (within the range of historical background incidence)
- Decrease in number of live fetuses and increase in post-implantation loss at 9 mg/kg/d due to the deaths of 10 fetuses in 2 of the 16 litters (considered incidental)
- Systemic exposure and validity of the study demonstrated by TK analysis
- No treatment-related increases in external and skeletal malformations, variations, and retardations in the fetuses at up to 9 mg/kg/d IV CG5503 given on gestation days 6-20 in rabbits

Study title: 14-Day Dose-Range-Finding Study to Determine the Dose Levels for an Examination of the Influence of CG5503 on the Pregnant Rat and the Fetus by Subcutaneous Administration Twice Daily

Key study findings:

- Injection site brown discoloration in 1/3 MD (15 mg/kg SC b.i.d.) and all HD (25 mg/kg SC b.i.d.) dams, with eroded/weeping hemorrhagic lesions (1/3 HD dams), eschar formation (1/3 HD dams), subcutaneous erythemas in all MD and HD dams, and injection site indurations in 2/3 HD dams
- Reduced food consumption at MD (-10%) and HD (-18%)
- Reduced motility at HD
- NOAEL = 5 mg/kg b.i.d. SC SC5503 in female rats
- The Sponsor selected the SC doses of 5, 15, and 25 mg/kg b.i.d. for the embryo-fetal toxicity study in rats, based on the results of this study

Study no.: TP2465

Conducting laboratory and location: []]

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Date of study initiation: November 4, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug CG5503 (tapentadol), lot # (Batch) CEWS112, and % purity: 97.9%

Methods

Doses: 0 (vehicle control), 5, 15, and 25 mg/kg b.i.d.

Species/strain [CD@] CD@_rat ([]] pages 7

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weeks, weights 182-200 g)

Number/sex/group: 3 non-pregnant females/dose

Route, formulation, volume, and infusion rate: Test article dissolved in 0.9% NaCl solution, administered by subcutaneous bolus injection (dorsal skin) at 2 ml/kg, twice daily (8 hours apart) for 14 consecutive days

Satellite groups used for toxicokinetics: Same animals used in the main study

Parameters and endpoints evaluated:

Local tolerance: injection site examined daily and at necropsy

Mortality: twice daily during dosing Days 1-14

Clinical signs: daily during dosing Days 1-14

Body Weights: daily for dose adjustment, weekly for toxicity evaluation

Food Consumption: daily during dosing Days 1-14, reported as weekly

mean

Necropsy: Sacrifice at the end of 2-week dosing period; macroscopic examination performed only, by pathologist

Toxicokinetics: Blood withdrawn (0.3 ml from retrobulbar venous plexus) at 0 (before first dose), 0.25, 1, 3, and 5 hours after the first dose on Day 14

Results

Local Tolerance: Injection site brown discoloration (approximately 8-12 mm x 8 mm) in 1/3 MD dams (Days 9-11), and local lesions (red/brown discolorations of 8-10 mm x 5 mm) at injection sites of all HD dams on several dosing days, with eroded/weeping hemorrhagic lesions (1/3 HDF) and eschar formation (1/3 HDF); Subcutaneous erythemas in all MD and HD females, with injection site indurations in 2/3 HDF

Mortality: No deaths during the study

Clinical signs: Reduced motility in all HDF throughout dosing period, starting 5 minutes and lasting 5-20 minutes after dosing

Body weight: No treatment-related effects; mean BWG values were +10%, +21%, +24%, and +12% at 0, and the LD, MD, and HD, respectively

Food consumption: Reduced at MD in Week 2 (-10%) and HD (in Weeks 1 and 2) (-18%); no treatment-related effects on water consumption

Toxicokinetics: The results of the toxicokinetic evaluation are presented in the following table (provided from the original NDA submission):

C_{max} and AUC_{0-4} values of CG5503 base

Dose CG5503 [mg/kg]	Study day	C_{max} [ng/mL]	AUC [h*ng/mL]
5	day 14	352	419
15	day 14	907	1144
25	day 14	2441	2565

There was a dose-proportional increase in plasma exposure (C_{max} and AUC).

Necroscopic evaluations: No treatment-related effects

Study title: CG5503 Preliminary Study for Tolerability in Pregnant Rats by Twice Daily Oral (Gavage) Administration

Key study findings:

- Hypersalivation (ptyalism) observed in most CD5503-treated rats (4/5 at HD of 200 mg/kg b.i.d. PO, 3/5 at MD of 150 mg/kg b.i.d. PO), in the second dosing week at the MD and throughout the dosing period at the HD
- Reduced body weight gains at the MD (-24%) and HD (-23%)
- No treatment-related effects in the macroscopic examination of the dams, the numbers of implantations, and dead and live fetuses
- NOAEL for maternal toxicity = <150 mg/kg b.i.d. (300 mg/kg/day) PO CG5503
- The Sponsor selected the doses of 150 and 200 mg/kg b.i.d. (300 and 400 mg/kg/day) by oral gavage for an embryo-fetal toxicity study in rats, based on the results of this study

Study no.: TP2767

Conducting laboratory and location []

Date of study initiation: March 22, 2006

GLP compliance: Yes

QA reports: yes (x) no ()

Drug CG5503 (tapentadol), lot # (Batch) E0001/10, and % purity: 100.2%

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Methods

Doses: 0 (vehicle control), 0, 150 and 200 mg/kg b.i.d. (0, 300 and 400 mg/kg/day)

Species/strain: Sprague Dawley [] CD(SD) IGS BR time-mated female rat [] Ages 10-11 weeks, mean weights 250 g

Number/sex/group: 5 non-pregnant females/group

Route, formulation, volume, and infusion rate: Test article dissolved in physiological saline (0.9% NaCl) administered by oral gavage, twice daily (5 hours apart) on gestation days 6 through 18, inclusive

Satellite groups used for toxicokinetics:

Parameters and endpoints evaluated:

Mortality: twice daily during dosing period

Clinical signs: daily during dosing period

Body weight: gestation days 2, 6, 9, 12, 15, and 19

Food consumption: gestation days 2-6, 6-9, 9-12, 12-15, and 15-19

Toxicokinetics: Not done

Necropsic evaluations (dams): gestation day 19; thoracic and abdominal organs examined, ovaries and uteri examined for numbers of corpora lutea, distribution of dead and live concepti, number of implantation sites, early and late resorptions, live and dead fetuses were counted; observations classified as following uterine scar = uterine implantation without implant, early resorptions = evidence of implant without recognizable embryo, later resorptions

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= dead embryo or fetus with external degenerative changes, and dead fetus = non live conceptus with discernible digits

Results

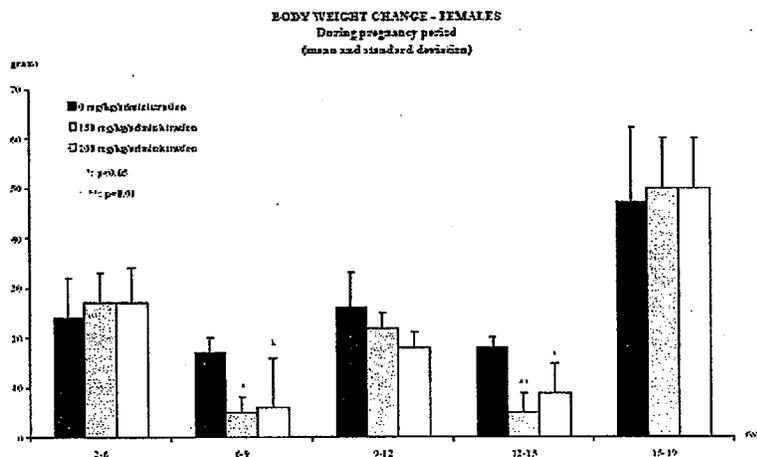
Mortality: No deaths were observed

Clinical signs:

- Hypersalivation (ptyalism) observed in most CD5503-treated rats (4/5 at HD, 3/5 at MD), in the second dosing week at the MD and throughout the dosing period at the HD

Body weight: Reduced body weight gain (BWG) compared to controls, at the MD (-24%) and HD (-23%) for the first 9 dosing days, statistically significant on gestation days 6-9 and 12-15 without relationship to dose; the results of the body weight gain measurements are presented in the following figure (provided from the original NDA submission):

**FIGURE 2. BODY WEIGHT CHANGE - FEMALES
(DURING PREGNANCY PERIOD) (MEAN AND STANDARD DEVIATIONS)**



Food consumption: Reduced, compared to controls, at the MD and HD throughout treatment, statistically significant, without relationship to dose

Necroscopic evaluations (dams):

- All females were pregnant
- No treatment-related effects in the macroscopic examination of the dams
- No treatment-related effects on numbers of implantations or live fetuses

Study title: CG5503: Combined Preliminary Study for Effects on Pre- and Post-Natal Development and Juvenile Toxicity by Once/Twice Daily Oral (Gavage) Administration in Rats

Key study findings:

- Maternal toxicity indicated by dose-related (25 (LD), 75 (MD), and 150 (HD) mg/kg PO (gavage) b.i.d., 50, 150, and 300 mg/kg/d PO) reduced body weight gains and food consumption
- No treatment-related effects on pregnancy and parturition
- Treatment-related F₁ toxicity (via transfer in milk through PND 8, and/or direct gavage treatment of the pups on PND 13-26 at 25 (LD), 75 (MD), and 150 (HD, reduced to 100 on post-natal day 15) mg/kg/day PO
 - HD pup deaths in first 4 PND (1 HD M at maternal HD), 2 HD M and 1 HD F
 - Reduced BWG, dose-related in F pups through end of maternal dosing (during lactation, up to PND 8), and in HD-treated M and F pups administered HD CG5503 from HD and control dams (PND 13-26)
 - Clinical signs in the pups were hypoactivity at 75 mg/kg/day PO and sedation and coldness at 150 mg/kg/day until reduction in pup dose to 100 mg/kg/day, more severe in pups from HD dams than in pups from control dams
- Systemic exposure to parent drug and to the glucuronide metabolite was demonstrated in maternal (F₀) rats and in the F₁ generation fetuses from treated dams, and treated F₁ pups and juvenile rats
 - Dose-linear increase in exposure in the F₁ fetuses and pups
 - No gender differences in pharmacokinetic parameters in the F₁ generation
 - No increases in exposure with repeated dosing vs. single dose (no accumulation) in the pups
- Transfer via placenta and via lactation likely, based on the toxicokinetic observations in the vehicle-treated F₁ fetal and juvenile rats from treated dams.
- Based on the results of this study, the maternal doses of 0, 10, 25, 75, and 150 mg/kg PO b.i.d. (0, 20, 50, 150, and 300 mg/kg/day PO) and juvenile rat doses of up to 100 mg/kg/day PO were selected for the definitive Pre- and Post-Natal Development Study in Rats, with treatment duration of up to PND21 in the maternal rats
 - Although decreased BWG and increased clinical signs were observed in the HD pups in this study, these signs of toxicity resolved with continued treatment beyond the first 9 days of dosing

Study no.: TP2772

Conducting laboratory and location: }

Date of study initiation: June 14, 2006

GLP compliance: Yes

QA reports: yes (x) no ()

Drug tapentadol (referred to as CG5503 in this study), lot # Batch E0001/10, and % purity: 100.2%

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Methods

Doses: F₀: 0, 0, 25, 75, and 150 mg/kg (0, 0, 50, 150, and 300 mg/kg/day)
 F₁: 0, 150/100*, 25, 75, and 150/100# mg/kg/day (*HD reduced from 150 mg/kg/day to 100 mg/kg/day on PND15, 3rd dosing day in the pups, due to severe clinical signs [sedation, coldness]); the two HD groups represent pups from dams given control vehicle and pups from dams given HD CG5503 during pregnancy through post-natal day (PND) 8

Species/strain: Sprague-Dawley [] CD(SD) IGS BR rats (ages 10 weeks)

Number/sex/group: 6 pregnant females/group, 12/sex/dose in the pups

Route, formulation, volume, and infusion rate: Test article dissolved in physiological saline (0.9% NaCl solution); administered by oral gavage at 5 ml/kg

Satellite groups used for toxicokinetics: 12F₀/group on GD6, 17, and 20; all culled pups on PND7; 12 /sex/group F₁ juvenile rats on PND 13 and 26

Study design: The F₀ rats were administered negative control and test articles twice daily from gestation day (GD) 6 through post-partum day (PPD) 8, inclusive. The F₁ pups were administered negative and control articles once daily from post-natal day (PND) 13-26, inclusive. One F₁ control group received vehicle control, and one F₁ control group received the high dose (150/100 mg/kg/day) to compare effects of pre-natal exposure and direct treatment. The rats were sacrificed as follows:

F₀ main study dams: after weaning of F₁ (PPD 22)

F₀ (toxicokinetic evaluation) TK dams: GD17 or 20

F₁ culled pups: PND 7 following TK sampling

F₁ extra pups: PND 13

F₁ Single-Dose administration pups: PND 13 after blood samples

F₁ Repeated-Dose administration pups: PND 26 after blood samples

Parameters and endpoints evaluated:**Maternal evaluations:**

Mortality: Once daily prior to treatment period; Twice daily from start of treatment; premature sacrifices to be examined macroscopically

Clinical signs: Once daily

Body weights: GD 2, 6, 9, 12, 15, and 20, and post-partum days 1, 4, 8, 10, 14, 18, and 22

Food consumption: Recorded for intervals GD 2-6, 6-9, 9-12, 12-15, and 15-20, and post-partum days 1-8, 8-14, and 14-22

Parturition: Monitored daily, day of parturition designated Day 1 post-partum (PPD1), gestation length recorded; normal littering and rearing of pups allowed through weaning

Toxicokinetics: Blood samples on GD 6 (0.25h after dosing), GD 17 (baseline, 0.25, 1, 2, and 4.5 hours after first daily dose and 0.25h after second daily dose), and GD 20 (0.25 and 4.5 h after first daily dose)

Post-mortem examination:

thoracic and abdominal organs (all dams)

numbers of corpora lutea

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numbers of implantations
implantation type (scars, early and late resorptions, live fetuses, dead fetuses
where appropriate)

F₁ Generation evaluations:

Litter size: Immediately after birth; litter adjusted on post-natal day 7 (culled to 5/sex/dose where possible), on post-natal day 9 (culled to 2/sex/litter/dose), and on post-natal day 13 (2/sex/litter chosen for pharmacokinetic evaluation)

Sex of pups: Immediately after birth

Numbers of live, dead and cannibalized pups: daily; pups found dead or sacrificed were examined macroscopically

Presence of Milk: After birth

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Post-natal days 1, 4, 8, and daily from days 12-26

Toxicokinetics: 3 separate groups of M and F pups from each litter were used for the following evaluations of tapentadol and tapentadol O-glucuronide pharmacokinetics, and were sacrificed after blood sampling:

Evaluation of exposure through milk: 0.5 ml blood sampled on post-natal day 7

Evaluation of single dose exposure: Following single dose on postnatal day 13, blood samples (0.5 ml) at baseline, 0.25, 2, and 4.5 hours after dosing (3pups/sex/timepoint)

Evaluation of repeated dose exposure: Following dosing in pups from post-natal days 13-26, blood sampled (0.5 ml) from the orbital sinus vein on post-natal day 26, at baseline (before last dose), and 0.25, 2, and 4.5 hours after dosing

Pups were sacrificed following blood withdrawal on all TK evaluation days (PND 7, 13, and 26)

Terminal examinations: Macroscopic examination of all pups found dead or sacrificed, culled pups (PND 7, however extra pups culled on PND 13 were not examined) and pups sacrificed on PND 26 after blood sampling; no histopathology examination performed in this study

Results

Toxicokinetics: The results of the toxicokinetic evaluation on tapentadol in maternal rats (F₀ generation, GD 17) and rat pups (F₁ generation, PND 13 and 26), are presented in the following table (provided from the original NDA submission):

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Relevant mean exposure data as average C_{max} (\pm SD) and $AUC_{0-4.5h}$ values of CG5503 base:

		Dose [mg/kg bid]			
Parameter		25	75	150	
F0	C_{max} [ng/mL]	254 \pm 111	601 \pm 270	810 \pm 172	
GD17	t_{max} [h]	0.25	0.25	0.25	
dams	$AUC_{0-4.5h}$ [h·ng/mL]	271	834	1273	
		Dose [mg/kg]			
Parameter		25	75	150 (Group 2) ¹	150 (Group 5) ²
F1	C_{max} [ng/mL]	129 \pm 27	1055 \pm 750	2459 \pm 1511	6804 \pm 6259
PND13	t_{max} [h]	2.0	4.5	4.5	2.0
male	$AUC_{0-4.5h}$ [h·ng/mL]	478	3266	4760	18971
F1	C_{max} [ng/mL]	159 \pm 71	4070 \pm 5943	2347 \pm 2358	5179 \pm 3702
PND13	t_{max} [h]	4.5	0.25	0.25	0.25
female	$AUC_{0-4.5h}$ [h·ng/mL]	628	6081	6764	9697
		25	75	100 (Group 2) ¹	100 (Group 5) ²
F1	C_{max} [ng/mL]	57 \pm 14	218 \pm 84	396 \pm 285	185 \pm 112
PND26	t_{max} [h]	0.25	0.25	0.25	0.25
male	$AUC_{0-4.5h}$ [h·ng/mL]	63	305	454	263
F1	C_{max} [ng/mL]	69 \pm 19	237 \pm 110	508 \pm 365	282 \pm 99
PND26	t_{max} [h]	0.25	0.25	0.25	0.25
female	$AUC_{0-4.5h}$ [h·ng/mL]	73	380	513	326

¹ naïve pups of control dams (Group 2)

² pups of high-dosed dams (Group 5)

The results of the toxicokinetic evaluation for the metabolite tapentadol O-glucuronide in maternal rats (F₀ generation, GD 17) and rat pups (F₁ generation, PND 13 and 26), are presented in the following table (provided from the original NDA submission):

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Relevant mean exposure data as average C_{max} (\pm SD) and $AUC_{0-4.5h}$ values of CG5503 glucuronide:

Parameter		Dose [mg/kg bid]		
		25	75	150
F0	C_{max} [ng/mL]	15699 \pm 1866	24467 \pm 3380	41795 \pm 13906
GD17 dam	t_{max} [h]	1.0	1.0	1.0
	$AUC_{0-4.5h}$ [h·ng/mL]	35492	84139	138947

Parameter		Dose [mg/kg]			
		25	75	150 (Group 2) ¹	150 (Group 5) ²
F1	C_{max} [ng/mL]	6791 \pm 789	41032 \pm 10754	55664 \pm 18761	65684 \pm 464
PND13 male	t_{max} [h]	2.0	4.5	4.5	4.5
	$AUC_{0-4.5h}$ [h·ng/mL]	23753	100239	112324	132753
F1	C_{max} [ng/mL]	9079 \pm 5247	36050 \pm 8596	59011 \pm 15466	59276 \pm 5039
PND13 female	t_{max} [h]	4.5	4.5	4.5	4.5
	$AUC_{0-4.5h}$ [h·ng/mL]	28992	85414	126066	125321

Parameter		Dose [mg/kg]			
		25	75	100 (Group 2) ¹	100 (Group 5) ²
F1	C_{max} [ng/mL]	10662 \pm 979	18894 \pm 7521	18879 \pm 2828	20336 \pm 8402
PND26 male	t_{max} [h]	0.25	0.25	0.25	4.5
	$AUC_{0-4.5h}$ [h·ng/mL]	18995	59344	68665	67005
F1	C_{max} [ng/mL]	8146 \pm 3530	14673 \pm 4932	19876 \pm 1209	18866 \pm 1689
PND26 female	t_{max} [h]	0.25	0.25	0.25	0.25
	$AUC_{0-4.5h}$ [h·ng/mL]	18581	56995	70826	63640

¹ naïve pups of control dams (Group 2)

² pups of high-dosed dams (Group 5)

The toxicokinetic evaluation showed continuous exposure to parent drug and the glucuronide metabolite, with nearly dose-linear increases. The tapentadol T_{max} was approximately 0.25 hour following Dose 1, and higher after subsequent doses at the MD and HD. No changes in exposure were observed with repeated dosing, suggesting absence of accumulation.

