

**Summary of pharmacokinetic parameters in monkeys after repeated i.v. administration of 5 mg/kg once daily (Mean  $\pm$  SD on day 14)**

Parameter	Mean $\pm$ SD
AUC [ng·h/mL]	1035 $\pm$ 230
C <sub>max</sub> [ng/mL]	862 $\pm$ 111
t <sub>max</sub> [h]	0.22 $\pm$ 0.09
t <sub>1/2,z</sub> [h]	1.0 $\pm$ 0.2
CL [mL/min/kg]	71.3 $\pm$ 14.8
V <sub>z</sub> [L/kg]	5.8 $\pm$ 0.2

### 3.3.4 Distribution

Tapentadol is rapidly and widely distributed in tissues. The volumes of distribution V<sub>d</sub> values are 5-11 L/kg across species and in humans. Protein binding was low in all species in a radiolabeled drug-binding assay *in vitro* (Study PK582), primarily to serum albumin. Protein binding across the concentration range of 42.9 to 687 ng/ml was 16.3%-16.7% in mouse, 17.2%-18.5% in rat, 9.2%-12.0% in rabbit, 15.6%-18.9% in dog, and 19.3%-20.7% in human plasma. The results of that study also showed tapentadol binding to sepiia melanin, at 26.7%-48.2%.

The results of a tissue distribution study using whole-body autoradiography in albino Sprague Dawley and pigmented Lister Hooded rats (Study PK432) given a single IV C<sup>14</sup>-tapentadol (10 mg/kg) showed distribution to the following tissues at 15 minutes after treatment, in decreasing order of tissue concentration: kidney medulla and cortex, preputial gland, intra-orbital lachrymal gland, exorbital lachrymal gland, salivary glands, liver, Harderian gland, pituitary, pancreas, spleen, adrenal, lung, bone marrow, mandibular lymph nodes, bulbo-urethral gland, thyroid, brain, spinal cord, and blood. Fat and muscle concentrations were very low. The patterns of distribution were similar in the albino and pigmented species, except for melanin binding in ureal tract and skin tissues in the Hooded rats. Tapentadol levels in most of the tissues were below the level of quantification at 24 hours after IV injection in that study, and melanin-bound radioactivity decreased over the next 1-3 days. CNS levels decreased from peaks of 9.40 and 6.97 mcg equiv/g in brain and spinal cord at 0.25 h, to 4 mcg equiv/g in the CNS at 1 h, 1 mcg equiv/g at 2 h, 0.13 mcg equiv at 4 h, and were below the level of detection at 8 hours. There is a low potential for penetration of tapentadol and its metabolites into red blood cells. Comparison of radioactivity in whole blood and in serum revealed lower concentrations in whole blood than in the serum in dogs (-36%) and humans (-90% to -95%) administered C<sup>14</sup>-tapentadol.

Tapentadol crosses the placenta, indicated by detection of the parent drug and the main glucuronide metabolite in albino rat fetuses in a dose range-finding study conducted prior to evaluation of Pre- and Post-Natal Development (Study TP2772, and PK432).

However, the concentrations measured in the fetuses were extremely low in that study, probably due in part to sampling times at 24 and 72 hours after the last maternal dose. A microdialysis study in Sprague Dawley rats administered non-labeled tapentadol at 6 oral gavage doses of 80 mg/kg each, 6 hours apart (Study PK664) demonstrated blood-brain penetration by the parent drug. Exposure to the parent drug in extracellular fluid in the corpus striatum was approximately half that in the peritoneal cavity samples collected to represent plasma concentration. Blood-brain penetration of the main glucuronide metabolite was lower, producing exposure of approximately 6% that in plasma following the first dose and 18% the plasma exposure after the last of the 6 doses. The results of that study are presented below (table provided from the original NDA submission):

After administration Tissues / organs	Pharmacokinetic parameters (mean ± Stand Dev)									
	AUC (h·ng/mL)		AUC ratio (rat/dog) <sup>a</sup>		C <sub>max</sub> (ng/mL)		t <sub>1/2</sub> (h)		MRT (h)	
	1st	6th	1st	6th	1st	6th	1st	6th	1st	6th
<b>CG5503 base</b>										
Peritoneal cavity	2220± 2001	2499± 1608			1024 ± 368	1046 ± 690	1.03 ± 0.55	1.57 ± 0.19	2.09 ± 0.58	2.34 ± 0.23
Extracellular fluid in the brain	979 ± 1284	1437± 1002	0.44 ± 0.23	0.62 ± 0.18	441 ± 659	491 ± 433	0.99 ± 0.29	2.13 ± 1.49	2.13 ± 0.29	3.15 ± 1.51
Plasma	604 ± 217	2688± 2762			473 ± 261	1602± 1484	1.21 ± 0.25	1.45 ± 0.50	2.06 ± 0.40	2.11 ± 0.42
<b>CG5503 glucuronide</b>										
Peritoneal cavity	47771±33147	67378±37524			16341±6334	31169±7343	0.57 ± 0.13	1.66 ± 1.11	2.43 ± 0.20	2.98 ± 0.15
Extracellular fluid in the brain	2634±1607	1248±7777	0.06 ± 0.03	0.18 ± 0.05	708.6±492.0	2464 ± 848	1.59 ± 0.57	2.45 ± 0.79	4.07 ± 0.52	4.07 ± 0.85
Plasma	45732±4872	61500±21755			12989±2696	16384±5715	1.49 ± 0.33	2.05 ± 1.08	3.14 ± 0.40	3.18 ± 1.24

### 3.3.5 Metabolism

Tapentadol is rapidly and extensively metabolized after oral administration. The results of *in vitro* evaluation in hepatic microsomes demonstrated that the main route of metabolism is by Phase II glucuronidation, in all species tested including humans (Study PKN233/A). The results of this study, showing the intrinsic clearance of tapentadol by O-glucuronidation and oxidation across species are presented below (table provided from the original NDA submission):

Species	Hamster		Minipig		Dog	Rabbit	Rat		Mouse	Cynomolgus		Guinea pig		Human
	n.s. <sup>a</sup>		M	F	M	n.s.	M	F	n.s.	M	F	M	F	n.s.
Intrinsic clearances (ml/min/kg) <sup>b</sup>	0.1555		0.0800	0.0929	0.0425	0.0350	0.0244	0.0331	0.0117	0.0128	0.0108	0.0108	0.0088	0.0019
Sex	n.s.		n.s.		n.s.	n.s.	M	F	n.s.	n.s.		n.s.		n.s.
Percentual losses <sup>c</sup>	78.9		99.2		9.6	26.7	28.5	14.3	20.4	51.5		78.3		0.6
UGT <sup>d</sup>	1A1		1A3	1A4	1A6	1A9	2B7	2B15						
Percentages of glucuronide <sup>e</sup>	0.17		0.52	0.15	1.19	1.98	1.64	0.32						

Additional Information: Human hepatic glucuronidation was catalysed by several isoforms but mainly by UGT1A6, UGT1A9 and UGT2B7.

The fact that several isoforms appear to be involved in the glucuronidation of tapentadol means that there is little risk that its metabolic clearance will be diminished in humans who are homozygous for a deficient allele of one or other of the isoforms.

a) not specified

b) V<sub>max</sub> / K<sub>m</sub> (ml/min/mg) microsomal protein determined for glucuronidations by microsomes

c) Percentual losses of the initial amount of tapentadol in oxidation assays performed with equivalent amounts of P450 (300 pmol/ml), an initial concentration of 10 μM tapentadol and incubating for 30 min.

d) deconbinant human glucuronyl transferase isoform

e) formed by various recombinant human glucuronyl transferase isoforms (UGTs) after 90 minutes

Tapentadol is more extensively glucuronidated in the animal species than in humans, based on the results of the study in hepatic microsomes. Oral bioavailability assessments in the nonclinical and clinical pharmacokinetic studies showed higher tapentadol bioavailability in human (32%) than in rat, dog and monkey (9%, 3% and <1%, respectively). The main metabolic enzymes involved in human tapentadol metabolism by glucuronidation are the UDP-glucuronosyltransferases UGT1A9, UGT2B7, and UGT1A6 (Study PK528). The percentages of glucuronide formed in human microsomes by the isoforms of UGT are shown in the following table (provided from the original NDA submission):