Placental transfer of parent drug was shown, with comparable exposure in the pups and dams on GD20 (41%, 23%, and 60% the exposure in the dams at 0.25h and 151%, 98%, and 129% the exposure in the dams at 4.5 h, at the LD, MD, and HD, respectively). T_{max} was also 0.25 h in the fetuses on GD20. Transfer of the drug in milk was revealed by pup plasma concentrations of approximately 5% that in the lactating dams on PND 4, without gender difference.

Plasma exposure in the juvenile rats administered tapentadol increased in a dose-linear manner (Tmax of approximately 0.25-4.5 h on PND13 and 0.25 h on PND 26). Lower plasma concentrations of both parent drug and metabolite were found in the pups on PND 26 than on PND 13. There were no differences in exposure in pups from control-treated vs. HD-treated dams, and no gender differences in exposure in the juvenile rats.

F₀ in-life:

Maternal toxicity:

- One dam sacrificed (PND 14), following deaths in all pups of the litter
- Dose-related reduction in body weight gains and food consumption
 - The mean body weights in the pregnant dams (GD20) are presented in the following table (provided from the original NDA submission):

Dose-level (mg/kg/administration)	0	0	25	75	150
Mean body weight on day 20 <i>post-coitum</i> (g)	386	359	364	362	345
Mean body weight change from day 6 to day 20 <i>post-coitum</i> (g)	+127	+102*	+109	+104*	+86#

*: p<0.05, #: p<0.001.

Mean body weights in the lactating dams (PPD8) are presented in the following table:

Dose-level (mg/kg/administration)	0	0	25	75	150
Mean body weight on day 8 <i>post-partum</i> (g)	337	344	318	298*	285**
Mean body weight change from day 1 to day 8 <i>post-partum</i> (g)	+35	+41	+29	+12**	+10**

*: p<0.05, **: p<0.01.

- Food consumption was significantly reduced at the HD throughout gestation, and at the MD on GD 6-15
- No treatment-related effects on pregnancy and parturition evaluations

F₀ necropsy: No treatment-related effects

F₁ physical development:

- Slight **increase in pup deaths** in first 4 PND at HD (150 mg/kg/d vs. control), within historical range: deaths in 1 HD M at 150, 2 HD M at 100 (following reduce dosing) and 1 HD F at 100 mg/kg/day
- **Sedation** in most of the HD M and F pups on first dosing day (PND 13)
- Sedation and **cold to touch** in most M and F pups in the repeated dose groups on second dosing day (PND 14); additionally hypersensitivity to noise in most pups

from litters not exposed in utero to tapentadol, but not in the pups from dams given HD tapentadol during pregnancy

- **Hypoactivity** on third dosing day in the HD pups from dams given HD and control vehicle during pregnancy (PND 15), with sedation and hypersensitivity to noise; clinical signs in both groups abated in most pups with reduction of the HD to 100 mg/kg/day, no clinical signs in any pups from PND 9-21
- **Reduced BWG** in F pups on PND 1, maternal dose-related through end of maternal dosing
- **Reduced BWG and BW** in HD-treated pups (PND 13-26) from HD and control dams
- The results of the pup BW and BWG evaluations on PND 26 (following dosing from PND 13-26) are presented in the following tables (provided from the original NDA submission):

Mean male body weights and body weight changes.

Dose-level (mg/kg/day)	0	150/100	25	75	150/100
Mean body weight on day 1 (g)	29	29	29	28	25#
Mean body weight on day 3 (g)	38	28#	37	34	26#
Mean body weight on day 14 (g)	87	70#	85	80	69#
Mean body weight gain day 1-14 (g)	+57	+42#	+56	+52	+44#

#: p<0.001

Mean female body weights and body weight changes.

Dose-level (mg/kg/day)	0	150/100	25	75	150/100
Mean body weight on day 1 (g)	29	26#	28	27**	25#
Mean body weight on day 3 (g)	39	28#	37	33#	27#
Mean body weight on day 14 (g)	83	66#	78	74**	68#
Mean body weight gain day 1-14 (g)	+53	+39#	+50	+47**	+43#

** : p<0.01. #: p<0.001

- **Pup clinical signs:**
 - hypoactivity at 75 mg/kg/day
 - sedation, coldness at HD of 150 mg/kg/day until reduction in pup dose to 100 mg/kg/day; more severe in pups of control dams than in pups of HD dams, suggesting some development of tolerance during prenatal tapentadol exposure

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Study title: CG5503: 14-Day Dose-Range-Finding Study To Determine The Dose Levels For An Examination Of The Influence Of BN-200 In the Pregnant Rabbit And The Fetus By Intravenous Administration

Key study findings:

- Reduced motility, flaccid position at all doses (3-15 mg/kg/day)
- Opisthotonus, increased respiratory rate, reduced food consumption and body weights at MD (7 mg/kg/day) and HD
- Reduced respiratory volume and exophthalmus at HD
- Clinical signs dose-related in severity and duration
- NOAEL in the F rabbits = <3 mg/kg/day IV
- The Sponsor proposed doses of 0, 1, 3, and 9 mg/kg/day IV for the embryotoxicity study in rabbits, based on the results of this study

Study no.: TP2061

Conducting laboratory and location: [redacted]

Date of study initiation: May 13, 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug tapentadol (referred to as BN-200 in the study report), lot # (Batch #) 06, and % purity: 98% (TLC)

b(4)

Methods

Doses: 0 (saline vehicle), 3 (LD), 7 (MD), and 15 (HD) mg/kg/day

Species/strain: Himalayan rabbit [redacted]

Age: ages 4 months, weights 2.4-2.6 kg

Number/sex/group: 2 non-pregnant F/dose

Route, formulation, volume, and infusion rate: Test article dissolved in physiological saline, administered by intravenous (IV) infusion at 3 ml/kg over 1 minute, once daily for 2 weeks

Satellite groups used for toxicokinetics: None

Study design:

Parameters and endpoints evaluated:

Local tolerance: injection site examined daily and at necropsy

Mortality: twice daily during dosing Days 1-14

Clinical signs: daily during dosing Days 1-14

Body Weights: daily for dose adjustment, weekly for toxicity evaluation

Food Consumption: daily during dosing Days 1-14, reported weekly

b(4)

mean

Necropsy: Sacrifice at the end of 2-week dosing period; macroscopic examination performed only, by pathologist

Toxicokinetics: Not done

Results

Local tolerance: No treatment-related effects at or near injection sites

Mortality (dams): No deaths

Clinical signs (dams): The results of the clinical signs observations are presented in the following table:

Incidence of Clinical Signs in Non-pregnant Female Rabbits Administered BN-200 by Intravenous Injection for 2 Weeks

Observation	Saline Control	3 mg/kg/day	7 mg/kg/day	15 mg/kg/day
Reduced mobility	0	2 (Days 1-14)	2 (marked, D1-14)	2 (4 days during Wk 1)
Flaccid position	0	2 (Day 1)	2 (marked (D1-14)	2 (D1-14)
Tendency toward flaccid position	0	2 (on 4 and 12 days) from 5-20 min after injection	-	-
Opisthotonus	0	0	2 (D1-14)	2 (D1-14)
Increased respiratory rate	0	0	2 (slight, on 1-4 days,)	2 (slight, D1)
Reduced respiratory volume	0	0	0	2 (several test days)

The clinical signs started approximately 5-20 minutes after dosing, and lasted for up to 20 minutes at 3 mg/kg/day, up to 60 minutes at 7 mg/kg/day, and up to 1-2 hours at 15 mg/kg/day. There was a dose-related increase in severity of clinical signs; severity of reduced motility and flaccid position was marked at the MD and HD.

Body weight (dams): No BW gain in 1 rabbit at 7 mg/kg/day, and BW reduced in both rabbits at 15 mg/kg/day (-12.3% compared to controls)

Food consumption (dams): Slight reduction after 2 weeks (-10%) at 7 mg/kg/day and pronounced reduction after test week 1 (-42%) and 2 (-13%)

Necropsy: No treatment-related effects

Study title: 14-Day Dose-Range-Finding Study to Determine the Dose Levels for an Examination of the Influence of CG5503 on the Pregnant Rabbit and the Fetus by Subcutaneous administration Twice Daily

Key study findings:

- HD (25 mg/kg b.i.d.) red/brown discoloration at the injection sites, with subcutaneous lesions and skin thickening
- Reduced body weight (-18%) at the HD
- Miosis and dose-related reduced food consumption at all doses (5-25 mg/kg/day)
- Dyspnea was found on the first dosing day at the HD
- Reduced motility, abdominal position and reduced body weights observed at the HD
- Pale liver (both rabbits) and urinary bladder gritty substance (one rabbit) observed at the HD in the necroscopic examination
- The NOAEL for maternal toxicity in rabbits was < 5 mg/kg b.i.d. SC tapentadol
- Based on the results of this study, the Sponsor selected the doses of 5, 15, and 25 mg/kg SC twice daily for an embryotoxicity study in rabbits

Study no.: TP2464

Conducting laboratory and location: []

Date of study initiation: November 4, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug tapentadol (referred to as CG5503 in the study report), lot # (Batch) CEWS112, and % purity: 97.9%

b(4)

Methods

Doses: 0 (vehicle control), 5, 15, and 25 mg/kg/day

Species/strain: Himalayan rabbit (ages 4-5 months, weights 2.45-2.99 kg)

Number/sex/group: 2 non-pregnant F/dose

Route, formulation, volume, and infusion rate: Test article dissolved in 0.9% NaCl solution, given by SC bolus injection (0.5 ml/kg, under dorsal skin) twice daily (8h apart) for 14 consecutive days

Satellite groups used for toxicokinetics: None

Parameters and endpoints evaluated:

Local tolerance: Injection site examined daily throughout dosing period

Clinical signs: Daily throughout dosing period

Mortality: Twice daily

Body weights: At first dose and weekly until sacrifice

Food and drinking water consumption: Daily throughout dosing period

Toxicokinetics: Blood sampled (0.3 ml, hind limb) at 0 (before dosing), 0.25, 1, 3, and 5 h after the first of the two doses on the last dosing day (Day 14)

Pathology: Macroscopic examination at the end of the 2-week dosing period

Results

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Local tolerance:

- No treatment-related effects at or near the injection site at LD and MD
- 10 mm diameter red/brown discoloration in one of the 2 HD dams (Days 8-14)
- Subcutaneous tissue lesions observed in all treated dams, with dose-related increase in severity (slight to severe erythemas) and size (1-20 mm), and thickened injection sites at MD and HD

Mortality (dams): No deaths were observed during the study

Clinical signs (dams):

- Miosis observed in all tapentadol-treated dams, slight at LD and MD from Day 2 until necropsy, and moderate at HD on all treatment days, starting 5 minutes after injection and lasting 1-2 hours
- Reduced motility at the HD throughout dosing, starting 5 minutes after dosing and lasting 1-2 hours after dosing
- Abdominal position with dyspnea at the HD after the first dose, starting within 1-2 hours after dosing and lasting for 5-20 minutes

Body weight (dams): The results of the body weight measurements are presented in the following table (provided from the original NDA submission):

Body weight increase at time point of dissection (compared to the start value)			
Control	5 mg/kg b.w.	15 mg/kg b.w.	25 mg/kg b.w.
plus 2%	minus 6%	0%	minus 18%

Food consumption (dams): There were no treatment-related effects on water consumption. The results of the food consumption measurements are presented in the following table (provided from the original NDA submission):

Food consumption (compared to the control value)			
Date	5 mg/kg b.w.	15 mg/kg b.w.	25 mg/kg b.w.
TW 1	minus 92%	minus 87%	minus 80%
TW 2	minus 73%	minus 83%	minus 88%

(TW = test week)

Toxicokinetics: The results of the toxicokinetic evaluation are presented in the following table (provided from the original NDA submission):

C_{max} and AUC_{0-4} values of CG5503 base

Dose CG5503 [mg/kg]	Study day	C_{max} [ng/mL]	AUC [h \times ng/mL]
5	day 14	593	1356
15	day 14	2099	4756
25	day 14	2845	7023

Exposure increased proportionally with dose.

Necroscopic evaluations: No treatment-related effects at LD and MD. Pale liver was observed in both rabbits and there were urinary bladder gritty substance in one rabbit, at the HD.

Fertility and early embryonic development

Study title: CG5503: Study of fertility and early embryonic development to implantation in the rat

Key study findings:

- Fo toxicity: slight body weight loss and decreased food consumption in the F at 6 (MD) and 12 (HD) mg/kg/day, and sedation, hunched posture and abnormal behavior all M and F rats at MD and HD
- All rats, except for one HD M mated, with a reduction in mean precoital time (by 0.8 – 1 day) at MD and HD
- There were no aborted or resorptions of litters
- The mean numbers of corpora lutea reduced in the F at MD (-4%) and HD (-6%)
- Decreased mean numbers of implantations at MD (12.9) and HD (12.5) compared to controls (14.0)
- Mean % preimplantation loss increased in a dose-related manner at 3 (LD) (0.5 %, not significant), MD (0.8%), and HD (0.8%), compared to controls (0.2%).
- Dose-related decrease in mean number of live conceptuses at LD (13.1, not significant), MD (12.2) and HD (11.2) compared to controls (13.7)
- Mean % post-implantation loss increased, dose-related at LD (0.6%, not significant), MD (0.7%) and HD (1.2%) compared to controls (0.3%)
- Sperm counts and pharmacokinetic measurements not conducted in this study.
- The adverse embryo-fetal development effects (preimplantation and postimplantation loss) at MD and HD could be attributed to treatment-related maternal toxicity, in agreement with the Sponsor, based on the observations of

dose-related adverse clinical signs and decreased prenatating body weights and food consumption in those dose groups

- NOAEL for maternal and paternal toxicity = 3 mg/kg/day IV
- NOAEL for adverse effects on fertility in rats = 12 mg/kg/day IV
- NOAEL for adverse effects on early embryonic development = 3 mg/kg/day IV
- Toxicokinetics analyses not conducted in this study; exposure ratios at the NOAEL, based on body surface area represented approximately 1.1 times the MRHD of 700 mg/day and 0.17 times the MRHD, in M and F rats, respectively
- Estimated systemic parent drug exposures based on the TK analyses in a 4-week IV study in rats (PT1966) given doses of 3, 7, and 15 mg/kg/day, represented approximately 0.41 times the clinical exposure at the MRHD in the M and 0.35 times in the F rats

Study no.: TP2445 () Study Report 843794

Conducting laboratory and location: () ()

b(4)

Date of study initiation: June 3, 2002

GLP compliance: Yes

QA reports: yes (x) no ()

Drug tapentadol (referred to as CG5503 in the study report), lot # (Batch) CE WS 140, and % purity: 97.7%

Methods

Doses: 0 (vehicle control), 3, 6, and 12 mg/kg/day

Species/strain: HanBrl: WIST (SPF) rat

Number/sex/group: 22/sex/group ()

ages: 8 weeks M and 10

b(4)

weeks F, weights: 235-318 g M and 176-252 g F)

Route, formulation, volume, and infusion rate: Test article dissolved in physiological saline (0.9% NaCl) and saline control were administered intravenously (IV) at 8 ml/kg (0.3 ml/minute) in a tail vein, once daily.

Satellite groups used for toxicokinetics: None

Study design: Food (Pelleted Standard () rat/mouse maintenance diet) and community tap water were available *ad libitum*. The animals were housed individually in temperature (22 ± 3 degC) and humidity (30%-70%) controlled room with 12 h light/12 h dark cycle. The treatment schedule is presented in the following table (provided from the original NDA submission):

Study sequences	Males	Females
Acclimatization	10 days prior to the first treatment	10 days prior to the first treatment
Prepairing	28 days	14 days
Treatment begins	Day 1 of prepairing	Day 1 of prepairing
Pairing (maximum)	9 days	9 days
After pairing/ gestation		14 days post coitum
Treatment ends	One day prior to the actual day of necropsy	On day 6 post coitum
Necropsy	After successful mating	On day 14 post coitum

Mating was confirmed by sperm-positive vaginal smear or observation of copulation plug.

Parameters and endpoints evaluated:

Mortality: twice daily

Clinical signs: twice daily

Body Weights: daily until necropsy

Food consumption: weekly in M and F during pre-pairing, Days 0-7 and 7-14 post coitum in F after pairing

Maternal necropsy: day 14 post-coitum; numbers of implantation sites, live or dead embryos and corpora lutea counted, macroscopic examination of organs including ovaries and uteri

Male termination (necropsy): after successful mating with F; macroscopic abnormalities, macroscopic examination of seminal vesicles, testes, epididymides

Results

Mating performance is summarized in the following table (provided from the original NDA submission):

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Group Dose (mg/kg)	1 (0)	2 (3)	3 (6)	4 (12)
Number of animals at initiation				
- males	22	22	22	22
- females	22	22	22	22
Number of animals at termination				
- males	22	22	22	20 (A)
- females	22	22	21 (B)	22
Number of mated females	22	22	22	22
Number of pregnant females (with live embryos)	21 (C)	21 (C)	21	22

(A) = One male (no. 67) died after dosing on day 13 of the preparing period and a further male (no. 85) after dosing on day 4 of the pairing period (after mating).

(B) = One female (no. 143) died after dosing on day 6 post coitum.

(C) = Female nos. 97 and 115 were not pregnant

The results of the assessments of time to mating are presented in the following table (provided from the original NDA submission):

NUMBER OF FEMALES MATED DURING THE FIRST PAIRING PERIOD

Day of the pairing period	GROUP 1 0 MG/KG	GROUP 2 3 MG/KG	GROUP 3 6 MG/KG	GROUP 4 12 MG/KG
1	4	3	6	7
2	1	5	7	1
3	6	5	3	7
4	8	4	5	6
6	-	1	-	-
7	1	1	-	-
8	2	-	1	1
9	-	3	-	-
Median precoital time	4	3	2	3
Mean precoital time	3.6	3.0	2.6	2.8
N	22	22	22	22

Mortality: There were deaths in 2 HD M (Dosing Days 13 [preparing] and 32 [day after confirmed mating]) and one MD F (last dosing Day) at 6 mg/kg.