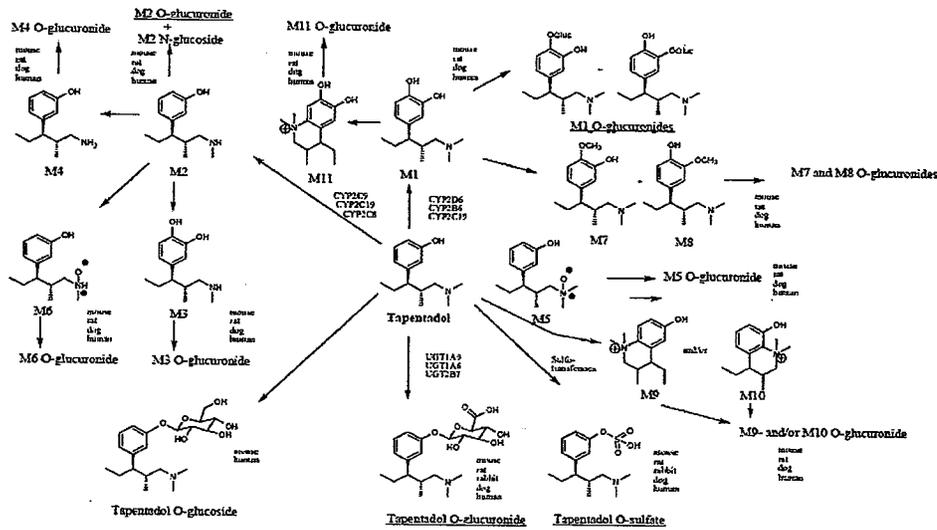
Table 4-2: Percentages of glucuronide formed by various recombinant human glucuronyl transferase isoforms (UGTs) after 90 minutes

UGT 1A1	UGT 1A3	UGT 1A4	UGT 1A6	UGT 1A9	UGT 2B7	UGT 2B15
0.17%	0.52%	0.15%	1.19%	1.98%	1.64%	0.32%

Additionally, tapentadol undergoes oxidation to a greater extent in the animal species tested than in humans. *In vivo* liver metabolism assay showed percent loss of tapentadol parent drug by hepatic microsomal P450 oxidation of 99.2% in minipig, 78.9% in hamster, 78.3% in guinea pig, 51.5% in Cynomolgus monkey, 28.5% in rat, 26.7% in rabbit, 20.4% in mouse, 14.3% in rat, 9.6% in dog, and 0.6% in human microsomes (Study PKN233/A). The Phase I metabolites were CYP2C9, CYP2C19, and CYP2C8 catalyzed N-demethyl tapentadol (M2), and hydroxy-tapentadol (M1) by CYP2D6, CYP2B6, and CYP2C19 catalysis, in microsomes from all species tested including human. Of all species tested, the metabolic profiles in rat and dog most resembled that in human.

The proposed tapentadol metabolic pathways in mice, rats, rabbits, dogs, and humans are diagrammed in the following figure (provided from the original NDA submission):

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The results of *in vivo* studies on tapentadol metabolism also showed qualitatively similar metabolic profiles in mice, rats, dogs and humans. HPLC analysis of urine samples collected over 48 hours in the animals and for 24 hours in humans after oral tapentadol administration (50 mg/kg in mice, 150 mg/kg in rats, 20 mg/kg in dogs and 100 mg in human volunteers, given by gavage in the animal species) revealed the following results (table provided from the original NDA submission):

Species	Sample	Sampling Time or Period (h)	% of Dose in Sample	% of Compound in Sample (mean values)				Study No.
				Parent	Parent conjugates	M1con.	M2con.	
Mice	Urine	0-48	82	4.9	44	20	2.8	PK581K/A
	Feces	0-48	6.6	-	-	-	-	PK581K/A
Rat male	Urine	0-48	69	0.8	25	18	14	PK581K/A
	Feces	0-48	26	-	-	-	-	PK581K/A
Rat female	Urine	0-48	94	3.2	39	39	5	PK581K/A
	Feces	0-48	5	-	-	-	-	PK581K/A
Dogs	Urine	0-24	81 <sup>f</sup>	<1	58	11	3.3	PK581K/A
	Feces	0-48	18	-	-	-	-	PK581K/A
Humans	Urine	0-24	99	4.5	70 <sup>b</sup>	2	13	PK581K/A
	Feces	0-24	1	-	-	-	-	PK581K/A

- a) quantitative investigations with HPLC and radiodetection of urine samples are from 3 males.
- b) total dose
- c) MBq/group
- d) MBq/animal
- e) MBq/kg body weight
- f) capsule of 100 mg CG3503 labeled with 1.85 MBq radiocarbon
- g) sampling Time is 0-48 h
- h) tapentadol O-glucuronide: 55% of dose; tapentadol O-sulfate: 15% of dose

Total radioactivity in urine collected for 48 hours after a single oral dose was similar across species, when comparing data from several excretion studies (87%, 82%, 91%, and 90% in mice, rats, dogs and humans, respectively). In another comparative study on tapentadol metabolic profiles (PK581/A), the following pharmacokinetic parameters including excretion data were seen (table provided from the original NDA submission):

Species	Terminal half-life	Total clearance (i.v.)	Volume of distribution (i.v.)	Absolute bio-availability	Excretion of		
	t <sub>1/2,z</sub> [h]	CL [mL/min·kg]	V <sub>z</sub> [L/kg]	F [%]	Total radioactivity [% of oral dose]	CG5503 base unchanged	
					Urine	Feces	Urine unchanged
Mouse (M)	0.29 (i.v.) 0.64 (p.o.)	223	5.6	40	82	6.6	4.1
Rat (M/F)	0.5 (i.v.) >4 (p.o.)	228	10	9	70 (M) 94 (F)	26 (M) 4.9 (F)	0.8 (M) 3.2 (F)
Dog (M)	0.9 (i.v.) 3.7 (p.o.)	145	11	3	81	18	<1.0
Monkey (M) <sup>1)</sup>	1.0 (i.v.)	71	5.8	<1	n.d.	n.d.	n.d.
Human (M)	4.1 (i.v.) 1.25 (p.o.)	21	7.3	32 (fasted)	99	1.2	3.2

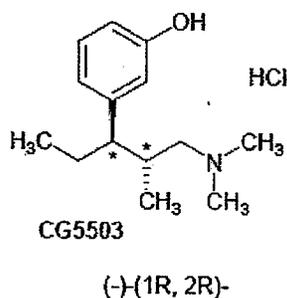
n.d. = not determined.

<sup>1)</sup> data after repeated i.v. or oral dosing

Minor metabolites (<5% total dose) are described in the figure on metabolic pathways of tapentadol, above. Nearly all (99.6% in dog and 96.6% in humans) of the eleven metabolites identified in plasma were found to be conjugated, with the remaining radioactivity associated with the parent drug.

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The potential for chiral interconversion of tapentadol's two chiral centers (pictured below in a figure from the Sponsor) 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 was evaluated in plasma samples from mice, rats, rabbits, dogs and humans (Study PK581K/A).



The percentages of tapentadol base found converted to the GRT4045Y+ enantiomer in the species tested are presented in the following table (provided from the original NDA submission):

Species	Dose	GRT4045Y+enantiomer [% of CG5503 base]
mouse	500 mg/kg daily p.o. 13 weeks	1.1
rat	300 mg/kg daily p.o. 26 weeks	0.43
rabbit	25 mg/kg daily s.c. 2 weeks	0.37
dog	80 mg/kg p.o. 13 weeks	0.67
human	50 mg q6h p.o. 6 days	0.38

Conversion of the chiral centers is unlikely to have taken place, because batch analyses showed up to  $\rightarrow$  of the diastereomer in the formulations administered.