Clinical signs:

- Sedation at ≥ 6 mg/kg/d in all M (throughout dosing) and F (Days 1-4 at MD and throughout dosing at HD)
- Hunched posture, and abnormal behavior (picking up bedding with mouth) from Day 25 until last dose in MD M and from Day 22 to end of dosing in HD F beginning on Day 2-4 and lasting until the final dosing at MD and HD

Body weight: Body weight loss in males (-6g at MD and -5g at HD, on Days 1 and 2) and reduced body weight gain in females at (+1% gain at MD and HD, vs. 2.9% in controls) on pre-pairing Days 1-8

Food consumption: Dose-related decrease in F at MD (-5.7% [during prepairing] to -2.7% [during gestation] compared to controls) and HD (9.4% [during pre-pairing] to 6.7% [during gestation], compared to controls) throughout dosing

Toxicokinetics: Not done

Necropsy:

- There were no treatment-related abnormal findings in the animals that died and in the surviving tapentadol-treated M and F
- The results of the necropsy examination in the M are presented in the following table (provided from the original NDA submission):

Necropsy observations				
Testes-one or both sides reduced in size	2	1	0	0
Testes-one side organ missing	0	0	1	0
Epididymides-one or both sides reduced in size	1	0	0	0
Epididymides-one side nodule(s), yellowish	0	0	1	0
Seminal vesicles-one or both sides reduced in size	0	1	0	0

- The results of the necropsy in the maternal rats are presented in the following table (provided from the original NDA submission):

Necropsy observations				
Prenatal body weight (g ^b)	218	+1.4	-0.9	-2.8
Gestation body weight (g ^b), days 0-14	280	-1.4	-1.1	-3.6
Prenatal food consumption (g ^b)	17.1	+0.6	-1.2	-5.8
Gestation food consumption (g ^b)	23.7	-1.3	0	-4.2
Mean pre-coital time (days)	3.6	3.8	2.6	2.8
No. of females mated	22	22	22	22
No. of pregnant females	21	21	21	22
No. aborted or with total resorption of litter	0	0	0	0
Mean no. corpora lutea	14.2	14.2	13.6	13.3

Fertility parameters

- The fertility index, conception rate, and gestation index values are presented in the following table (provided from the original NDA submission):

FEMALES SCHEDULED FOR CAESAREAN SECTION

	GROUP 1 0 MG/KG	GROUP 2 3 MG/KG	GROUP 3 6 MG/KG	GROUP 4 12 MG/KG
Percentage mating	100.0	100.0	100.0	100.0
Fertility index (%)	95.5	95.5	95.5	100.0
Conception rate (%)	95.5	95.5	95.5	100.0
Gestation index (%) (Caesarean section)	100.0	100.0	100.0	100.0

Percentage mating = (Females mated / Females paired) * 100
 Fertility index = (Females achieving a pregnancy / Females paired) * 100
 Conception rate = (Females achieving a pregnancy / Females mated) * 100
 Gestation index = (Number of females with living pups / Number of females pregnant) * 100

/ ## : Fisher's Exact Test significant at 5% (#) or 1% (##) level

- Reproduction data are summarized in the following table (provided from the original NDA submission):

	GROUP 1 0 MG/KG	GROUP 2 3 MG/KG	GROUP 3 6 MG/KG	GROUP 4 12 MG/KG
NUMBER OF DAMS	21	21	21	22
CORPORA LUTEA	299	299	286	292
MEAN (+)	14.2	14.2	13.6	13.3
ST.DEV.	1.7	2.4	1.8	1.7
PRE-IMPLANTATION LOSS	5	11	16	18
% OF CORP. LUTEA (#)	1.7	3.7	5.6 ##	6.2 ##
MEAN (+)	0.2	0.5	0.8	0.8
ST.DEV.	0.5	0.8	1.6	1.8
NUMBER OF DAMS AFFECTED	4	8	7	8
IMPLANTATION SITES	294	288	270	274
% OF CORP. LUTEA (#)	98.3	96.3	94.4 ##	93.8 ##
MEAN (+)	14.0	13.7	12.9	12.5
ST.DEV.	1.6	2.5	2.6	2.8
POST-IMPLANTATION LOSS	7	13	14	27
% OF IMPL. SITES (#)	2.4	4.5	5.2	9.9 ##
MEAN (+)	0.3	0.6	0.7	1.2
ST.DEV.	0.5	1.2	0.8	2.3
NUMBER OF DAMS AFFECTED	7	8	10	16
IMPLANTATION SITE SCARS	0	0	0	0
EMBRYONIC/FETAL DEATHS TOTAL	7	13	14	27
EMBRYONIC RESORPTIONS	7	13	14	27
FETAL RESORPTIONS	0	0	0	0
FETUSES				
TOTAL FETUSES	287	275	256	247
% OF IMPL. SITES (#)	97.6	95.5	94.8	90.1 ##
MEAN (+)	13.7	13.1	12.2	11.2
ST.DEV.	1.5	2.5	2.6	3.1
LIVE FETUSES	287	275	256	247

*/** : Dunnett-Test based on pooled variance significant at level 5% (*) or 1% (**)
 #/## : Fisher's Exact Test significant at level 5% (#) or 1% (##)
 + : Steel Test significant at level 5%

- Dose-related increased pre-implantation losses and reduced numbers of implantations at MD and HD
- Dose-related increased post-implantation losses, and reduced number of fetuses/embryos, statistically significant at HD
- The results of the fertility parameter evaluations in the F are presented in the following table (provided from the original NDA submission):

Daily Dose (mg/kg)	0 (Control)	3	6	12
No. evaluated	22	22	22	22
Mean no. implantations	14.0	13.7	12.9	12.5
Mean % preimplantation loss	0.2	0.5	0.8	0.8
Mean no. live conceptuses	13.7	13.1	12.2	11.2
Mean no. resorptions	0	0	0	0
Mean no. dead conceptuses	0	0	0	0
Mean % postimplantation loss	0.3	0.6	0.7	1.2

- = no noteworthy findings, n.d. = not determined

a) at the end of dosing period. For controls, means are shown. For treated groups, percentage differences from controls are shown. Statistical significance is based on actual data, not on percent differences

b) at the end of the respective period

Embryofetal development

Study title: Study of Embryo-Fetal Development in Rats with CG5503 (BN200) by Subcutaneous Administration

Key study findings:

- There was no evidence of tapentadol-induced teratogenicity in rats at maternally toxic doses of up to 20 mg/kg b.i.d. (40 mg/kg/day, HD) SC, under the conditions of this study
- Maternal toxicity observed at all dose levels from 10 (LD) - 40 (HD) mg/kg/day SC, and included dose-related severity and duration of reduced body weight gain, and abdominal position lasting 1-2 hours
- Local toxicity: eschar formation (at MD of 20 mg/kg/day and HD) and hemorrhagic foci (HD)
- No treatment-related malformations or variations were observed at maternally toxic doses up to the HD
- Tapentadol was embryotoxic at the HD, resulting in developmental delay (skeletal retardation) with increased incidence of incomplete/missing ossification of sternbra and caudal vertebral bodies when compared to control incidence
- The NOAEL for maternal toxicity was less than 5 mg/kg b.i.d (10 mg/kg/day) SC in rats, under the conditions of this study
- The NOAEL for embryo-fetal toxicity was 10 mg/kg b.i.d. (20 mg/kg/day)
- The systemic exposure to the parent drug at the highest dose studied represented approximately 3 times the clinical exposure at the MRHD, on an AUC basis.
- Exposure to the glucuronide metabolite at the highest dose studied was approximately equivalent to clinical exposure at the MRHD, on an AUC basis.

Study no.: TP2510

Conducting laboratory and location: []

Date of study initiation: Mating: October 7, 2003; and Treatment: October 13, 2003

GLP compliance: Yes

b(4)

QA reports: yes (x) no ()

Drug CG5503 (BN200, Tapentadol), lot # (Batch) CEWS112, and % purity: 98.3%

Methods

Doses: 0 (vehicle control), 5, 10, and 20 mg/kg b.i.d. (0, 10, 20, and 40 mg/kg/day)

Species/strain: [CD-1 D@; pregnant female (F) rats ([]] ages 8-9 weeks, weights 204-254 g

b(4)

Number/sex/group: 20 pregnant F/dose

Route, formulation, volume, and infusion rate: Test article dissolved in 0.9% NaCl solution, administered subcutaneously by bolus injection under the dorsal skin at 2 ml/kg, twice daily 8 hours apart, from gestation Days 6 through 17, inclusive

Satellite groups used for toxicokinetics: 9 F for TK analyses (3/group)

b(4)

Study design: The dose levels were selected based on a 14-day dose range-finding study ([] study 16078/02, TP2464) in non-pregnant F rats given SC doses of 5, 15, and 25 mg/kg SC b.i.d. 8 hours apart (10, 30, and 50 mg/kg/day). The results of the preliminary study showed reduced food consumption at 30 mg/kg/day and reduced motility at 50 mg/kg/day, with no treatment-related pathology (NOAEL 10 mg/kg/day SC). In the main embryo-fetal toxicity study, confirmed-mated (by vaginal smear) dams were administered control vehicle and test article on gestation days 6-17 inclusive. C-sections were performed at necropsy on gestation day 20.

Parameters and endpoints evaluated:

Mortality (dams): Twice daily during treatment period

Clinical signs, including local tolerance (dams): Daily immediately after dosing and periodically for 8.75 hours after dosing

Body weight (dams): Baseline, prior to the first dose, and daily during dosing period; reported for the intervals for gestation days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, and 18-20.

Food consumption (dams): Daily during dosing period; included measurement of water consumption

Toxicokinetics: 0.3 ml blood withdrawn from the retrobulbar venous plexus on Gestation Days 6 (after first dose) and 17, at 0 (before dosing), 15 minutes, and 1, 2, 5, and 7 hours 50 minutes (prior to second daily dose)

Terminal and necroscopic evaluations:

- Gross necropsy examination of the dams, including internal organs and placentae
- C-section data:
 - ovaries, uterus and carcass weights
 - numbers (per dam, group and mean per group) of corpora lutea in the ovaries

- implantation sites (numbers per dam, distribution in uterine horns, absolute and mean numbers per group)
- resorptions (numbers per dam, distribution in uterine horns, absolute and mean numbers per group, mean percent per group, early and late resorptions)
- weight and number of fetuses alive and dead (absolute, mean and mean % numbers per dam, per sex, and group), numbers of runts per dam and per group, location of fetuses in the uterus, fetal sex and viability, placental weights

Offspring (malformations, variations, etc.): External examination, skeletal (50% litter, numbers and types of retardations, variations, malformations) and visceral (remaining 50% litter, thoracic and peritoneal cavity anomalies), size and condition of internal organs) examinations for variations, retardations, and malformations (numbers and percents per group)

Results: The numbers of animals that were examined and or evaluated are presented in the following table (provided from the original NDA submission):

Summary of animals examined/evaluated

Text Table 4:

CG5503 (BN200) dose in mg/kg b.w./administration s.c, twice daily.	Animal nos. of mated rats	Animal nos. of evaluated pregnant rats	Deceased rats (animal nos.)	Dams not pregnant (animal nos.)	Reserve animals, not examined (animal nos.)
Control	1 - 25	1, 3 - 14, 16 - 22 (N = 20)	none	2, 15	23 - 25
5 (10 mg/kg b.w./day)	26 - 50	26 - 36, 38 - 46 (N = 20)	none	37	47 - 50
10 (20 mg/kg b.w./day)	51 - 75	51 - 70 (N = 20)	none	none	71 - 75
20 (40 mg/kg b.w./day)	76 - 100	76 - 83, 85 - 96 (N = 20)	none	84	97 - 100

Mortality (dams): No mortality observed

Clinical signs (dams):

- **Abdominal position** at ≥ 20 mg/kg/day (18/20 MD and 18/20 HD dams, on 1 or more test days), starting at 20-60 minutes after dosing, and lasting 1-2 hours
- Local, injection site toxicity: Slight-moderate **eschar formation** at injection sites at ≥ 20 mg/kg/day (7/20 MD dams and all HD dams) from gestation day 11 MD or 7 at HD until end of study) and **injection site hemorrhagic foci** at 20-40 mg/kg/day (1 MD dam and 6 HD dams, which also showed eschar formation)

Body weight (dams):

- Dose-related **reduced body weight gain** at 10 mg/kg/day and higher (all dose groups) on gestation days 6 and 9 compared to controls, persisting to the end of treatment at the MD and HD
- Statistically significant on gestation Day 18 and at necropsy at the HD (-5% compared to controls by absolute BW, and -18% compared to controls by BWG)

Control body weights: 220.9 g on Day 0, 354.2 g on Day 18, +133.3 g BWG

HD body weights: 228.7 g on Day 0, 337.4 g on Day 18, +108.7 g BWG

Food consumption (dams): Reduced compared to controls at 40 mg/kg/day; no treatment-related effects on water consumption

Toxicokinetics: The results of the toxicokinetic evaluation are presented in the following tables (provided from the original NDA submission):

Table: Serum concentrations of CG5503 base in pregnant rats from the subcutaneous Segment II study (mean values ± standard deviation (S.D.))

Dose	[mg/kg]	5 bid	10 bid	20 bid
Day 6 of gestation				
C _{max}	[µg/L]	252	468	764
S.D.		± 52	± 104	± 359
AUC	[µg·h/L]	373	868	1707
S.D.		± 28	± 133	± 179
Day 17 of gestation				
C _{max}	[µg/L]	298	764	1169
S.D.		± 62	± 80	± 157
AUC	[µg·h/L]	407	882	1563
S.D.		± 26	± 89	± 96

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Table: Serum concentrations of CG5503 glucuronide in pregnant rats from the subcutaneous Segment II study
(mean values \pm standard deviation (S.D.))

Dose	[mg/kg]	5 bid	10 bid	20 bid
Day 6 of gestation				
C_{max}	[μ g/L]	1629	4010	6462
S.D.		\pm 207	\pm 1110	\pm 1988
AUC	[μ g-h/L]	3894	9619	19151
S.D.		\pm 540	\pm 1837	\pm 653
Day 17 of gestation				
C_{max}	[μ g/L]	1689	4783	7128
S.D.		\pm 119	\pm 248	\pm 569
AUC	[μ g-h/L]	4018	10734	18514
S.D.		\pm 269	\pm 411	\pm 2153

Terminal and necroscopic evaluations:

- There were no treatment-related macroscopic pathology findings in the dams and placentae in any dose group at necropsy; enlarged spleen (1.2 g) in 1 HD dam is considered to be incidental, in agreement with the Sponsor
- No treatment-related effects on uterus weights, number of corpora lutea, implantation sites, resorptions, fetal and placental weights and numbers of live fetuses.
- There were no runts and no dead fetuses in any test-article-treated group; there was one malformed runt found dead in the control group.

The results of the C-section observations are presented in the following table (provided from the original NDA submission):

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Parameter		Group 1 Control (n = 20)	Group 2 5 mg/kg b.w./day twice daily (N = 20)	Group 3 10 mg/kg b.w./day twice daily (N = 20)	Group 4 20 mg/kg b.w./day twice daily (N = 20)
Corpora lutea	total	299	312	299	329
	per dam	15.0	15.6	15.0	16.5
Implantation sites	total	297	310	296	318*
	per dam	14.9	15.5	14.8	15.9
Resorptions	total	24	8**	13*	12*
	per dam	1.2	0.4	0.7	0.6
Early resorptions	total	19	5**	11	8*
	per dam	1.0	0.3	0.6	0.4
Late resorptions	total	5	3	2	4
	per dam	0.3	0.2	0.1	0.2
Live fetuses	total	272	302**	283*	306**
	per dam	13.6	15.1	14.2	15.3
Dead fetuses	total	1	0	0	0
Pre-implantation loss	mean %	0.7	0.8	1.4	3.3
Post-implantation loss	mean %	9.0	2.5	4.4	3.9

* Significantly different from the controls at $p \leq 0.05$
 ** Significantly different from the controls at $p \leq 0.01$

There were no treatment-related effects in the C-section data, when compared to concurrent controls. Although there were statistically significant treatment-related findings of increased implantation sites, decreased resorptions and early resorptions, and increased number of life fetuses, the changes were based on comparison with concurrent controls that showed relatively high or low values, and all values fell within the range of historical control values for the conducting laboratory. The historical control values from 20 embryotoxicity studies conducted by [redacted] from 2000-2003 using Sprague-Dawley rats, for the parameters in which treatment-related differences were found in the present study are presented below:

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- Number of corpora lutea/dam: Range 13.6-17.7
- Implantations/dam: Range 13.3-17.4
- Resorptions/dam: Range 0.2-6.4
- Early resorptions mean %: Range 0.5%-87.5%
- Mean # dead fetuses/dam: Range 0.00-0.05
- Pre-implantation loss mean %: Range 0.0%-9.6%
- Post-implantation loss mean %: Range 1.5%-14.3%

Offspring:

- There were no treatment-related effects on sex distribution of the fetuses, location in the uterine horn, mean placental weights and mean fetal weights
- **Malformations:** There were no treatment-related external and skeletal malformations in the fetal rats in any dose group; two vehicle control fetuses showed malformations (1 runt with omphalocele, malrotated hind limbs, and short abdomen, and one control fetus with malrotated hind paws), that are considered to be incidental findings
- **Variations:** There were no treatment-related external and soft tissue variations; however, decreased incidence of wavy ribs, accessory 14th ribs, and sternbrae bipartite were observed in the LD and HD fetuses
- There was a treatment-related increase in the incidence of **skeletal retardations**, observed in the following:
 - skull (incomplete ossification of frontal, parietal, interparietal and supraoccipital)
 - hyoid (ossification missing)
 - sternum (incomplete, missing ossification or reduced in size)
 - thoracic vertebral bodies (bipartite, dumbbell-shaped)
 - lumbar vertebral bodies (<6 ossified)
 - caudal vertebral bodies (<2 ossified)
 - the increases in the incidences of incomplete ossification of sternbrae and missing caudal vertebral bodies at the high dose of 40 mg/kg/day were statistically significant and considered to be treatment-related, although within the range of historical data and observed in litters of dams showing maternal toxicity
- The results of the fetal examinations are presented in the following table (provided from the original NDA submission):

Study Type	Study On Embryo-Foetal Developmental Toxicity in Rats			GLP:	Yes
Dose [mg/kg]	0 (Control)	5 bid	10 bid	20 bid	

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Summary of foetal anomalies				
Total malformations				
— Foetal Incidence [N] (%)	2 (0.7)	0	0	0
— Litter Incidence [N] (%)	2 (10)	0	0	0
Total variations				
— Foetal incidences [N] (%)	23 (16.7)	13* (8.6)	28 (19.7)	13* (8.5)
— Litter incidences [N] (%)	11 (55)	6 (30)	12 (60)	6 (30)
Total retardations				
— Foetal incidences [N] (%)	123 (89.1)	131 (86.8)	125 (88.0)	136 (88.9)
— Litter incidences [N] (%)	20 (100)	20 (100)	20 (100)	20 (100)
Caudal vertebral bodies				
— Foetal incidences [N]	3	9	2	19**a
— Litter incidences [N]	1	3	1	10**a
Sterna bra(e) incompletely ossified				
— Foetal incidences [N]	29	26	31	63**a
— Litter incidences [N]	11	12	14	16
Soft tissue variations				
— Foetal incidences [N] (%)	9 (6.7)	13 (8.6)	15 (10.6)	19 (12.4)
— Litter incidences [N] (%)	8 (40)	10 (50)	12 (60)	14 (70)

* = $p \leq 0.05$ ** = $p \leq 0.01$; a = incidences were still within the range of background data.

Study title: Study of Embryo-Fetal Development in Rabbits with CG5503 (BN200) by Subcutaneous Administration

Key study findings:

- **Maternal toxicity** observed at all doses (2-12 mg/kg SC b.i.d.), that was marked and considered to be excessive at the mid-dose (MD, 5 mg/kg b.i.d.) and high dose (HD, 12 mg/kg b.i.d.), including miosis, abdominal position, and reduced body weight gain and food consumption (LD, MD and HD) and body weight loss (MD and HD)
- **Fetal weights** in the M and F fetuses were reduced significantly at the MD (-10%) and HD (-13%)
- CG5503 treatment was associated with increased embryo-fetal toxicity, including **decreased fetal and placental weights, fetal viability, variations and malformations**; considered by the Sponsor to be secondary to increased maternal toxicity, although a direct treatment-related effect on embryo-fetal malformations cannot be ruled out, as there were fetuses without observable malformations born to dams showing treatment-related toxicity
- The incidence of variations and malformations observed were within the upper limit of historical range for the laboratory

- The internal malformations included **multiple malformations** (gastroschisis or thoracogastroschisis, prolapsed organs, amelia, and phocomelia, with incidence of 0.9% at the MD and 1.7% at the HD)
- Also observed were **encephalocele** in 1 HD runt, **spinal bifida** in 1 HD runt, **kyphosis** in 1 HD runt, **ablepharia** in 3 HD fetuses from 1 litter, and **cleft palate** in 1 MD fetus [0.9%] and 3 fetuses from 1 HD litter and 1 additional fetus from another HD litter [3.4%]
- The incidence of **post-Caesarian fetal deaths** during 24-hour incubator stay was increased at the MD (9 fetal deaths, compared to 3 in each of the control and LD groups) and HD (7 deaths)
- The NOAEL for maternal toxicity was <2 mg/kg SC b.i.d.
- The NOAEL for teratogenicity by CG5503 in rabbits was 2 mg/kg SC b.i.d. in this study
- The systemic exposures to the parent drug and the O-glucuronide metabolite at the NOAEL of 4 mg/kg/d SC were both approximately equivalent to clinical exposure to the parent and metabolite at the MRHD on an AUC basis.