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### 3.3.6 Excretion

Tapentadol is primarily excreted in urine as the glucuronide and sulfate metabolites, with minor excretion in feces. In several mass balance studies animals and humans were administered radiolabeled oral tapentadol, and urine and feces were collected for 48 hours post-dose (Studies PK586/A, PK583, PK480/A, and HP5503/05). The methods and results are shown in the following table (provided from the original NDA submission):

Species	Mice	Rats	Rats	Dogs	Humans										
Gender (M/F) / Number of animals	25M <sup>a</sup>	5M	5F	3M	4M										
Feeding condition:	Fasted	Fasted	Fasted	Fasted	Fasted										
Vehicle/Formulation:	Aqua bidest / Solution	Capsule													
Method of Administration:	Gavage	Gavage	Gavage	Gavage	Oral										
Dose (mg/kg)	50	150	150	20	100 mg <sup>b</sup>										
Analyte:	TRA <sup>c</sup>	TRA <sup>c</sup>	TRA <sup>c</sup>	TRA <sup>c</sup>	TRA <sup>c</sup>										
Assay:	LSC <sup>d</sup>	LSC <sup>d</sup>	LSC <sup>d</sup>	LSC <sup>d</sup>	LSC <sup>d</sup>										
Excretion route	Urine <sup>e</sup>	Feces	Total	Urine <sup>e</sup>	Feces	Total	Urine <sup>e</sup>	Feces	Total	Urine <sup>e</sup>	Feces	Total	Urine	Feces	Total
Time 0 - 48 hr	82	7	89	69	26	95	94	5	99	81	18	99	99	1	100
Mean excretion balance of urine:faeces	12:1			3:1			19:1			4.6:1			76:1		
Study number	PK586/A			PK583			PK583			PK480/A			HP5509/05		
Location in CTD															

- a) 5 groups of 5 each
- b) total dose
- c) total radioactivity; percent of dose (mean values)
- d) liquid scintillation counting
- e) the amount of radioactivity found in rinsing water being added to the urine.

Excretion in milk was demonstrated in the pre- and post-natal development study in rats (Study TP2772) by plasma levels measured in the pups of lactating dams.

### 3.3.7 Pharmacokinetic drug interactions

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 by glucuronidation. Drugs that inhibit the UGT enzymes, particularly subtypes involved in tapentadol glucuronidation (UGT1A9 and UGT2B7) such as probenecid, chloramphenicol and naproxen, and thus could potentially increase exposure to parent drug, the were examined in a study in liver microsomes (Study PK681). The results of that study showed only slight inhibition of tapentadol glucuronidation, with highest inhibition of 27% identified by naproxen and 45% by probenecid. The results of the assessment of glucuronidation by UGT in pooled human liver microsomes are presented in the following table (provided from the original NDA submission):

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Inhibitor	C <sub>max</sub> (μM) <sup>a</sup>	Ref	Test concs (μM)	I <sub>max</sub> (%) <sup>b</sup>	K <sub>i</sub> (μM)	I <sub>in</sub> (%) <sup>c</sup>
Amitriptyline	1	G	1, 10, 50, 100	38	30	3
Nortriptyline	0.5	G	1, 10, 50, 100	0	n.e.	0
Imipramine	1.5	G	1, 10, 50, 100	40	30	7
Desipramine	0.5	G	1, 10, 50, 100	10	n.e.	0
Codeine	0.3	G	1, 10, 50, 100	5	n.e.	0
Morphine	0.4	G	1, 10, 50, 100	0	n.e.	0
Naproxen	217	V	0.1, 0.5, 1, 2 mM	65	600	27
Diclophenac	4	V	50, 100, 250, 400	90	56	6
Ketorolac	1.2	V	1, 10, 50, 100	2	n.e.	0
Fenoprofen	0.3	V	1, 10, 50, 100	30	20	1
Ketoprofen	1.2	V	1, 10, 50, 100	12	n.e.	0
Ibuprofen	48	V	500	n.e.	980	5
Niflumic acid	0.5	V	1, 10, 50, 100	10	n.e.	0
Meclofenamate	16	V	1, 50, 100, 250	90	29	36
Salicylic acid	2000	V	0.1, 0.5, 1, 2 mM	10	n.e.	12
Valproic acid	500	G	1 mM	30	4400	10
Paracetamol	2000	P	0.1, 0.5, 1, 2 mM	Activation	n.e.	#
Indomethacin	10	V	10, 50, 200, 500	40	50	9
Lidocaine	20	G	10, 50, 200, 500	16	n.e.	0
Verapamil	2	G	1, 10, 50, 100	37	30	8
Chloramphenicol	50	G	10, 50, 200, 500	38	80	15
Ketoconazole	10	G	1, 10, 50, 100	63	25	21
Miconazole	10	R	10, 50, 200, 500	70	30	23
Zidovudine	5	R	1, 10, 50, 100	11	840	1
Probenecid	700	R	0.25, 0.75, 1, 2 mM	67	840	45

a) maximal clinical concentrations (C<sub>max</sub>) were derived from either Goodman and Gilman (ref G), Prescott (ref P), Rajanarison et al. (ref R), or Vietri et al. (ref V)

b) apparent maximal extent of inhibition (I<sub>max</sub>) is estimated, as is the IC50/K<sub>i</sub>

c) approximated maximal extent of inhibition in the clinic (I<sub>in</sub>) is the best estimate from the available data, but does not take any known protein binding into account.

Additional *in vitro* investigations demonstrated potentiation of the UGT metabolic rate by acetaminophen by 20%, suggesting a potential decrease in tapentadol exposure with co-administration. The potential for metabolic interactions of tapentadol with drugs that inhibit or potentiate UGT metabolism was also addressed in the clinical studies (see Studies PAI-1011 and PAI-1013) in the dose ranges relevant to those indicated for treatment. Increased tapentadol exposure of approximately 17% by naproxen and 57% by probenecid were found in those studies, but there were no effects on tapentadol exposure by co-treatment with acetaminophen. *In vitro* studies conducted to investigate tapentadol inhibition and induction of the cytochrome P450 metabolic enzymes found inhibition of the isoenzyme, CYP2D6 only (K<sub>i</sub> = 181 μM for competitive inhibition and 1410 μM for noncompetitive inhibition), at concentrations that were 180 to 1400 times the C<sub>max</sub> for tapentadol in clinical treatment (studies PK680 and PK679). There was no effect of co-incubation of tapentadol with dextromethorphan in the presence of the NADPH regenerating system *in vitro*. No induction of the cytochrome P450 isoenzymes CYP1A2, CYP2C9, and CYP3A4 were found in human hepatocytes (Study PK679), except for a 1.5X induction at a concentration of 200 times the highest plasma concentration (C<sub>max</sub>) in clinical treatment in the indicated dose range.

Potential metabolic drug interactions with tapentadol was examined *in vivo* in rats and dogs using assessments of metabolic enzyme activities in microsomes from liver samples taken at necropsy to measure several of the markers for induction of CYP isoenzymes and Phase II glucuronidation (see studies TP2397, TP2415, TP2441, TP1968/A, and TP2593 [PK268], below under Toxicology). Thyroxine UDP glucuronosyltransferase