Study no.: TP2511

Conducting laboratory and location: [] [] [] []

Date of study initiation: September 29, 2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug tapentadol (referred to as CG5503 in the study report), lot # (Batch) CEWS112, and % purity: 98.3%

Methods

Doses: 0 (solvent vehicle), 2 (LD), 5 (MD), and 12 (HD) mg/kg b.i.d. (0, 4, 10, and 24 mg/kg/day)

Species/strain: Himilayan rabbit (ages 4-5 months, weights 2.13-3.00 kg)

Number/sex/group: 20 pregnant F/dose (of 24/dose group that were treated)

Route, formulation, volume, and infusion rate: Test article dissolved in 0.9% NaCl, administered by subcutaneous (SC) bolus injection under dorsal skin at 0.5 ml/kg

Satellite groups used for toxicokinetics: 3/dose group

Study design: Doses were selected based on the results of a dose range-finding study in rabbits (Study 16079/02) given 5, 15, and 25 mg/kg SC tapentadol twice daily (b.i.d.) (10, 30, and 50 mg/kg/d SC) for 14 days. The results of the dose-finding study showed dose-related miosis and reduced food consumption at all doses, and HD effects of reduced motility, abdominal position, and reduced body weights. Dyspnea was observed following the first high dose administration.

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In the definitive embryo-fetal toxicity study, food and water were available *ad libitum*. The test and vehicle control articles were administered twice daily, 8 hours apart on gestation days (GD) 6-20 inclusive (day of mating = Day 0). Abortions and prematurely delivered fetuses were examined for abnormalities.

Parameters and endpoints evaluated:

Maternal observations:

Mortality: Twice daily

Clinical signs and local tolerance: At least once daily, and immediately after dosing

Food and water consumption: Once daily

Body weights: Day 0 and daily, with calculations for intervals of GD 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24, 24-27, 27-29 (day of necropsy and Caesarian section), and change from GD 6-29.

Macroscopic examination: GD 29: internal organs, ovaries, uteri, and placentae evaluated (weights and examinations) at necropsy

Toxicokinetics: Blood withdrawn from the vena saphena (0.5 ml) on GD 6 (first dosing day) and GD 20 (last dosing day), at 0 (before dosing), 15 minutes, and 1, 3, 5, and 7 hours + 50 minutes (10 minutes prior to second daily dose).

Reproduction Data: Evaluated at necropsy (GD 29)

Number of dams pregnant

Abortions/premature births

Corpora lutea: Numbers per dam and per group, and mean per group

Implantations: Numbers per dam and per group, mean per group, and distribution in uterine horns

Resorptions: Numbers per dam and per group, mean per group, mean % per group, distribution in uterine horns, early (<2g) and late (>2g) resorptions

Weight of placenta: Per fetus, means per litter and per group, litter means per group and per sex and group

Locations of fetuses in uterus

Weights and sex of fetuses: Per fetus, per litter and per sex and litter, litter mean per group and per sex and group

Numbers of live and dead fetuses: Per dam, per sex and dam, distribution in uterine horns, absolute and mean numbers alive per group, mean % alive per group and per sex and group

Numbers of Runts (weights <70% mean litter weight), number per dam and mean per group

Fetal Data: Means per fetus and per group, and types of malformations, retardations, and variations evaluated at necropsy on GD 29:

External anomalies

Skeletal anomalies

Soft tissue anomalies (including brain)

Results

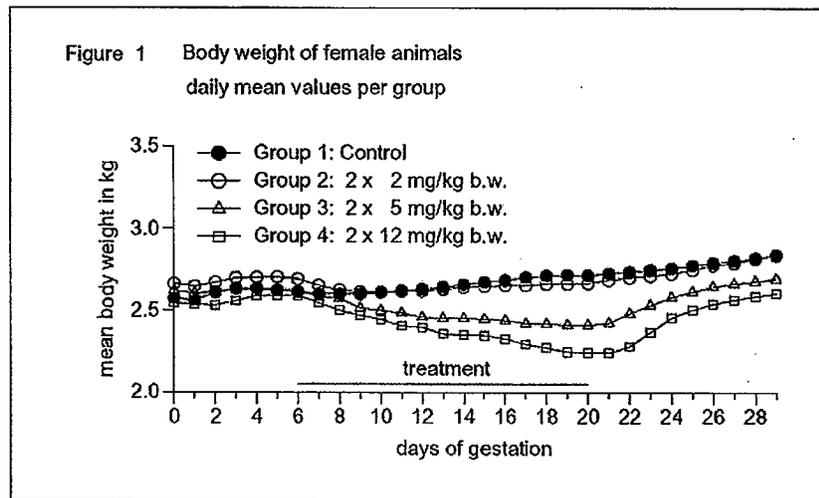
Mortality: No deaths observed during this study

Clinical signs:

- No treatment-related effects at the injection sites

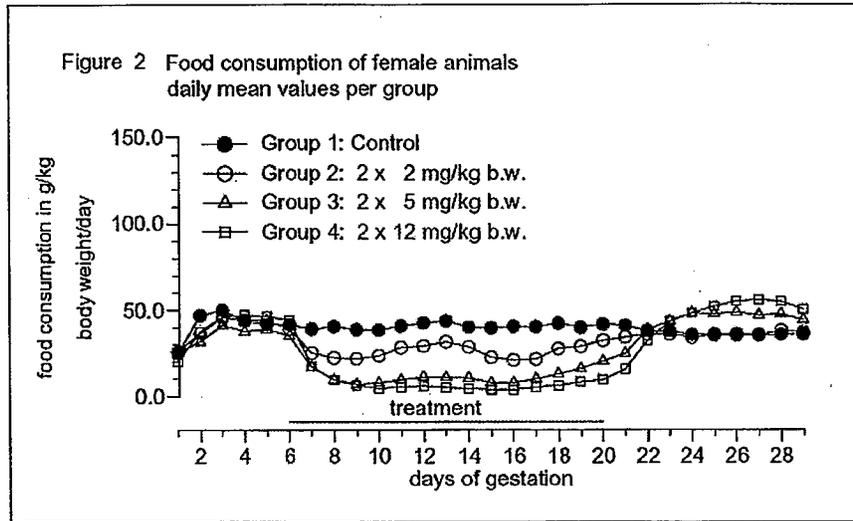
- **Miosis** (starting 5 minutes after dosing, lasting 1-2 hours) and **abdominal position** (starting 20-60 minutes after dosing, lasting 20-60 minutes) in 3/21 dams (GD 8 and 13) at MD, and all dams (GD6 onward in several and from GD 13 onward in all dams) at HD

Body weight: Statistically significant dose-related reduction in body weight gain and reduced body weights at MD (11%) and HD (18%). The daily mean body weights are presented in the following figure (provided from the original NDA submission):



Food consumption: Statistically significant reduction in food consumption at LD (up to -49%), MD (-77%) and HD (-92%), from GD 6 throughout dosing, with some recovery of food consumption after dosing period, from GD23-29; no treatment-related effects on water consumption. The daily group mean food consumption values are presented in the following figure (provided from the original NDA submission):

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Toxicokinetics: The results of the toxicokinetic evaluations in the maternal rabbits are presented in the following table (provided from the original NDA submission):

Dose [mg/kg]	Mean Concentrations		
	2x2	2x5	2x12
Day 6 of gestation			
C _{max} [ng/ml]	131	519	1221
S.D.	13	95	97
Daily AUC [ng/mL*h]	598	1894	5010
Day 20 of gestation			
C _{max} (ng/mL)	149	582	1513
S.D.	21	141	401
Daily AUC [ng/mL*h]	615	1920	5743

Terminal and necroscopic evaluations:

Maternal: No macroscopic findings at the injection sites and in the standard macroscopic examination.

Reproduction Data: There were 2 dams not pregnant in the control and LD groups, 2 not pregnant in the MD group and no dams not pregnant in the HD group. Reserve pregnant dams replaced the non-pregnant dams for evaluation. The results (group means) of the Caesarian section data are presented in the following table (provided from the original NDA submission):

Study Type	Dose [mg/kg]	Study On Embryo-Foetal Developmental Toxicity in Rabbits GLP: Yes			
		0 (Control)	2x2	2x5	2x12
Litters evaluated	[N]	20/20	20/20	21/21	20/20
Aborted	[N]	0	0	0	0
Dams with	[N]				
— viable fetuses		20	20	20	20
— all resorptions	[N]	0	0	1	0
Total live fetuses	[N]	114	129	115**	118
Foetuses died within 6 h after laparotomy	[N]	3	3	9	7
Foetuses died within 6- 24 h after laparotomy	[N]	2	1	2	9*
Mean placental weights	[g]	5.37	5.08	4.74**	4.59**
Mean foetal body weight	[g]	40.1	39.5	36.0*	34.9*

*= p<0.05

**=p<0.01

- Uterus weights were reduced at the MD and HD due to low fetal and placental weights
- There were no treatment-related effects on numbers of corpora lutea, implantation sites, resorptions and live fetuses.
- Post implantation loss (due to early and total resorptions and ratio of viable fetuses to implantation sites) was due to total post-implantation loss in one MD dam in early pregnancy, and is considered to be without relationship to tapentadol treatment. Another dam was added to the number evaluated in the MD group, to obtain the full number of evaluable litters.
- The results of the reproduction evaluation are presented in the following table (provided from the original NDA submission):

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Parameter		Group 1 Control (n=20)	Group 2 2 x 2 mg/ kg b.w./day (n=20)	Group 3 2 x 5 mg/kg b.w./day (n=21) ¹⁰	Group 4 2 x 12 mg/kg b.w./day (n=20)
Corpora lutea	total	132	147	140	140
per dam		6.6	7.4	6.7	7.0
Implantation sites	total	118	132	132	126
per dam		5.9	6.6	6.3	6.3
Resorptions	total	4	3	16**	8
per dam		0.2	0.2	0.8	0.4
Early resorptions	total	3	2	11*	6
per dam		0.2	0.1	0.5	0.3
Late resorptions	total	1	1	5	2
per dam		0.1	0.1	0.2	0.1
Live fetuses	total	114	129	115**	118
per dam		5.7	6.5	5.8 ¹¹	5.9
Dead fetuses at laparotomy	total	0	0	1	0
Pre-implantation loss	mean %	12.3	8.7	5.3	8.6
Post-implantation loss	mean %	3.4	1.8	10.3	7.0

* Significantly different from the controls at $p \leq 0.05$

** Significantly different from the controls at $p \leq 0.01$

Fetal Observations:

- No treatment-related effects on sex distribution of the fetuses
- Dose-related decrease in mean placental weights, statistically significant at MD and HD, considered to be treatment-related
- Treatment-related decreased mean fetal weights at MD and HD
- One fetus was found dead at necropsy at MD; there was an increase in numbers of fetuses that died after 6 hours incubator stay following Caesarian section, at the MD (9 fetuses compared to 3 fetuses in the control and LD groups) and HD (7 fetuses), and from 6-24 hours after Caesarian section at the HD (statistically significant, in 9 fetuses compared to 2 in the control group)
- There were 4 runts at the HD compared to 2 in the control group; all runts died during incubator stay
- External Examination:
 - No treatment-related variations
 - Slight but not statistically significant increase in fetal incidence and percent incidence of external malformations, including malrotated fore and hind limbs, malrotated fore paws, and short neck in all treated groups compared to controls

- **Skeletal Examination:**
 - Statistically significant increase in skeletal variations at the HD, in rib (accessory 13th rib or shortened ribs), caudal vertebral bodies (misaligned, fused), skull (not ossified in parietal area), and sternum (fused or misaligned)
 - Skeletal retardations were found in the skull (incomplete ossification in frontal, parietal, interparietal, supraoccipital), hyoid (not ossified), sternum (incomplete or not ossified, small size) and vertebral bodies (reduced in size, 7 not ossified, dumbbell-shaped)

- The fetal malformations are summarized in the following table from the Sponsor:

Malformed Fetuses				
Group	Dam No.	Fetus No.	Type of malformation	Fetal death after laparotomy
1 (Control)	7	2	malrotated fore and hind limbs short neck	30 min
		3	malrotated fore and hind limbs short neck	30 min
		9	malrotated fore paws	-
		12	malrotated fore paws	-
2 (2x2 mg/kg)	39	4	malrotated right fore paw	-
3 (2x5 mg/kg)	49	5 (runt)	cleft palate multiple malformations gastroschisis with prolapse of organs amelia of forelimbs phocomelia of left hind limb	30 min
		58	Im-plant No. 3 late resorption: fetus 15.9 g; with visible malrotated fore paws, placenta: 3.7 g with a few dark-red foci (diameter approx. 1-2 mm)	-
		69	5 malrotated fore paws short neck	1 h
4 (2x12 mg/kg)	74	3 (runt)	multiple malformations thoracogastroschisis with prolapse of organs amelia of right fore limb phocomelia of left fore limb encephalocele with prolapse of red-dish tissue, diameter approx. 3 mm spina bifida (cervical region) approx. 10 x 5 mm kyphosis (convex deformation of cervical region)	10 min
		1	ablepharia (both eyes) cleft palate	24 h
		3 (runt)	cleft palate malrotated right fore paw	24 h
		4	ablepharia (right eye)	-
		5	ablepharia (right eye)	-
		6	cleft palate	-

Malformed Fetuses (continued)				
Group	Dam No.	Fetus No.	Type of malformation	Fetal death after laparotomy
4 (2x12 mg/kg)	86	1 (nut)	cleft palate short neck multiple malformations thoracogastroschisis with prolapse of organs phocomelia of right forelimb spina bifida (thoracic region), spinal cord visible spina bifida (cervical region), diameter approx 10 mm left kidney small	1 h

The fetal and litter incidence (number and %) values for external and skeletal variations, retardations, and malformations are summarized in the following tables (provided from the original NDA submission):

External Malformations						
Number of fetuses with malformations		Group 1 Control	Group 2 2 x 2 mg/kg	Group 3 2 x 5 mg/kg	Group 4 2 x 12 mg/kg	
fetal incidence	N	4	1	3	7	
	%	3.5	0.8	2.6	5.9	
litter incidence	N	3	1	3	3	
	%	15.0	5.0	15.0	15.0	

External Variations						
Number of fetuses with variations		Group 1 Control	Group 2 2 x 2 mg/kg	Group 3 2 x 5 mg/kg	Group 4 2 x 12 mg/kg	
fetal incidence	N	0	0	0	0	
	%	0.0	0.0	0.0	0.0	
litter incidence	N	0	0	0	0	
	%	0.0	0.0	0.0	0.0	

Skeletal Malformations						
Number of fetuses with malformations		Group 1 Control	Group 2 2 x 2 mg/kg	Group 3 2 x 5 mg/kg	Group 4 2 x 12 mg/kg	
fetal incidence	N	0	0	1	2	
	%	0.0	0.0	0.9	1.7	
litter incidence	N	0	0	1	2	
	%	0.0	0.0	5.0	10.0	

Skeletal Variations						
Number of fetuses with variations		Group 1 Control	Group 2 2 x 2 mg/kg	Group 3 2 x 5 mg/kg	Group 4 2 x 12 mg/kg	
fetal incidence	N	3	7	8	19**	
	%	2.6	5.4	6.9	16.1	
litter incidence	N	3	5	6	11*	
	%	15.0	25.0	30.0	55.0	

* Significantly different from the controls at $p \leq 0.05$

** Significantly different from the controls at $p \leq 0.01$

Skeletal Retardations						
Number of fetuses with retardations		Group 1 Control	Group 2 2 x 2 mg/kg	Group 3 2 x 5 mg/kg	Group 4 2 x 12 mg/kg	
fetal incidence	N	72	83	77	82	
	%	63.2	64.3	66.4	69.5	
litter incidence	N	19	20	20	20	
	%	95.0	100.0	100.0	100.0	

* Significantly different from the controls at $p \leq 0.05$

** Significantly different from the controls at $p \leq 0.01$

The historical incidence range for selected malformations observed in this study, and historical range values at comparable levels of maternal toxicity for the laboratory, are presented in the following table (provided from the original NDA submission):

Findings	Fetal Incidence (%)		
	Study 17027/03	Range of Background Data	Range observed at a comparable level of maternal toxicity ¹²
Multiple malformations including gastroschisis with prolapse of organs, amelia, phocomelia	0.9	0.0 - 1.2	1.1-1.8
Multiple malformations including thoracogastroschisis with prolapse of organs, amelia, phocomelia, encephalocele, spina bifida, kyphosis	1.7	0.0 - 2.1	2.2-2.8
Ablepharia	2.5	0.0 - 2.6	
Cleft palate	3.4	0.0 - 4.5	

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The malformations included multiple malformations (0.9% at the MD and 1.7% at the HD) with gastroschisis (fissure of abdominal wall) and/or thoracogastroschisis (fissure of trunk involving thoracic and abdominal cavities), prolapsed organs, amelia (absence of limb(s)), and phocomelia (limb defect such that feet are attached close to the body). Also observed at the high dose were encephalocele (gap in skull with brain substance herniation, in 1 HD runt), spina bifida (failure of fusion of 1 or more vertebral arches), kyphosis (anteriorly concave curvature of vertebral column), ablepharia (absence of eyelids, 3 HD fetuses from 1 litter), and cleft palate (incomplete merging or fusion, 1 fetus/0.9% at 10 and 3 fetuses from 1 litter and 1 fetus from another litter/3.4% at the HD).

The results (group means) of the fetal anomalies observations are presented in the following table (provided from the original NDA submission):

Total malformations				
— Foetal Incidence [N]	4	1	3	7
— Litter Incidence [N]	3	1	3	3
— Affected foetuses/litter in %	5.7	1.0	3.3	6.7
Total variations				
— Foetal incidences [N]	3	7	8	19**
— Litter incidences [N]	3	5	6	11*

*= p<0.05

**=p<0.01

The percent incidences of fetal malformations with historical range data are presented for comparison in the following table (provided from the original NDA submission):

	Current Study				Range of historical data	
	Control	2x2 mg/kg	2x5 mg/kg	2x12 mg/kg	Control groups	Test item groups
Total malformations						
Fetal incidence [%]	3.5	0.8	2.6	5.9	0 - 5.1	0 - 6.3
Litter incidence [%]	15.0	5.0	15.0	15.0	0 - 20.0	0 - 20.0

Maternal toxicity in the dams with fetuses showing embryo-fetal malformations is presented in the following table for comparison:

Observation	10 mg/kg/d				24 mg/kg/d			
	Dam 49	Dam 58	Dam 64	Dam 69	Dam 74	Dam 75	Dam 77	Dam 86
Maternal miosis & abdominal position	-	+(GD10-13)	+(GD 8-11)	-	+(GD 13-20)	+(GD 13-20)	+(GD 12-19)	+(GD 7-13, 16-20)
Maternal BW change ^a	-7%	-8%	-7%	+2%	-13%	-14%	-17%	-9%
Reduced food consumption ^b	-49%	-89%	-13%	-13%	-92%	-62%	-78%	-98%
Mean fetal weight for the litter in g ^c	36.13 (-10%)	17.52 (-56%)	37.58 (-6%)	36.28 (-9%)	25.41 (-37%)	33.02 (-18%)	24.63 (-39%)	35.62 (-11%)
Runts	Fetus #5	-	-	-	Fetus#3	Fetus#3	Fetus#7	Fetus#1
Multiple malformations, external	Fetus#5	Fetus#3	-	Fetus#5	Fetus#3	Fetus#3	-	Fetus#1
Skeletal malformations	Fetus#5	-	-	-	Fetus#3	-	-	Fetus#1
Cleft Palate	Fetus#5	-	-	-	-	Fetus#1 Fetus#2 Fetus#6	-	Fetus#1
Amelia	Fetus#5	-	-	-	Fetus#3	-	-	-
Spinal bifida	-	-	-	-	Fetus#3	-	-	Fetus#1
Ablephonia	-	-	-	-	-	Fetus#1 Fetus#4 Fetus#5	-	-

- = not observed

+ = observed

^a = GD21 vs. GD0 (baseline); BW loss observed in 3/21 in the MD group and all animals in HD group

^b = overall group means -49%, -77%, -92% at the LD, MD, and HD, respectively; the figures presented represent % difference in food consumption on GD20 vs. GD5 (baseline)

^c = % difference from control mean fetal weight/litter 40.06g in parentheses

Prenatal and postnatal development

Study title: CG5503: Study for Effects on Pre- and Post-Natal Development by Twice Daily Oral (Gavage) Administration to Rats

Key study findings:

- **Treatment-related maternal toxicity:**
 - Deaths in 6 HD dams (300 mg/kg/d PO, 2 found dead, 4 sacrificed for cannibalizing pups, difficult delivery and clinical signs, and/or dead litter), 1 HMD dam (150 mg/kg/d, sacrificed due to dead litter), 1 LMD dam (50 mg/kg/d, sacrificed *in extremis*), 2 LD dams (20 mg/kg/d, sacrificed for difficult delivery)
 - Ptyalism at the HD, piloerection and round back at the HD and HMD (150-300 mg/kg/d)
 - Reduced BWG at the HMD (-22%, to gestation day [GD] 13) and HD (-24%, to GD 16); reduced BW at the HMD and HD
 - Reduced food consumption at the HMD and HD (GD6-20 and post-partum days [PPD] 1-7)
 - No treatment-related effects on pregnancy and parturition
- **Increase in pup mortality during lactation:** treatment-related reduction in viability index
 - complete litter deaths in 1 litter at HMD and 2 litters at HD
 - increased # pup deaths at HMD (16 pups) and HD (18 pups)
- **Significant reduction in pup body weights and body weight gains** (from PPD1 throughout lactation) at the HD, and body weights at the HMD (PPD4-end of lactation)
- **Unossified 6th centrum of cervical vertebrae** in 50% HD pups found dead (PPD1-4), and non- or incomplete ossification in other bones in several pups, likely due to lower body weights
- **F₁ post-weaning physical development: Reduced BW and BWG** in HD (300 reduced to 200 mg/kg/day) M (PPD22-end of study) and HD (PPD22-ages 10-11 weeks) and MD (150 mg/kg/day) PPD22-37) females compared to controls
- Slight (but not significant) **increase in horizontal movements** and rearing at MD and HD in M (+7%-+10%)
- Slight dose-related (but not significant) increase in horizontal movements and rearing in the LD (50 mg/kg/day), MD and HD F (+2%-+20%)
- No treatment-related effects on sexual development, auditory function, pupil constriction
- Slight (but not significant) **increase in T-maze learning test time** in M (in seconds to complete T-maze, learning phase 4th test) at MD (150 mg/kg/day, +63%) and HD (300 mg/kg/day, +52%) **and T-maze 2nd memorization test** (memory phase, +12% at the HD compared to controls)
- F₁ reproduction: No treatment-related effects on M and F mating, mean numbers of days to mate, fertility data pregnancy status, hysterectomy data (e.g., corpora lutea, implantations, concepti)
- Approximately dose-related linear increases in exposure to parent drug and glucuronide metabolite in the pregnant dams, with increased exposure to the

parent drug with repeated dosing at all but the HD, and to the glucuronide at all doses, suggesting accumulation.