may be involved in thyroid tumor formation by non-genotoxic CYP enzyme induction, via stimulation of thyroxine glucuronidation and biliary excretion, resulting in decreased serum thyroxine and triiodothyronine, with increased serum thyroid stimulating hormone, which during chronic stimulation results in thyroid follicular cell hyperplasia that may progress to follicular cell tumors. Cytochrome P450 and thyroxine UDP glucuronosyltransferase activities were measured in Wistar rats (Study TP2593 [redacted] report 5335] administered oral gavage tapentadol doses of 75-300 mg/kg/day for 4 weeks. There were significant dose-related increases in hepatic microsomal total CYP content (+143% in the males at 300 mg/kg/day and 11%-117% in the females at 150-300 mg/kg/day), predominantly on CYP2B-dependent activity by 7-pentoxoresorufin O-depentyase (429%-3219% in the males given 75-300 mg/kg/day, and 542%-3159% in the females given 150-300 mg/kg/day), compared to control values. Additionally, there were dose-related, statistically significant increases in hepatic microsomal 7-ethoxyresorufin O-deethylase activity (176%-362% all doses in the males, 188% in the females at 300 mg/kg/day), 7-pentoxoresorufin O-depentyase activity (429%-3219% at all doses in the males, 542%-3159% at 150-300 mg/kg/day in the females), and 4-nitrophenol hydroxylase activity (143%-179% at 150-300 mg/kg/day in the males, 131% at 300 mg/kg/day in the females). There were no tapentadol treatment-related changes in microsomal activities of testosterone 6B-hydroxylase, lauric acid 11-hydroxylase, lauric acid 12-hydroxylase, and thyroxine UDP-glucuronosyltransferase. These results suggest tapentadol-related induction of CYP2B isoforms in male and female rats, and similarity to other CYP2B inducers including phenobarbital, and therefore potential interactions with drugs that are metabolized by CYP2B-dependent 70-pentoxoresorufin O-depentyase, but not CYP1A, CYP2E, CYP3A and CYP4A forms. The effects on the other CYP isoenzymes were relatively minor in comparison. The results of liver metabolic enzyme activity in the 4-week oral gavage study in rats are presented in the following tables (provided from the original NDA submission, means  $\pm$  SD, \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001):

b(4)

Treatment <sup>a</sup>	Cytochrome P450 content	
	(nmol/mg protein)	
	Male rats	Female rats
Control (vehicle only)	0.54 $\pm$ 0.061 <sup>b</sup> (100) <sup>c</sup>	0.46 $\pm$ 0.015 (100)
CG5503 75 mg/kg/day	0.61 $\pm$ 0.068 (113)	0.51 $\pm$ 0.013 (111)
CG5503 150 mg/kg/day	0.60 $\pm$ 0.063 (111)	0.51 $\pm$ 0.057* (111)
CG5503 300 mg/kg/day	0.77 $\pm$ 0.104*** (143)	0.54 $\pm$ 0.030** (117)

Treatment <sup>a</sup>	7-Ethoxyresorufin O-deethylase activity	
	(pmol/min/mg protein)	
	Male rats	Female rats
Control (vehicle only)	21 ± 2.1 <sup>b</sup> (100) <sup>c</sup>	17 ± 2.6 (100)
CG5503 75 mg/kg/day	37 ± 10.2* (176)	19 ± 3.6 (112)
CG5503 150 mg/kg/day	44 ± 9.9** (210)	17 ± 4.7 (100)
CG5503 300 mg/kg/day	76 ± 15.3*** (362)	32 ± 4.1*** (188)

Treatment <sup>a</sup>	7-Pentoxoresorufin O-depentylase activity	
	(pmol/min/mg protein)	
	Male rats	Female rats
Control (vehicle only)	21 ± 5.0 <sup>b</sup> (100) <sup>c</sup>	6.5 ± 2.00 (100)
CG5503 75 mg/kg/day	90 ± 39.4*** (429)	8.9 ± 3.65 (137)
CG5503 150 mg/kg/day	242 ± 62.1*** (1152)	35.2 ± 9.85*** (542)
CG5503 300 mg/kg/day	676 ± 191.8*** (3219)	205.3 ± 71.79*** (3159)

Treatment <sup>a</sup>	4-Nitrophenol hydroxylase activity	
	(umol/min/mg protein)	
	Male rats	Female rats
Control (vehicle only)	0.56 ± 0.086 <sup>b</sup> (100) <sup>c</sup>	0.55 ± 0.080 (100)
CG5503 75 mg/kg/day	0.67 ± 0.069 (120)	0.57 ± 0.063 (104)
CG5503 150 mg/kg/day	0.80 ± 0.181* (143)	0.56 ± 0.068 (102)
CG5503 300 mg/kg/day	1.00 ± 0.159*** (179)	0.72 ± 0.071** (131)