- The NOAEL for maternal toxicity was 50 mg/kg/day PO, due to reduced BWG and food consumption (exposure represented approximately 1.5 times the clinical exposure to the parent drug and approximately 35 times the exposure to the tapentadol O-glucuronide metabolite at the MRHD, on an AUC basis).
- The NOAEL for pup development was 20 mg/kg/day PO, due to pup deaths from PPD 1-4 (exposure of approximately 0.3 times the clinical exposure at the MRHD on an AUC basis)
- The NOAEL for the F₁ generation was 300 mg/kg/day; although body weights were reduced, there were no effects on mating, fertility, and neurobehavioral parameters (representing exposure of approximately 10X the clinical exposure at the proposed MRHD, on an AUC basis)

Study no.: TP2834

Conducting laboratory and location:

Date of study initiation:

GLP compliance: Yes

QA reports: yes (x) no ()

Drug CG5503 (tapentadol), lot # (Batch) E0001/10, and % purity: 100.2%

Methods

Doses: 0 (vehicle alone), 10 (LD), 25(LMD), 75 (HMD), and 150 (HD) mg/kg b.i.d. (0, 20, 50, 150, and 300 mg/kg/day)

Species/strain: Sprague-Dawley CD(SD) IGS BR sexually mature, primigravid F rats (ages 10 weeks)

Number/sex/group: 24 F/dose

Route, formulation, volume, and infusion rate: Test article dissolved in physiological saline (0.9% NaCl), given by oral gavage at 5 ml/kg.

Satellite groups used for toxicokinetics: 15 F/group for TK evaluation

Study design: The b.i.d. doses were administered 5 hours apart, daily from gestation day (GD) 6 through post-partum day (PPD) 21, inclusive; GD 0 was defined as the day of confirmed mating by detection of vaginal plug. Food and water was provided *ad libitum*. The progeny were littered and reared normally by the maternal rats until weaning. Pups were culled to 4/sex/dose on PPD 4. The maternal rats were sacrificed the day after final dose.

Parameters and endpoints evaluated:

Maternal parameters (F₀ generation):

Mortality: Twice daily

Clinical Signs: Once daily

Body weights: Daily from GD 2 through the day before necropsy (PPD 22 or 23)

Food consumption: Recorded for intervals of GD2-6, 6-9, 9-12, 12-15 and 15-20, and PPDs 1-7, 7-14, and 14-21

Parturition: Monitored four times daily from GD21 through delivery of last F; difficulty/prolonged parturition, completion of parturition, length of gestation were recorded

Macroscopic examination: Day after weaning of F₁ (PPD 22 or 23), PPD 25 in F₀ F that didn't deliver by PPD 25, all F₀ F with deaths of entire litters, all F₁ F that were found dead or sacrificed prematurely (numbers of corpora lutea, implantation sites recorded, uterine scars counted in uterine horns without visible implantation sites after positive mating)

Organ weights: Not performed

Histopathology: Not performed

Toxicokinetics: GD 6 and 17, 0.5 ml blood sampled from 3F/group/timepoint, at pre-dose, 0.25, 1, 2, 3; and 4.75 hours after the first dose, and at 0.25, 1, 3, and 19 hours after the second daily dose

Offspring parameters (F₁ generation):

Observations during lactation:

litter size

number and sex of pups

numbers of live, dead and cannibalized pups throughout lactation

gross malformations

pup body weights (PPD 1, 4, 7, 14, and 21)

clinical signs (daily)

physical development:

pinna unfolding (PPD 5)

hair growth (PPD 5)

tooth eruption (PPD 13)

auditory canal opening (PPD 17)

eye opening (PPD 17)

reflex development:

surface righting reflex (PPD 5)

cliff avoidance (PPD 11)

air-righting reflex (PPD 17)

Clinical examinations after weaning: 1-2 pups/sex/litter selected for the F₁ generation post-weaning examination (PND 22):

Mortality: Twice daily

Clinical signs: Once daily

Body weights: First post-weaning day and weekly thereafter

Sexual development: M from ages 32-47 days (D11-26 of the F₁ generation), until cleavage of balanopreputial groove/preputial separation; F from PND 28-40 (D 7-19 of the F₁ generation) until vaginal opening

Neurobehavioral tests (F₁ generation):

Auditory function: 4 weeks of age: startle response (Preyer reflex)

Pupil constriction reflex: 4 weeks of age

Learning and Memory: Ages 6-7 weeks: water multiple (3T) T-maze with 4 learning trials followed one week later with the memory phase (2 consecutive trials)

Positive/negative test result

Time for positive test

Numbers of right and wrong directions

Spontaneous locomotor activity: 8 weeks of age, with infra-red sensor equipment for 1 hour periods

Movements within front and back of cage

Back and forth movements

Vertical movements

Mating of F₁ Generation: 10-11 weeks of age, pairing with animals from different litters within same dose group

Day of confirmed mating (vaginal plug or sperm in vaginal lavage, designated post-coitum day 0)

Pre-coital time (up to 10 days per mate)

Necropsy on post-coitum day 15

Pathology: PPD 4 pups not selected, PPD 22 or PPD 23 pups not selected at weaning, all pups from dams that died, F₁ M after F₁ F hysterectomies, F₁ F on post-coitum day 15, all pups found dead or prematurely sacrificed

Macroscopic examination of thoracic and abdominal organs

Skeletal examination

Hysterectomies of F₁ generation (post-coitum day 15): ovaries and uterus

Numbers of corpora lutea

Numbers/distribution of dead/live concepti

Numbers/distribution early/late resorptions (implant without recognizable embryo/dead embryo with external degenerative changes)

Numbers/distribution of implantation sites/uterine scars (implantation without implant)

Organ weights: not performed

Histopathology: Not performed

Results

F₀ in-life:

Pregnancy status: The results of the pregnancy status observations are presented in the following table (provided from the original NDA submission):

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Dose-level (mg/kg/administration)	0	10	25	75	150
Number of mated females	24	24	24	24	24
Number of non-pregnant females	0	1	3	1	0
Number of pregnant females	24	23	21	23	24
. prematurely sacrificed due to difficulties to deliver	0	2	1	0	1
. pregnant but did not deliver	2 ^b	0	1 ^b	0	0
Number of females that delivered	22	21	19	23	23 ^a
. prematurely sacrificed during lactation	0	0	0	0	1
. found dead	0	0	0	0	1
. with dead litter	0	0	0	1	2
Number of principal females with a litter on day 21 <i>post-partum</i>	22	21	19	22	19

a: one female (M29369) was observed to be in mid-parturition but at the next observation the parturition was finished and all the pups were missing.

b: a low body weight gain was recorded for these females during gestation so an unnoticed delivery was considered unlikely, although a small number of pups may have been born and cannibalized.

Mortality:

2 HD F found dead

 1 on PPD 16 during lactation with enlarged liver and dilated cecum

 1 TK F on GD6

4HD F sacrificed

 1 on GD23 after delivering and cannibalizing pups

 1 on GD23 for difficult delivery, with piloerection, pallor, cold skin, 12

dead fetuses in uterine horns

 1 on PPD 3 for clinical signs (piloerection, round back, pallor, cold skin,

hypoactivity, abdominal breathing, without necroscopic abnormalities

 1 on PPD 3 due to dead litter

 1 HMD F on PPD 1 with dead litter

 1 LMD F on GD23 for difficult delivery with piloerection, pallor, abdominal breathing, red vaginal discharge, with 5 live and 8 dead fetuses in uterine horns and small spleen

 2 LD F, on GD 23 and 24

 1 with difficult delivery, pallor of eyes/extremities, 1 dead fetus in vagina,

3 live and 10 dead fetuses in uterine horns, pale liver, grey/green kidneys)

 1 difficult delivery, piloerection, round back, pallor, abdominal breathing,

12 dead fetuses in uterine horns, pale liver and kidneys, enlarged and red adrenal glands

Clinical Signs:

 Treatment-related **ptyalism** in 28/39 HD F, beginning at mid-gestation and lasting through lactation

Piloerection, round back in 3 HD F at beginning of lactation, 1 HD F at end of lactation, 1 HMD F from GD14-20 and PPD 0-1, 1 HMD F from GD14-20 and PPD 0-1

Body weights: Statistically significant reduced mean body weights (BW) and body weight gains (BWG) at the HMD (-22%, until GD13) and the HD (-24%, until GD 16); statistically significant reduction in BW during lactation at the HMD and HD compared to controls; the results of the body weight measurements and body weight gain calculations are presented in the following figures (provided from the original NDA submission):

FIGURE 1. F0 GENERATION - MEAN BODY WEIGHT - FEMALES (DURING PREGNANCY PERIOD)

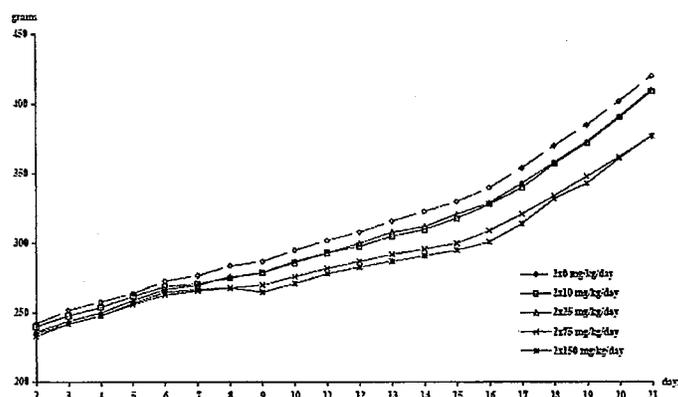


FIGURE 2. F0 GENERATION - BODY WEIGHT CHANGE - FEMALES (DURING PREGNANCY PERIOD) (MEAN AND STANDARD DEVIATIONS)

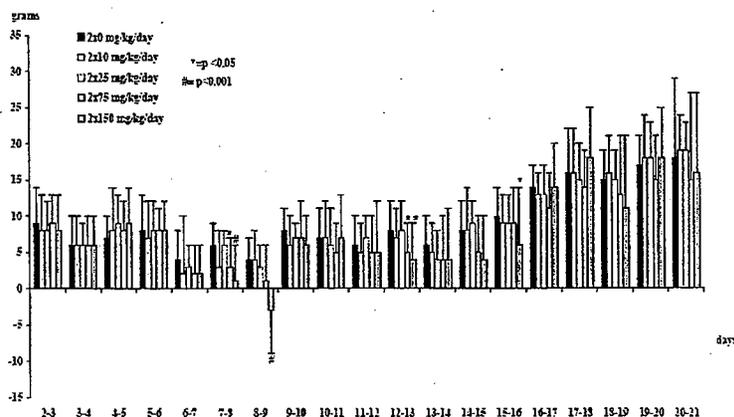


FIGURE 3. F0 GENERATION - MEAN BODY WEIGHT - FEMALES (DURING LACTATION PERIOD)

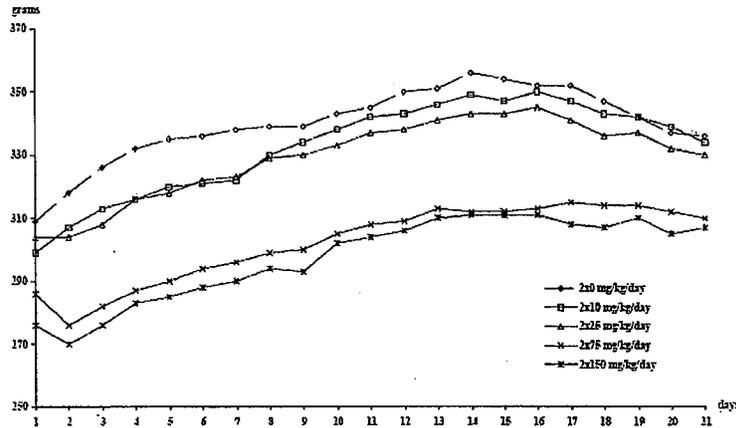
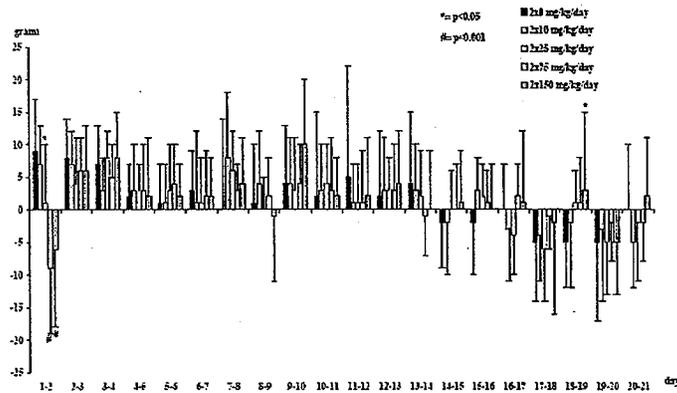


FIGURE 4. F0 GENERATION - BODY WEIGHT CHANGE - FEMALES (DURING LACTATION PERIOD) (MEAN AND STANDARD DEVIATIONS)



Food consumption: Statistically significant reduced food consumption at the HMD and HD from GD6-20 and PPD 1-7, but no treatment-related effects for remaining lactation period; the results of mean food consumption during pregnancy and lactation are presented in the following tables (provided from the original NDA submission):

F0 GENERATION
FOOD CONSUMPTION (Mean values/grams per day/Females/Pregnancy period)

Dose: (mg/kg/day)		2x0	2x10	2x25	2x75	2x150
DAYS 3 TO 6	MEAN	24 d	23	24	23	24
	S.D.	4	3	2	3	2
	N	22	23	20	23	24
DAYS 6 TO 9	MEAN	25 d	23	24	20#	16#
	S.D.	2	6	2	3	3
	N	22	23	20	23	24
DAYS 9 TO 12	MEAN	27 d	25	25	23#	22#
	S.D.	3	4	2	3	3
	N	22	23	20	23	24
DAYS 12 TO 15	MEAN	27 d	26	26	23#	23#
	S.D.	3	5	2	2	3
	N	22	23	20	23	24
DAYS 15 TO 20	MEAN	29 d	29	28	25#	25#
	S.D.	2	4	3	3	3
	N	22	23	20	23	24

Statistical key: d-RMCOVA + Dunnett-test # - p<0.001

F0 GENERATION
FOOD CONSUMPTION (Mean values/grams per day/Females/Lactation period)

Dose: (mg/kg/day)		2x0	2x10	2x25	2x75	2x150
DAYS 1 TO 7	MEAN	45 d	44	42	38#	40*
	S.D.	8	2	5	5	6
	N	22	21	19	22	20
DAYS 7 TO 14	MEAN	62 d	64	62	59	60
	S.D.	8	4	7	7	7
	N	22	21	19	22	20
DAYS 14 TO 21	MEAN	74 d	75	74	71	72
	S.D.	5	6	6	7	9
	N	22	21	19	22	19

Statistical key: d-RMCOVA + Dunnett-test * - p<0.05 # - p<0.001

Parturition:

- No treatment-related effects on duration of gestation
- No treatment-related effects on duration of parturition (2-4 hours across groups), and on failure to deliver or difficulty of delivery (2, 2, 2, 0, and 1 F at 0, 10, 25, and 150 mg/kg b.i.d., respectively)

F0 necropsy:

Macroscopic examination: mammary gland mass (firm, homogenous) in 1 HMD F; no other macroscopic findings in any group

The results of the maternal reproduction observations (group means) are summarized in the following table (Figures represent means in the control group, and percent differences from controls in the treated groups, provided from the original NDA submission):

**APPEARS THIS WAY
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Daily dose CG5503 (mg/kg/d): Control 20 50 150

Gestation body weight (g) ^a	420	-2.6	-2.4	-10.2
Lactation body weight (g) on lactation day 21	336	-0.6	-2.4	-7.7
Gestation food consumption (g ^a)	29	-3.4	-3.4	-13.8
Food consumption (g) on lactation days 15-21	74	+1.3	0	-5.3
Mean duration of gestation (days)	21.3	21.3	21.3	21.3
Mean no. of corpora lutea	15.3	15.9	14.7	15.0
Mean no. of implantation scars	14.2	14.9	14.0	14.0
Mean % pre-implantation loss	7.4	5.7	4.7	6.3

^a = end of dosing period

Litter data during lactation:

- No treatment-related effects on mean numbers of corpora lutea, implantation sites and pups born
- % M pups 46.8%-50.3% on PPD 0, 47.1%-52.4% on PPD 21 (no treatment-related differences compared to controls)
- **Pup mortality:** complete litter deaths in 1 litter at HMD and 2 litters at HD during PPD 0-4
- **Pup clinical signs:** Cold to touch on PPD2 in pups of one litter at the HD (observations in that dam were piloerection, round back, emaciated appearance), with deaths in 3 M and 2 F pups in that litter
- **Pup body weight:** Statistically significant reduction in pup body weights on PPD1 and body weights and body weight gains throughout lactation at the HD compared to controls, and body weights in pups at the HMD from PPD 4 to end of lactation
- **Pup physical and reflex development:** No treatment-related effects during lactation
- **Pup necropsy** (non-selected pups sacrificed after weaning, pups found dead): No treatment-related effects
- **Pup skeletal examination** (pups found dead, PPD 1-4): Unossified 1st to 6th centrum of cervical vertebra in approximately 50% pups at the HD, with several observations of non-ossified bones or incomplete ossification in other bones, likely due to lower body weight

The results of the pre-weaning observations are summarized in the following table (provided from the original NDA submission):

**APPEARS THIS WAY
ON ORIGINAL**

Daily dose (mg/kg)	<u>0 (Control)</u>	<u>20</u>	<u>50</u>	<u>150</u>
F₁ litters preweaning				
No. litters evaluated	22	22	22	24
Mean no. pups/litter	13.5	13.6	13.3	12.4
Mean no. of liveborn pups/litter	13.5	13.6	13.3	12.4
Mean no. of stillborn pups/litter	0	0	0	0
Postnatal survival to day 4 (%)	99.3	99.0	95.2	94.4
Change in pup body weight ^b (g)	+48.9	+49.0	+48.2	+44.4
Pup sex ratios (% males) at birth	47.3	50.4	47.0	48.9
Pup clinical signs				
cold to touch	0	0	0	0
Pup necropsy observation				
scabs on forelimb (no. of pups)	1	0	0	0
malformation on forelimb (no. of pups)	1	0	0	0
increased size of hindlimb	0	1	0	0
wound on hindlimb	0	0	0	0
scabs on hindlimb	0	0	0	0
short tail	0	0	0	0

F₁ physical development (from PPD 22, after weaning):

Mortality: No treatment-related effects (1 HMDF sacrificed after delivering)

Clinical signs: No effects of maternal treatment on F₁ clinical signs

Body weight (BW) and Body weight gain (BWG): Statistically significant reduction in mean M BW and slight reduction in M BWG at the HD (PPD22-end of study), Days 1-43) and HMD (Days 1-15) compared to controls; Statistically significant reduction in BW and slight reduction in BWG in the HD F compared to controls from Day 1-pairing (PPD 22-ages 10-11 weeks), and statistically significant reduction in BW in HMD F from Days 1-15; the results of the mean F₁ generation BW measurements and BWG calculations are presented in the following figures (provided from the original NDA submission):

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ON ORIGINAL**

FIGURE 6. F1 GENERATION - MEAN BODY WEIGHT - MALES (DURING PREMATING PERIOD)

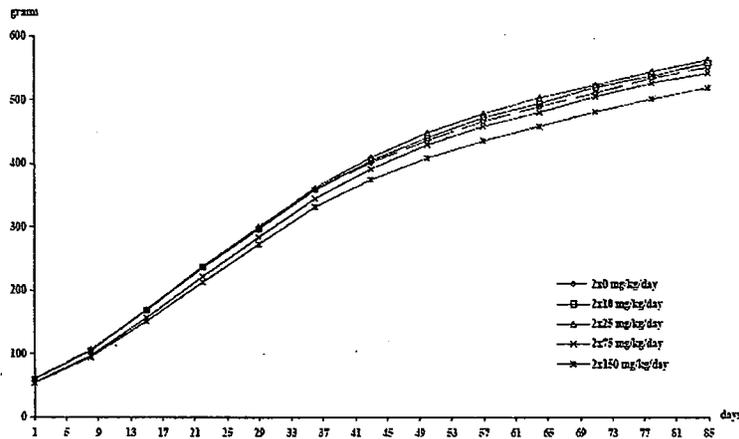
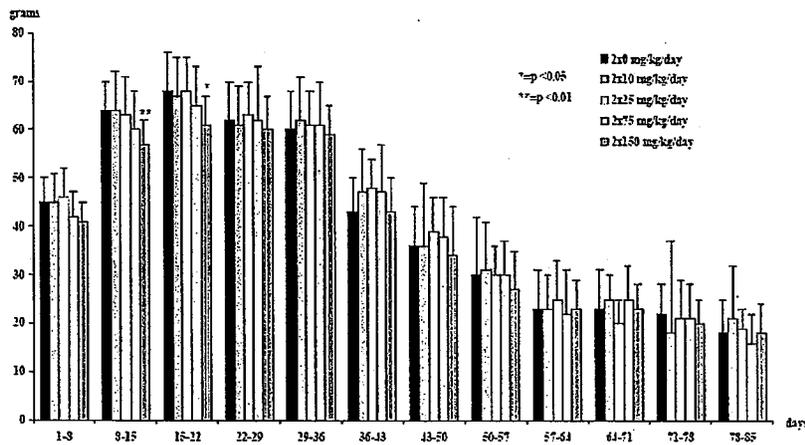
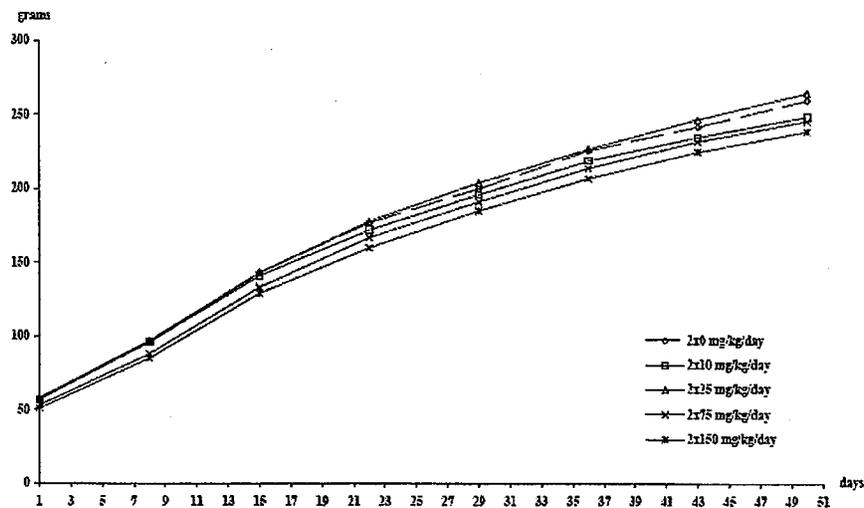


FIGURE 7. F1 GENERATION - BODY WEIGHT CHANGE - MALES (DURING PREMATING PERIOD) (MEAN AND STANDARD DEVIATIONS)



**FIGURE 8. F1 GENERATION - MEAN BODY WEIGHT - FEMALES
(DURING PREMATING PERIOD)**



**FIGURE 9. F1 GENERATION - BODY WEIGHT CHANGE - FEMALES
(DURING PREMATING PERIOD) (MEAN AND STANDARD DEVIATIONS)**

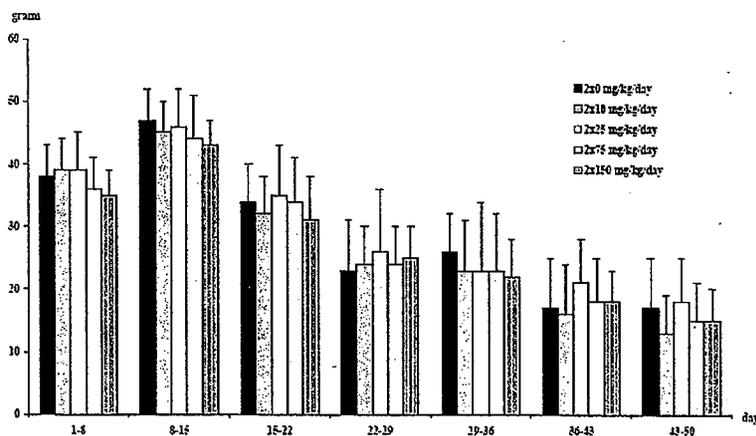


FIGURE 10. F1 GENERATION - MEAN BODY WEIGHT - FEMALES (DURING PREGNANCY PERIOD)

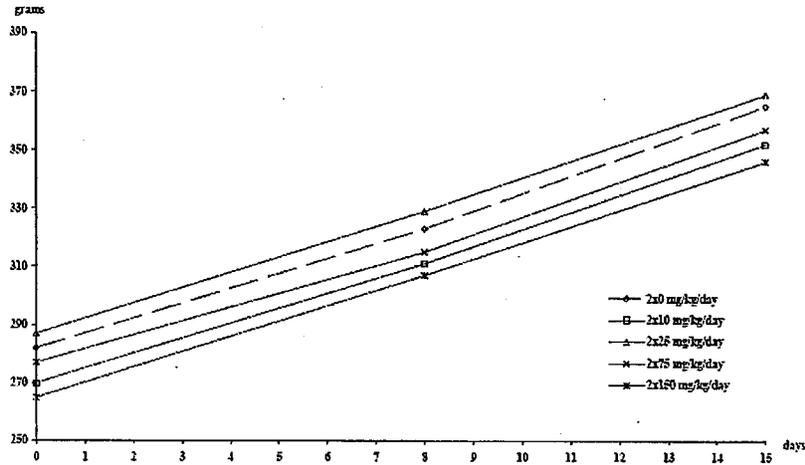
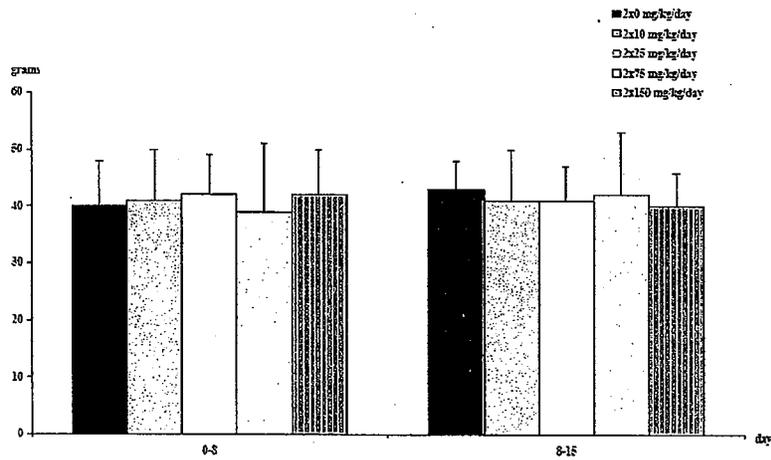


FIGURE 11. F1 GENERATION - BODY WEIGHT CHANGE - FEMALES (DURING PREGNANCY PERIOD) (MEAN AND STANDARD DEVIATIONS)



Sexual development:

- Slightly delayed age of balanopreputial separation in pups of dams in all dose groups, compared to controls
 - No relationship to dose
 - No relationship to body weights
 - Within historical control range
 - Not considered to be treatment-related, in agreement with Sponsor

- Slightly earlier vaginal opening in F pups at all doses, compared to controls
 - Not considered to be treatment-related, in agreement with Sponsor

F₁ behavioral evaluation (PPD 22 after weaning, onward):

- **Auditory function:** No treatment-related effects in all pups ages 4 weeks
- **Pupil constriction:** No treatment-related effects in all pups ages 4 weeks
- **Learning and Memory:** No treatment-related effects on mean time to complete maze, numbers of errors, numbers of failures
- **Spontaneous locomotor activity:** No treatment-related effects

F₁ reproduction:

- **Pairing and mating data**
 - All F₁F successfully mated
 - Failure to mate in 2, 1, and 1 M offspring at LD, MD, and HD
 - No treatment-related effects in mean numbers of days to mate
- **Fertility data/pregnancy status:** No treatment-related effects

F₁ Necropsy: No treatment-related effects

Hysterectomy data:

- No treatment-related effects on corpora lutea, implantations, concepti, early and late resorptions

The F₁ post-weaning observations in the M and F are summarized in the following table (provided from the original NDA submission):

Daily dose CG5503 (mg/kg/d):	<u>Control</u>	<u>20</u>	<u>50</u>	<u>150</u>
<u>F₁ males</u> <u>postweaning</u>				
No. litters evaluated	20	20	20	20
No died or sacrificed moribund	0	0	0	0
Clinical observations				
hair loss on forelimb	2	1	1	0
emaciated appearance and loud breathing	0	1	0	0

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Necropsy observations				
kidney: dilated pelvis	2	0	0	1
testis: translucent and reduced size, bilateral; epididymis: reduced size, bilateral	0	0	0	1
Body weight change until day 92	510	+1.0	+2.4	-0.6
Balanopreputial separation	47.8	49.1	50.3	50.5
Sensory function (acoustic startle reflex) % of positive responses	100	100	100	100
Motor activity (horizontal movements / rearing)	632.0 / 317.5	627.1 / 312.2	676.4 / 314.9	694.0 / 324.3
Learning and memory (T-maze) 4 th learning test (mean s)	11.25	9.60	18.35	17.10
Learning and memory (T-maze) 2 nd memorization test (mean s)	13.85	14.30	12.80	15.50
Pupil constriction reflex % of positive responses	100	100	100	100
Mean no. days prior to mating	2.8	2.7	4.0	3.4
No of males that mated	20	20	20	19
No of fertile males	20	20	20	19
<u>F₁ females postweaning</u>				
No. evaluated postweaning	20	20	20	20
No died or sacrificed moribund	0	0	0	0
Clinical observations				
hair loss on forelimb	1	0	0	0
Necropsy observations				
kidney: dilated pelvis	3	0	0	0
kidney: enlarged	1	0	0	0
Gestation body weight change to day 15 (g)	83.0	-1.2	-1.2	-3.6
Prenatal food consumption (% ^a)				
Gestation food consumption (% ^b)				
Mean age of vaginal patency (days)	36.5	35.2	34.6	35.6
Motor activity (horizontal movements / rearing)	795.0 / 356.5	841.7 / 395.2	839.4 / 364.9	956.3 / 421.5
Learning and memory (T-maze) 4 th learning test (mean s)	16.55	10.95	19.40	18.15
Learning and memory (T-maze) 2 nd memorization test (mean s)	16.45	12.10	20.90	14.45
Pupil constriction reflex % of positive responses	100	100	100	100
Mean no. days prior to mating	2.8	2.7	4.0	3.4
No of females sperm positive	20	20	20	19
No of pregnant females	20	20	20	19
Mean no. of corpora lutea	18.5	16.9	18.3	17.5
Mean no. of implantations	16.8	15.2	16.3	16.1
Mean % preimplantation loss	8.6	11.4	10.5	7.7
<u>F₂ litters</u>				
Mean no of live conceptuses/litter	15.5	14.4	15.4	14.9
Mean no of resorptions	1.3	0.8	0.9	1.2
No of dead conceptuses	0	0.1	0.1	0
Mean no of postimplantation loss	8.1	7.6	5.7	7.4

Fisher's Exact test or Chi-square test: # p<0.05, ## p<0.01

G = gestation day

a) at the end of dosing period. For controls, means are shown. For treated groups, percent differences from controls are shown.

b) from birth to weaning

c) from weaning to mating (postnatal day 22 to day 57)

d) during postweaning period. For controls means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on percentile differences).

Toxicokinetics: The results of the toxicokinetic evaluation of CG5503 in the pregnant dams on GD 6 and 17 are presented in the following table (provided from the original NDA submission, means \pm SD):

Parameter		Dose [mg/kg/administration]			
		10	25	75	150
GD6	C _{max} [ng/mL]	16.0 \pm 5.6	112.3 \pm 33.6	447.0 \pm 34.8	2043 \pm 532.5
	t _{max} [h]	5.50 \pm 0.43	5.25 \pm 0.00	5.25 \pm 0.00	5.25 \pm 0.00
	AUC _{0-24h} [h·ng/mL]	44.0 \pm 8.5	275.3 \pm 59.2	1693 \pm 453.9	5839 \pm 723.9
GD17	C _{max} [ng/mL]	48.2 \pm 3.7	354.8 \pm 217.5	1186 \pm 752.2	1441 \pm 1061
	t _{max} [h]	5.50 \pm 0.43	5.25 \pm 0.00	5.25 \pm 0.00	3.83 \pm 3.13
	AUC _{0-24h} [h·ng/mL]	154.9 \pm 38.3	760.3 \pm 352.0	3875 \pm 1477	5224 \pm 458.4

The results of the tapentadol O-glucuronide toxicokinetic evaluation in the pregnant dams on GD 6 and 17 are presented in the following table (provided from the original NDA submission, means \pm SD):

Parameter		Dose [mg/kg/administration]			
		10	25	75	150
GD6	C _{max} [ng/mL]	7801 \pm 745.0	12334 \pm 1494	22150 \pm 1476	38303 \pm 4895
	t _{max} [h]	2.17 \pm 3.32	5.75 \pm 0.43	4.67 \pm 2.31	6.00 \pm 0.00
	AUC _{0-24h} [h·ng/mL]	28496 \pm 2228	73669 \pm 6158	217883 \pm 35813	477112 \pm 88205
GD17	C _{max} [ng/mL]	10301 \pm 498.7	14679 \pm 1000	36852 \pm 10528	52657 \pm 3067
	t _{max} [h]	5.75 \pm 0.43	5.50 \pm 0.43	5.50 \pm 0.43	5.00 \pm 1.73
	AUC _{0-24h} [h·ng/mL]	32682 \pm 3552	90395 \pm 4928	304507 \pm 69746	575109 \pm 105547

The toxicokinetic evaluation revealed approximately dose-related linear increases in exposure to parent drug and O-glucuronide metabolite in the pregnant dams, with increased exposure to the parent drug with repeated dosing at all but the HD, and increased exposure to the glucuronide at all doses, suggesting accumulation.

3.4.7 Local tolerance

Injection site effects were evaluated in the intravenous (IV) and subcutaneous (SC) injection toxicity studies in rats, rabbits, and dogs. The results of 14-day feasibility studies in M and F Sprague-Dawley rats (Studies TP2471 and 602548) given daily injections of tapentadol at 15-120 mg/kg/day IV and 30 and 45 mg/kg/day SC showed

infusion site swellings in the SC-treated rats. SC tapentadol in pregnant female rats (Study TP2465) produced injection site discoloration and erythemas, with weeping eroded hemorrhagic lesions, eschar formation and injection site indurations at doses of 15 mg/kg b.i.d. or higher given daily for 2 weeks. SC tapentadol injections in a dose range-finding study in pregnant rabbits at doses of 5-25 mg/kg/day for 14 days produced subcutaneous tissue lesions with dose-related increases in severity from slight to severe erythemas, of approximately 1-20 mm in size (Study TP2464). Injection site swelling was observed in Beagle dogs given SC injections at twice daily doses of 7.5-10 mg/kg for up to 9 consecutive days (Study TP2564). When administered for 3 months by SC injection (10-40 mg/kg b.i.d.) in dogs, tapentadol produced local tissue findings indicating severe scratching of the sites throughout the study, and necropsic findings of ecchymoses with hemorrhages, edema and gelatinous consistency of the underlying tissues at the injection sites (Study TP2455). A second 3-month SC toxicity study in dogs demonstrated similar local effects, with hemorrhages, inflammatory infiltrates, partially reversible fibrosis, phlebitis and thrombophlebitis, with chronic focal or multifocal perivasculitis at the highest dose of 16 mg/kg/day SC (Study TP2559).

There were no adverse treatment-related effects at the injection sites in the dose range-finding IV studies in pregnant rats given 3-15 mg/kg/day for 12 days (Study TP2060) and in pregnant rabbits given daily IV tapentadol injections at 1-9 mg/kg/day for 15 days (Study TP2062) and at 3-15 mg/kg/day for 14 days (Study TP2061). In Beagle dogs, IV injections at 7.5 mg/kg/day for 10 days resulted in red foci at the injection sites (Study TP1967), but no local toxicity was found in dogs given comparable IV doses in a second 4-week study (Study TP1968).

3.4.8 Special toxicology studies

NEUROTOXICITY

Studies reported in the published literature have shown a characteristic pattern of lesions, including vacuolation and necrosis in specific brain regions following administration of the NMDA receptor antagonist drugs, such as MK(+)-801 and others (See Fix et al. 1993. Neuronal Vacuolization and Necrosis Induced by the Noncompetitive N-methyl-D-aspartate (NMDA) Antagonist MK(+)-801 (Dizocilpine Maleate): A Light and Electron Microscopic Evaluation of the Rat Retrosplenial Cortex. *Experimental Neurology* 123: 204-215; Olney et al. 1989. Pathological Changes Induced in Cerebrocortical Neurons by Phencyclidine and Related Drugs. *Science* 244: 1360-1362). Based on the results of the receptor binding assays on tapentadol, showing weak inhibition of the glutamate PCP receptor in rat cerebral cortex (Study MP30), further investigation of potential NMDA-receptor induced neurotoxicity in brain was conducted during drug development.

The Sponsor performed separate histopathological examinations (Study TP2384, in addition to the standard histopathology evaluations in these studies) of rat brain from the 4-week gavage (Study TP1971) and 4-week intravenous (Study TP1966) studies, focusing specifically on the brain regions, including the retrosplenial cortex and hippocampal CA1 region, known to be sensitive to NMDA receptor antagonist-induced

neuronal injury. Male and female rats were administered tapentadol at 3, 7, and 15 mg/kg/day IV or 300, 425, and 600 mg/kg/day PO once daily for 4 weeks. Standard histopathology at the end of the dosing period included examination of brain sections (4 mcM slices, paraffin wax embedded, H&E stained) of medulla/pons, cerebellum and cerebrum. Separate microscopic examinations of the retrosplenial cortex and hippocampus CA1 region failed to detect evidence of vacuolation and necrosis. This study is considered to be incompletely adequate to detect a vacuolation response, best observed within a 12-hour window following an initial dose of an NMDA receptor antagonist agent. It is doubtful that necrosis and/or gliosis, optimally observed within days of the drug administration, would have been detected after 4 weeks of drug exposure. Therefore, the results of this study provide only limited evidence for an absence of NMDA receptor-induced neurotoxicity by tapentadol. However, due to the low affinity for the NMDA receptor by tapentadol, concern regarding this effect is minimal.

IMMUNOTOXICITY

Potential immune system effects by tapentadol were investigated in a supplementary 4-week study in Wistar rats (Study TP2593. [] Study 857847) given doses from 75-300 mg/kg/day by oral gavage. Leukocyte populations were examined, with particular focus on morphology, distribution, and function of the T-lymphocyte, B-lymphocyte, monocyte and granulocyte populations (e.g., CD3+/CD4+ T-lymphocytes, CD3+/CD8+ T-lymphocytes, CD45 RA+ and CD11b+). There were no treatment-related effects compared to controls at any dose in the males and females, and variations were within historical control ranges for the laboratory. Therefore, there was no evidence of potential immunotoxic response by oral tapentadol under the conditions of this study. The results of this study are presented in the following tables (provided from the original NDA submission):

b(4)

Dose *	CD3 ⁺ -Cells				CD3 ⁺ /CD4 ⁺ -Cells				CD3 ⁺ /CD8 ⁺ -Cells			
	Mean	SD	S**	P	Mean	SD	S**	P	Mean	SD	S**	P
1	56.5	5.9		0.721	40.7	5.3		0.900	16.2	1.8		0.202
2	59.1	5.4	-		41.6	3.7	-		18.2	2.1	-	
3	58.6	4.3	-		42.2	4.7	-		16.8	2.0	-	
4	58.3	4.6	-		41.4	3.3	-		17.1	1.7	-	

Dose *	CD45RA ⁺ -Cells				CD11b ⁺ -Cells			
	Mean	SD	S**	P	Mean	SD	S**	P
1	22.0	2.7		0.317	31.5	4.3		0.593
2	22.7	4.2	-		28.9	5.7	-	
3	24.8	4.4	-		30.1	5.8	-	
4	25.1	4.6	-		28.5	3.8	-	

*: Dose groups: 1 = control group, 2-4 = groups with 75, 150 and 300 mg/kg b.w./day, respectively
 **: statistical analysis: -, = not significant; +, ** = significant; P = p-value

Impurities

The impurity profiles of tapentadol HCl drug substance registration batches manufactured by the proposed commercial process are presented in the following table (provided from the original NDA submission):

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5

b(4)

The impurities [] were qualified, however, in the nonclinical studies listed in the following table (provided from the original NDA submission):

1.3. Toxicological Qualification of Impurities During Development

7

b(4)

3.6 OVERALL SUMMARY AND DISCUSSION

Background

Tapentadol HCl, a new molecular entity is a centrally acting analgesic for the treatment of moderate to severe, acute pain at oral doses of 100 mg up to 6 times daily (600 mg/day), and up to 7 times on the first day of treatment (700 mg). Tapentadol is a pure enantiomer without observable enantiomeric interconversion and with no clinically-relevant active metabolites, and is freely soluble in water, hydrochloric acid 0.1N, and intestinal fluid. The rationale for development of this drug product is a potential for enhanced mu-opioid induced analgesic activity by interaction with norepinephrine transmission with fewer adverse effects that are usually associated with treatment using more selective mu-agonist agents, such as morphine and fentanyl.

Primary and Secondary Pharmacology

The results of *in vitro* receptor-, ion channel-, and neurotransmitter transporter-binding studies on tapentadol showed highest affinity for the mu-opioid and sigma (S2) receptors and norepinephrine transporter, with minimal binding to all other sites tested. Mu-opioid agonist activity was demonstrated *ex vivo* by inhibition of the twitch reaction in isolated guinea pig ileum, that was reversed by the opioid antagonist naloxone.

Tapentadol analgesic activity was investigated *in vivo*, using models of acute antinociception and inflammatory and neuropathic pain models in mice, rats, and rabbits. The results of these studies demonstrated efficacy in the tail-flick, phenylquinone writhing, hot-plate and tooth pulp stimulation tests with approximately 1/4X-1/2X the potency of morphine and 3X-5x that by tramadol. By the oral (PO) route, tapentadol was approximately equipotent to morphine analgesic effects in these assays. The oral activity of tapentadol was 2%-8%, with a maximum effect at approximately 20 minutes and duration of action of 60-90 minutes. In *in vivo* models of inflammatory and neuropathic pain using the paw-pressure test, formalin test and chronic constriction injury models in rats, tapentadol showed approximately 1/2 the potency of morphine and 1/4X-1X that of tramadol by the intraperitoneal (IP) and intravenous (IV) routes. Support for a mu-opioid receptor mechanism of action was shown by naloxone blockade, but not alpha2-receptor antagonist (yohimbine) and the serotonin-2A-C antagonist (ritanserin) blockade of the acute antinociceptive effects.

Tapentadol treatment produced secondary pharmacodynamic effects in animals typically observed by mu-opioid receptor agonist and norepinephrine reuptake inhibitor drugs. Treatment-related emesis, retching and vomiting were seen in ferrets and dogs, although with lower potency than that by morphine where compared. Antitussive action was demonstrated in rats by potent, dose-dependent inhibition of NH₃-induced cough. Additionally, intradermal injections in guinea pigs reduced the dermal twitch response to mechanical stimuli in a concentration-dependent manner.

Safety Pharmacology

Central nervous system (CNS) safety pharmacology studies in mice and rats showed tapentadol-induced general depressant effects, indicated by dose-related decreases in exploration activity and prolonged hexobarbital sleeping time, with potency less than that of morphine and barbiturate anesthesia. Tapentadol had little effect on motor coordination in a rota-rod performance study in mice, showing potency of 1/10 that of diazepam. Conscious Wistar rats administered single IV tapentadol injections in a standard Irwin test showed mydriasis, exophthalmus, increased muscle tone, and insensitivity to tail pinch, and at high doses decreased awareness, increased excitability, and loss of reflexes including corneal, pinna and hindlimb. Clonic convulsions were seen at the highest dose in that study. Tapentadol was pro-convulsant in conscious male Wistar rats pre-treated with single IV doses prior to IP pentylenetetrazole injection. Convulsions, which were also observed in two single IV dose studies in Sprague-Dawley rats were prevented by pre-treatment with diazepam and phenobarbital, but not by naloxone. However, naloxone given after tapentadol injection, as opposed to naloxone pretreatment as in the previous study, dose-dependently reduced the incidence of convulsions and Straub tail induced by tapentadol, with abolishment of these neurological effects at the highest dose in a second IV study in Sprague-Dawley rats.

The results of one CNS Safety Pharmacology study in rat (15 mg/kg IV), conducted to explore the timecourse of tapentadol-induced respiratory depression, convulsions and deaths in relation to plasma and CSF parent drug, O-glucuronide and sulfate metabolite concentrations showed treatment-related respiratory depression starting 15-20 minutes after infusion, with cyanosis and convulsions in 3/14 rats and deaths in 4/14 rats, respectively. There were also late peaks in the incidences of convulsions with cyanosis and deaths at ≥ 12 hours after dosing, and after parent drug and glucuronide metabolite were nearly completely cleared from plasma and CSF (below level of detection). Therefore, the late convulsions, cyanosis and deaths could not be attributed to concurrent plasma and CSF parent or glucuronide and sulfate metabolite concentrations. The Sponsor suggested that potential products of parent drug or metabolite enrichment in deeper CNS compartments, not detected in plasma and CSF, may have produced these effects, but this issue was not further addressed in the submission. The findings in this study are noted, but not of great concern at this time and will not be considered to be an approvability issue, because there were no clear or repeatable findings of delayed onset or continued findings of neurobehavioral toxicity after drug and metabolite clearance from plasma in the toxicology studies. Also, whole body autoradiography after single IV (10 mg/kg) tapentadol in a tissue distribution assay in rat showed blood/brain barrier crossing of radioactivity, with brain radioactivity levels decreasing rapidly from a peak at 0.25 h, to below detection levels at 8 hours. The results of the microscopic examinations in brain in the toxicology studies including the special histopathology evaluation of potential NMDA receptor target site neurotoxicity found no morphological signs of neurotoxicity considered to be treatment-related. Finally, there were no findings in the clinical studies of convulsions or results indicating a potential for delayed or extended adverse CNS and respiratory effects by tapentadol after clearance of the drug in humans.

Tapentadol cardiovascular safety was assessed nonclinically *in vitro*, *ex vivo* and *in vivo*. There was a concentration-dependent reduction of the outward potassium tail current amplitude at all concentrations studied from 1 to 100 mcM in a hERG assay (IC₅₀ = 36.14 mcM, 7978 mcg/L, approximately 68 times the maximum clinical plasma concentration [C_{max}] at steady state at the MRHD), with 66% recovery after wash-out indicating partial reversibility. Tapentadol also showed concentration-dependent negative chronotropic effects in an isolated guinea pig atrial muscle preparation (EC₅₀ = 408 mcM) and reversible positive inotropic effects in electrically driven, isolated Guinea pig papillary muscle, although negative inotropic effects were seen at higher concentrations (EC₅₀ = 508 mcM). In comparison, the EC₅₀ in these preparations for quinidine sulfate negative chronotropic effects was 120 mcM and for the negative inotropic effects was 80 mcM. There was a dose-dependent prolongation of the action potential duration (APD₉₀) in isolated New Zealand White rabbit papillary muscles (NOEL = 10 mcM). Tapentadol shortened the action potential duration (APD₉₀) at 10 and 100 mcM, and reduced upstroke velocity (V_{max}) and action potential amplitude (APA) at 100 mcM (up to 130 times the therapeutic plasma concentration at the clinical dose of 100 mg PO) in isolated Guinea pig papillary muscles. A volume-conducted electrocardiogram assessment in Langendorff heart preparations using spontaneously beating Guinea-Pig hearts showed a tapentadol concentration-dependent reduction of heart rate at 3-30 mcM (corresponding to 663-6630 mcg/L, up to -30%), with atrio-ventricular conduction and ventricular depolarization slowing, indicated by reversible increase in PR interval (up to 22%) and QRS width broadening (up to 31%). There were no treatment-related effects on QTc interval at any concentration tested in that study. Additionally, concentration-dependent inhibition of calcium-dependent isometric aortic contractions were observed in potassium-depolarized solution in isolated rat thoracic aortic strips bathed in tapentadol solutions a concentrations of 100-316 mcM, using sodium channel blockade by lidocaine.

Tapentadol cardiovascular effects were studied *in vivo*, in mice, rats, rabbits and dogs. Tapentadol generally increased heart rate and systolic, diastolic, and mean arterial blood pressures in conscious, male Sprague-Dawley rats. In anesthetized rabbits, IV tapentadol decreased cardiac contractility, output, and stroke volume and heart rate, and increased central venous and systolic, diastolic and mean arterial blood pressures. The ECG measurements showed treatment-related reduced, eliminated or negative T-waves and prolonged PQ time (10%), QRS time 14% and 19% (lasting 2 minutes after injection), and prolonged SxT time (22%) in the IV-treated rabbits.

Conscious male (M) Beagle dogs administered tapentadol by short-term IV infusion (3-9 mg/kg) showed reversible (complete at 60 minutes after start of infusion), dose-related increases in arterial blood pressure (up to +43%), heart rate (up to +77%), and cardiac output, and decreased left ventricular ejection fraction. The PQ interval was shortened at the highest dose administered, and the QT interval was decreased at all doses for up to 30 minutes after initiation of the infusion. There were no effects on stroke volume and QRS complex. IV tapentadol (0.5-4.5 mg/kg) in anesthetized male and female Beagle dogs, produced dose-related cardiac depression that resulted in decreased blood pressures and associated peripheral arterial vasoconstriction. There were treatment-related decreases in

systolic left ventricular pressure (LVP), dLVP(+), dLVP(-), left ventricular work, cardiac output and stroke volume, that were statistically significant at the highest dose tested. A compensatory increase was seen in total peripheral resistance, indicating peripheral vasoconstriction. Reduced renal resistance and renal blood flow were also observed at the high dose, and decreased femoral blood flow, indicated by increased femoral resistance was found at all doses. The QRS interval was increased (+4%), suggesting decreased rate of ventricular depolarization. There were no treatment-related effects on heart rate, pulmonary arterial pressure, coronary flow and resistance, end-diastolic left ventricular pressure, blood gas measures, and the PR (-10%) and QT intervals, including corrected QT interval (Van de Water's) at the doses tested. Sinoatrial node depression and junctional rhythm development were suggested by decreased or eliminated P wave amplitude. Treatment-related T wave changes at these doses also suggested ventricular repolarization effects, involving either negative inotropic effect or electrophysiological effects on ion channel conductance.

Pulmonary effects: Respiratory safety pharmacology was explored in Wistar and Sprague Dawley rats administered tapentadol by single and repeated IV and IP injections, and in New Zealand White rabbits by the IV route. The results of these studies showed pulmonary effects typically observed following treatment with mu-opioid receptor agonists as a class. Single dose tapentadol (4.64-14.68 mg/kg IV) decreased spontaneous respiratory frequency in conscious M Wistar rats by up to -38% immediately after HD injection (ED50 0.71 mg/kg vs. 0.23 mg/kg IV for morphine and 10 mg/kg for tramadol HCl). There was a slight dose-dependent inhibition of CO₂-induced respiratory stimulation in that study. Plethysmography in unrestrained male Wistar rats given single IV tapentadol injections (2-18 mg/kg), showed a respiratory stimulant effect at lower doses and rapid-onset, decreased respiratory rate, peak inspiratory and expiratory flows and minute volume with increased inspiration and expiration times indicating a depressant effect at the high dose. There were no treatment-related effects on airway resistance in that study. Repeated IV tapentadol injections in male Wistar rats at 15 mg/kg/day induced respiratory depression starting 15-20 minutes after infusion, with cyanosis and convulsions (observed for up to 12 hours after injection) in 3/1 rats and deaths in 4/14 rats, respectively. Peak tapentadol concentration in both serum and CSF was approximately 1000 ng/ml (8.5 times the C_{max} at the MRHD).

Blood gas assessments in male Sprague Dawley rats given single IV doses of 4.64-14.7 mg/kg revealed increased arterial blood pCO₂ and decreased pO₂ at doses of ≥ 10 mg/kg. The ED25 in that study, indicating an increase in pCO₂ by 25% compared to baseline was 14.4 mg/kg for tapentadol, vs. 7.9 mg/kg for morphine.

Some tolerance to the respiratory depressant effects of tapentadol was found in male rats given repeated IP tapentadol injections (21.5 mg/kg/d) from dosing day 8 to the end of the 22-day study, indicated by decreasing magnitude of the increased pCO₂ effect. The rate of tolerance development to tapentadol respiratory depressant effects in that study followed observed rate of tolerance development to analgesic effects in a separate study using the tail flick test, in which complete abolishment of analgesia was seen by Dosing Day 51.

The cardiohemodynamic and respiratory assessments in anesthetized New Zealand White rabbits given IV bolus injections (1-10 mg/kg, up to 3 times the ED50 for efficacy in tooth pulp analgesia in rabbits) showed dose-related reduced (-59% to -64%) respiratory frequencies within one minute of injection, which remained reduced by 20% to 40% for the duration of the one-hour observation period. There was one death in a high dose rabbit 5 minutes after dosing, which was attributed to respiratory depression.

Gastrointestinal effects: Inhibition of intestinal fluid transport and motility are well known class effects of the mu-opioid receptor agonists, and therefore would be expected by tapentadol administration. Tapentadol was weakly emetic and induced a dose-related increase in incidence of retching in ferrets. Tapentadol inhibited gut motility in mice, measured by a dose-dependent decrease in charcoal transit distance along the gastrointestinal tract. For comparison, the ED25 values, or dose that induces 25% decrease in charcoal distance, were 28 mg/kg IP for tapentadol, 5.91 mg/kg IP for morphine, and 66.7 mg/kg IP for tramadol. Anti-diarrheal activity was demonstrated in another study using prostaglandin-induced diarrhea in mice; the results showed an ED50 of 10.3 mg/kg IP for tapentadol, compared with 1.12 mg/kg IP for morphine and 27.1 mg/kg IP for tramadol.

Tapentadol inhibited acetylcholine-induced isotonic contractions in isolated guinea pig ileum *in vitro*, at concentrations of 0.1-2.15 mcM, while morphine had no effect on the isotonic contractions at up to 100 mcM, and atropine was 30 times more potent in this assay (0.001 – 0.01 mcM).

Renal Effects: There were no effects on urine volume, and sodium, potassium and chloride excretion in conscious male rats given single IV tapentadol injections (1-10 mg/kg) at the lowest dose tested, but electrolyte excretion was transiently reduced during the one hour observation period after administration at the HD.

Other Organ Systems: Tapentadol (0.1-2.15 mcM) interaction with acetylcholine (ACh)-induced effects in guinea pig smooth and skeletal muscle were studied *in vitro*. Dose-dependent isotonic contractions, induced by acetylcholine in isolated ileal preparations, were inhibited in a concentration-related manner by tapentadol, indicated by a right shift of the concentration-response curve for ACh at all doses tested. The pA2 value for tapentadol was 6.2, and 300 times weaker than that for atropine (pA2 = 9.7). In comparison, morphine had no effect on ACh-induced contractions in the isolated guinea pig ileum preparations.

Tapentadol and the main metabolite, tapentadol O-glucuronide metabolite had no effects on the extent and incidence of muscle relaxation in a traction test study in male NMRI mice given single IV bolus injections (4.64-31.6 mg/kg). Further, there were no interactive effects on the muscle relaxation scores between tapentadol and diazepam or tetrazepam treated mice.

Pharmacokinetics

The results of the pharmacokinetic studies and toxicokinetic analyses in the toxicology studies indicated rapid oral tapentadol absorption, but low bioavailability across species. Tapentadol is rapidly and widely distributed in tissues indicated by a large volume of distribution across species including humans. Tapentadol crosses the blood-brain barrier and placenta, and is weakly protein bound, primarily to plasma albumin. Tapentadol undergoes rapid and extensive metabolism after oral administration, primarily by direct glucuronidation and to a lesser extent by sulfate formation, with some oxidative P450 metabolism by N-demethylation and hydroxylation. The main circulating metabolite is tapentadol O-glucuronide, resulting in systemic exposure to the glucuronide metabolite of up to 14 times compared to parent drug exposure. Elimination is primarily via renal excretion of tapentadol O-glucuronide. The metabolic profile of tapentadol was similar in bioassays in rat, dog and human liver microsomes. No active metabolites were found. Tapentadol half-life is approximately 0.5-1 hour by the IV route and approximately 4 hours by the oral route across doses and across species. No major differences in exposure were found with repeated dosing, indicating a low potential for accumulation. No effects were observed on hepatic microsomal cytochrome P450 content, 7-ethoxy O-deethylase activity, 2-aminophenol glucuronyltransferase activity, and 4-aminopyrine N-demethylase activity in the animal studies, and no inhibition of cytochrome P450 was found in an *in vitro* assay. Therefore the potential for pharmacokinetic drug interactions is low.

From the results of the study in hepatic microsomes, it can be concluded that tapentadol is more extensively glucuronidated in the animal species, particularly in rat, than in humans. The results of oral bioavailability measurements across species and in the clinical pharmacokinetic assessments corroborated this finding, showing higher tapentadol bioavailability in human (32%) than in rat, dog and monkey (9%, 3% and <1%, respectively). The metabolic enzymes most involved in human tapentadol metabolism by glucuronidation are the high capacity UDP-glucuronosyltransferases UGT1A9, UGT2B7, and UGT1A6. Additionally, tapentadol undergoes oxidation to a greater extent in the animal species tested, compared to that in humans. The percent loss of tapentadol parent drug by hepatic microsomal P450 oxidation in an *in vitro* liver metabolism study showed 99.2% loss in minipig, 78.9% loss in hamster, 78.3% loss in guinea pig, 51.5% loss in Cynomolgus monkey, 28.5% loss in rat, 26.7% loss in rabbit, 20.4% loss in mouse, 14.3% loss in rat, 9.6% loss in dog, and 0.6% loss in human microsomes. The Phase I metabolites were N-demethyl tapentadol (M2) by CYP2C9, CYP2C19, and CYP2C8 catalysis, and hydroxy-tapentadol (M1) by CYP2D6, CYP2B6, and CYP2C19, in microsomes from all species tested including human. Metabolism in rat and dog most resembled that in human, supporting species selection in the main toxicology studies. The results of *in vivo* studies on tapentadol metabolism, however, showed qualitatively similar metabolic profiles in mice, rats, dogs and humans. Minor metabolites represented <5% total dose, although nearly all (99.6% in dog and 96.6% in humans) of the eleven metabolites identified in plasma were found to be conjugated.

The potential for chiral interconversion was identified at two of tapentadol's chiral centers to form the diastereomer (-)-(1S,2R)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol (GRT4045Y) and its enantiomer (+)-(1R, 2S)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol. Plasma sample analyses showed GRT405Y+enantiomer levels of 1.1% in mouse, 0.43% in rat, 0.37% in rabbit, 0.67% in dog, and 0.38% in human plasma samples after tapentadol administration. Also, because batch analyses of the drug formulations administered showed up to — of the diastereomer, conversion of the chiral centers is unlikely to have taken place.

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The potential for drug-drug interactions with tapentadol was investigated *in vitro* and *in vivo*, with additional information provided by the results of the primary and secondary pharmacology, pharmacokinetics and toxicology studies. *In vitro* evaluations of tapentadol metabolism revealed that conjugation by uridine diphosphate (UDP) glucuronyl transferase is primarily responsible for clearance of the parent drug from plasma. The high capacity of the UGT system reduces the likelihood of saturation in the presence of other drugs also cleared via glucuronidation. Drugs that inhibit the UGT enzymes and could increase exposure to the parent tapentadol molecule, particularly the subtypes involved in tapentadol glucuronidation (UGT1A9 and UGT2B7) such as probenecid, chloramphenicol and naproxen were examined in a study in liver microsomes. The results of that study showed only slight inhibition of tapentadol glucuronidation, with highest inhibition of 27% by naproxen and 45% by probenecid.

Enzyme induction, of P450 content, and N-dealkylation (aminopyrine N-demethylase), O-dealkylation (7-ethoxycoumarin O-deethylase) and UDP glucuronyltransferase (2-aminophenol-glucuronyltransferase) activity, was also investigated in a 26-week oral gavage toxicity study in the male and female Wistar rats to assess the potential for interactive effects with other drugs that induce or inhibit these enzyme systems. The measurements were made in microsomes isolated from the rat livers at necropsy. The results showed statistically significant increases in 2-aminophenol glucuronyltransferase activity in the tapentadol-treated female rats, but not in the male rats. There were no tapentadol-related effects on the other metabolic enzyme systems tested in rat.

Liver metabolic enzyme activities by cytochrome P450, N-dealkylation (aminopyrine N-demethylase), O-dealkylation (O-ethoxycoumarin O-deethylase) and glucuronyltransferase (2-aminophenol-glucuronyltransferase) activity were also examined in microsomes isolated at necropsy from the livers of dogs administered tapentadol by oral gavage for 13 and 52 weeks, and by IV injection for 4 weeks. The results of the 13-week oral gavage study found significant induction of aminopyrine N-demethylase activity in the male dogs, and glucuronyltransferase activity in the male and female dogs, but no effects on P450 content and O-deethylase activity by tapentadol treatment. There were dose-related increases in ethoxycoumarin O-deethylase activity in dogs evaluated at 52 weeks. There were no changes in cytochrome P450 content, and glucuronyltransferase activity was decreased. The results of enzyme activity in microsomes from the livers in dogs given IV tapentadol for 4 weeks showed no effects on cytochrome P450 content, N-dealkylation (aminopyrine N-demethylase), O-dealkylation

(O-ethoxycoumarin O-deethylase), and aminophenol glucuronyltransferase activities. Greater activity by tapentadol on glucuronyltransferase activity when given orally is likely due to extensive clearance in the liver by that route.

Single Dose Toxicology:

The single oral (PO) and intravenous (IV) dose toxicology assessments revealed LD50 values of 47 mg/kg IV and 300-350 mg/kg PO in mouse (approximately 17 times the MRHD for a single 100 mg oral dose in a 70 kg patient, on a body surface area basis), and 46 mg/kg IV and >1000 mg/kg PO in rat (approximately 113 times the single MRHD on a body surface area basis). The deaths occurred within 15 minutes of IV administration, and within several hours of oral administration, and were attributed to respiratory depression resulting from pharmacological activity by tapentadol in regulatory centers in the brain stem, a characteristic class effect by of mu-opioid analgesic effect. The clinical signs of acute toxicity in the rodents were irritability, hyperactivity, cyanosis, agents, Straub tail, lateral recumbency, tremor, increased sensitivity to touch and noise, increased escape response, irregular respiration, and convulsions. The clinical signs, also characteristic of acute opioid agonist effect in rodents, were reversible in the surviving animals, resolving several hours after drug administration. The histopathology examination showed increased incidence of respiratory tract discoloration characterized by hyperemia.

Repeated dose toxicology:

Repeated dose toxicology studies on tapentadol were conducted in CD-1 and NMRI mice, Wistar and Sprague Dawley rats, Beagle dogs, and Cynomolgus monkeys, by IV, SC and/or oral routes. The main repeated dose toxicology studies used oral dosing to support the safety of the intended clinical route. The results of the non-pivotal studies conducted for dose-selection in the main toxicology evaluations are summarized below for additional insight on timing and severity of emerging toxicity in the longer duration studies, and potential reversibility or tolerance development to adverse tapentadol effects.

Mouse:

The main target organ of toxicity in the mouse studies was the liver. Two-week oral toxicity was examined in male (M) and female (F) CD-1 mice, given tapentadol by dietary admixture (50-250 mg/kg/day) and by oral gavage (50-200 mg/kg/day). Treatment-related organ weight effects were found, including increased relative (to body weights) adrenal, liver and prostate weights and decreased relative kidney weights in the male mice, and increased relative spleen, thyroid, parathyroid, and liver weights in the female mice in the gavage study, and increased relative pituitary weights in female mice in the feeding study. The toxicokinetic evaluation showed higher systemic exposure, with higher C_{max} and AUC values after gavage than after dietary administration. The no adverse effect level (NOAEL) was <50 mg/kg/day PO, based on organ weight changes at all doses.

In a dietary administration study in M and F NMRI mice administered higher doses (50-1000 mg/kg/day) for 13-weeks, the clinical biochemical analyses showed reduced albumin and A/G ratios in the M at ≥ 150 mg/kg/day and in the high dose (HD) F, increased beta-globulin in the M at ≥ 250 mg/kg/day and in the HD F, increased urea and ALP in the HD M and cholesterol in M at ≥ 500 mg/kg/day, compared to controls. Bilirubin was reduced in both the M and F mice at ≥ 250 mg/kg/day. Liver weights were increased in the M at ≥ 250 mg/kg/day and in the F at ≥ 500 mg/kg/day. The microscopic examinations revealed hepatocellular hypertrophy and group cell necrosis in the M at ≥ 500 mg/kg/day and in the HD F. Systemic exposure to the parent drug and the main metabolite tapentadol-glucuronide were higher in the M than in the F mice. A second 13-week toxicity study in M and F CD-1 mice administered oral gavage tapentadol (10-200 mg/kg/day) revealed dose-related decreased body weights and food consumption. Alanine aminotransferase was increased in the M and F at the HD and aspartate aminotransferase was increased in a dose-related manner in all treated mice. There was a slight increase in liver weights in all groups, which was statistically significant in the HD F, without microscopic correlates in the histopathology examination. The liver findings and low oral tapentadol bioavailability seen in the toxicokinetic analyses may suggest that the treatment-related effects in liver reflect adaptive changes due to extensive metabolic activity and treatment-related changes in hepatic protein synthesis, fat metabolism, and hemoglobin product breakdown.

Rat:

The repeated dose studies in rat identified target organ toxicity in the central nervous system (CNS) and liver, with minor gastrointestinal (GI) system findings.

IV and SC tapentadol toxicity was compared in rats treated for 14 days (15-120 mg/kg/day IV and 30-45 mg/kg/day SC). The results showed dose-related increases in mortality in the SC arm, and deaths at the IV HD. Swellings were found in the SC infusion sites, and clinical signs of exophthalmus, subdued behavior, and reduced body weight gain in the IV treated rats. Systemic exposure was similar by the two routes, and increased in a dose-related manner. All treated rats showed reduction in body weights and body weight gains. Enlarged, reddened lymph nodes were noted at the higher IV doses, and subcutaneous dorsal cavity swelling with fluid exudate were found in most of the SC treated rats in the necropsy examination. The IV NOAEL was 90 mg/kg IV, but was not determined for the SC route. Four-week IV tapentadol administration in rats (3-15 mg/kg/day) revealed deaths at the HD, and clinical signs that were comparable to those in the 2-week study and consistent with mu-opioid receptor agonist CNS effects. There were dose-related increases in the incidence and severity of fearfulness, sedation, excitability and hunched posture, with reduced food consumption in the HDF. Circulating liver enzymes (ALAT, ALP) suggestive of increased liver metabolic activity were increased in the HD M. Microscopic examination found red foci in the stomach and hyperemia in the lamina propria of the gastric mucosa in HD M, and adrenal congestion in the HD F. Hepatotoxicity was evident by single cell hepatocellular necrosis in 1M and 1F at the high dose. Toxicokinetic evaluation showed a dose-

proportional increase in exposure, with no differences in exposure after 4 weeks treatment compared to exposure on Day 1, indicating absence of accumulation.

Preliminary palatability testing in M and F Wistar rats given tapentadol (250-1000 mg/kg/day) by dietary admixture for 7 days showed no treatment-related clinical signs or mortality. The HD M and F rats showed reduced food consumption and body weights compared to the control rats, which was more severe in the M than in the F. This would be expected because systemic exposure was found to be higher in the M than in the F. Food consumption and body weights recovered to a greater extent in the F during the 7-day dosing period, suggesting that palatability issues may have played a greater role in the M feeding. The necropsy examination showed decreased absolute and relative spleen weights in the M, and absolute and relative thymus weights in the F, compared to controls, but no effects in liver. As observed in the 13-week mouse dietary study, tapentadol exposure and toxicity was higher in the M than in the F rats. This finding is not clear in light of the observation that tolerance to potential palatability effects may have developed more quickly in the F, with reversibility of the treatment-related decrease in food consumption and the implication of increased exposure to test article in the F compared to the M rats in this study.

Gavage dosing in M and F Wistar rats for 10 consecutive days (100 increased to 400, and 300 increased to 600 mg/kg/day on Day 6) resulted in treatment-related clinical signs upon dose escalation on Day 6, that included frightened appearance, sedation, and reduced food consumption characteristic of high dose opioid agonist effect. Oral gavage treatment in a subsequent 4-week study in rats at the same dose range (300-600 mg/kg/day) revealed similar dose-related sedation and decreased body weight gain and food consumption, and additionally treatment-related deaths, increased white blood cell counts and lymphocytes at ≥ 400 mg/kg/day. At the HD, there were treatment-related increases in serum glucose, bilirubin, ALAT, LDH, and electrolytes (calcium, phosphorus, and chloride), and ocular findings (corneal opacities).

Oral tapentadol toxicity was evaluated using dietary (250-1000 mg/kg/day) and gavage (60-400 mg/kg/day) administration for 13 weeks in Wistar rats to support dose selection in the 2-year carcinogenicity study. Behavioral signs were noted in the gavage-treated rats, only, and included treatment-related increases in the incidence, severity and duration of exophthalmus, mouthing of the bedding material, soft feces and hunched posture. The clinical sign observations in the gavage, but not in the dietary study, reflect higher systemic tapentadol exposure (up to 4828 ng.h/ml in the males and 11829 ng.h/ml in the females) compared to the dietary exposures (up to 1891 and 2373 ng.h/ml in males and females, respectively). Additionally, there was considerable exposure to the tapentadol O-glucuronide metabolite in the gavage, but not in the dietary study. Body weight gains were reduced to similar magnitudes in the treated males in each study, but were reduced only in the HD F administered tapentadol by dietary admixture, probably reflecting the higher exposure in the females in the dietary study. Treatment-related hematology changes included increased hemoglobin, reticulocytes and middle reticulocyte fluorescence ratio (MFR), and decreased low reticulocyte fluorescence ratio (LFR) in the females given tapentadol in the diet, and increased white blood cell counts, lymphocytes

and large unstained cell counts in the HDF dosed by gavage. Treatment-related changes in circulating liver enzymes were observed in both studies. In the dietary study, GGT levels were increased by tapentadol (+105% at the MD and +95% at the HD) in male rats and ALP was increased (+84% at the MD and +116% at the HD) in the female rats. In the gavage study, there were slight increases in ASAT (+23% in the M, +22% in the F), and increased ALAT (+35% in the M and +17% in the F) at the HD. Relative liver weights (to body weight, +9% to +35% in all dietary treated M groups, non-significant increases in all gavage treated groups except for significant in MD gavage-treated F at +14.3%) and absolute liver weights (all gavage-treated M and F) were increased in both studies. Additionally, in the dietary study, there were treatment-related increases in absolute and relative brain, testes, and ovary weights. Heart, thymus and spleen weights showed dietary treatment-related reductions. Necropsy examination revealed gastrointestinal findings in both studies, with dilation and discoloration in the duodenum in the HD F given dietary tapentadol, and isolated dark red foci in the stomach fundus mucosa in the HD M dosed by gavage. Microscopic indices of tapentadol-related liver toxicity were hepatocellular hypertrophy in 30% of the dietary MD M and F, and 100% of the M and 90% of the F at the dietary HD, and mild hepatocellular centrilobular hypertrophy in one rat each in the LD M, MD M, and MD F, and in all HD M and 3/5 HD F treated by gavage. Dose-related increased incidence and severity (compared to controls) of fatty change was also seen in the livers of dietary administered rats at the MD (M) and HD (M&F).

Chronic (26-week treatment with 8-week recovery period) oral tapentadol toxicity in rodents was studied in M and F Wistar rats (75-300 mg/kg/day by gavage). The initial HD of 450 mg/kg/day was terminated after 13 weeks due to excessive mortality, and a new HD of 300 mg/kg/day was initiated, extending the dosing period for the new HD group to 26 weeks. The necropsic evaluation included additional assessment of microsomes from the livers of the M and F for P450 content, N-dealkylation activity, O-dealkylation activity and glucuronyltransferase activity. There was a dose-related increase in deaths during the dosing period (0/20, 3/20, 7/20, and 16/30 M and 1/20, 1/20, 8/20, and 17/30 F at 75, 150, 300, and 450 mg/kg/day, respectively, versus no deaths in the control rats [30/gender]). The deaths were associated with treatment-related respiratory depression, a known class effect of mu-opioid receptor agonist agents. The treatment-related clinical signs were dose-related in incidence and severity, and similar in the animals that died prematurely and in those that survived to the end of the dosing periods. The clinical signs of toxicity, characteristic of mu-opioid agonist effects were excitability, recumbency, hunched posture, labored respiration, and general poor condition during the first 13 weeks of the dosing period at doses of ≥ 150 mg/kg/day. Body weights were reduced at 300 mg/kg/day in the M (-6% to -7%), and at 450 mg/kg/day in the M (-6% to -7%) and F (-4% to -7%), although there were only small reductions in food consumption in these groups early in the study. The behavioral signs and body weight changes were reversible during the 8-week recovery period.

Chronic oral tapentadol treatment induced the following hematology changes: increased leukocyte counts (lymphocytes and segmented neutrophils) in the F at ≥ 300 mg/kg/day and fibrinogen in the HD M and F, and decreased PT and APTT in the HDM, and RBCs

in all treated M groups. Liver enzymes ASAT (+660%) and ALAT (+149%) were increased in the M at the HD in Week 13, and ALP at ≥ 150 mg/kg/day (dose-related) in Weeks 13 and 26. LDH was increased in M and F at the HD, and albumin in all treated groups in Week 26.

The results of the necropsy examination at the end of dosing showed dose-related increased liver weights in the M at ≥ 150 (+11% to +45% at 450 mg/kg/d in the early termination group) and F at 300 (+15%) and 450 (+31%, early termination group) mg/kg/day compared to controls. Microscopic pathology was noted only in liver. There were dose-related increases in the incidence of centrilobular or diffuse hepatocellular hypertrophy in all M and F groups given ≥ 150 mg/kg/day (up to 6/20 M and 2/20 F at 450 mg/kg/day), accompanied by fatty change in the groups that received ≥ 300 mg/kg/day. No evidence of liver necrosis was found at any dose, and no hepatocellular hypertrophy was found after the 8-week treatment-free recovery period, suggesting reversibility upon withdrawal of drug treatment. The microscopic changes are considered to be adaptive to increased liver enzyme activity, based on the clinical laboratory findings of increased serum liver enzymes and increased glucuronyl transferase activity in the examination of liver microsomes. Fatty change may be attributed to increased transport of fatty acids from adipose tissue and use of lipids for energy due to reductions in food consumption and body weights. The toxicokinetic analyses demonstrated dose proportional increases in plasma tapentadol and the metabolite tapentadol O-glucuronide at Week 25, with higher exposure to the parent drug but not to the metabolite in the F rats, and increased exposure in Week 26 compared to exposure on Day 1. Plasma exposure to the parent drug at the NOAEL in this study (75 mg/kg/day) represented 0.8 times in the M and 1.7 times in the F rats, the clinical exposure at the MRHD of 600 mg/day in a 70 kg patient on an AUC basis. Exposure to the glucuronide metabolite at the NOAEL represented approximately 9 times the clinical exposure to the metabolite at the MRHD, on an AUC basis.

In summary, the repeated dose studies in rat identified target organ toxicity in the central nervous system and liver. Treatment-related toxicity was more severe by the SC than by the IV routes, indicated by treatment-related mortality, clinical signs and decreased body weights at lower SC than IV doses, although systemic exposures were similar. The observations in the oral toxicity studies in rat, mortality, clinical signs such as sedation, and reduced body weights were evident at lower doses in the gavage than in the dietary experiments, probably due to higher systemic exposure with gavage treatment. Some tolerance development to tapentadol CNS effects was suggested by the higher doses required to elicit the clinical signs with increased treatment duration across studies. Increased hepatic activity, indicated by increased circulating liver enzymes (e.g., ALAT, ASAT, ALP, etc.), and hepatotoxicity revealed by increased liver weights and microscopic findings of hepatocellular hypertrophy, were found with increasing severity and at lower doses in the longer duration studies. Fatty changes were also found after longer duration treatment (13 and 26 weeks) by the dietary and gavage methods. Treatment-related liver necrosis was observed in the 4-week IV study in single M and F rats at the highest dose administered only, and Kupffer cell activation was not found in the rats administered tapentadol by any route or duration. The treatment-related liver