Treatment <sup>a</sup>	Testosterone 6 $\beta$ -hydroxylase activity	
	(nmol/min/mg protein)	
	Male rats	Female rats
Control (vehicle only)	1.00 $\pm$ 0.315 <sup>b</sup> (100) <sup>c</sup>	0.08 $\pm$ 0.034 (100)
CG5503 75 mg/kg/day	1.06 $\pm$ 0.252 (106)	0.07 $\pm$ 0.039 (88)
CG5503 150 mg/kg/day	1.14 $\pm$ 0.377 (114)	0.08 $\pm$ 0.011 (100)
CG5503 300 mg/kg/day	1.14 $\pm$ 0.168 (114)	0.10 $\pm$ 0.015 (125)

Treatment <sup>a</sup>	Lauric acid 11-hydroxylase activity	
	(nmol/min/mg protein)	
	Male rats	Female rats
Control (vehicle only)	0.34 $\pm$ 0.084 <sup>b</sup> (100) <sup>c</sup>	0.35 $\pm$ 0.031 (100)
CG5503 75 mg/kg/day	0.36 $\pm$ 0.066 (106)	0.31 $\pm$ 0.066 (89)
CG5503 150 mg/kg/day	0.37 $\pm$ 0.064 (109)	0.34 $\pm$ 0.077 (97)
CG5503 300 mg/kg/day	0.43 $\pm$ 0.096 (127)	0.37 $\pm$ 0.048 (106)

Treatment <sup>a</sup>	Lauric acid 12-hydroxylase activity	
	(nmol/min/mg protein)	
	Male rats	Female rats
Control (vehicle only)	0.44 $\pm$ 0.158 <sup>b</sup> (100) <sup>c</sup>	0.50 $\pm$ 0.032 (100)
CG5503 75 mg/kg/day	0.42 $\pm$ 0.122 (96)	0.46 $\pm$ 0.105 (92)
CG5503 150 mg/kg/day	0.36 $\pm$ 0.029 (82)	0.56 $\pm$ 0.161 (112)
CG5503 300 mg/kg/day	0.37 $\pm$ 0.111 (84)	0.53 $\pm$ 0.068 (106)

Treatment <sup>a</sup>	Thyroxine UDPglucuronosyltransferase activity	
	(pmol/min/mg protein)	
	Male rats	Female rats
Control (vehicle only)	5.2 ± 1.20 <sup>b</sup> (100) <sup>c</sup>	5.4 ± 1.16 (100)
CG5503 75 mg/kg/day	6.0 ± 1.59 (115)	5.6 ± 0.71 (104)
CG5503 150 mg/kg/day	4.3 ± 1.35 (83)	6.8 ± 1.12* (126)
CG5503 300 mg/kg/day	5.0 ± 0.55 (96)	5.4 ± 0.28 (100)

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

P450 content and enzyme induction of N-dealkylation (aminopyrine N-demethylase), O-dealkylation (7-ethoxycoumarin O-deethylase) and UDP glucuronyltransferase (2-aminophenol-glucuronyltransferase) activity, was assessed in the 26-week oral gavage toxicity study in the male and female Wistar rats given control vehicle and tapentadol at 75 mg/kg/day (Study TP2397 [Study PKN253]) to explore 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, presented in the following tables from the Sponsor, 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.

Dose [mg/kg]	P450 [pmol/mg]	ECOD [pkat/mg]	AND [pkat/mg]	GT [pkat/mg]
Control	1283 ± 91	24.7 ± 3.1	40.9 ± 1.9	16.2 ± 3.7
75	1368 ± 178	30.1 ± 6.7	48.1 ± 12.9	15.2 ± 3.9
Induction [%]	107	122	118	94
ANOVA p-values	not significant	not significant	not significant	not significant

Induction is expressed as 75 mg/kg vs. control ratio

Table 3.2: Mean (± S.D.) P450 contents and enzyme activities in female rats

Dose [mg/kg]	P450 [pmol/mg]	ECOD [pkat/mg]	AND [pkat/mg]	GT [pkat/mg]
Control	1024 ± 200	11.4 ± 1.7	22.1 ± 2.9	8.3 ± 2.2
75	1041 ± 216	9.3 ± 1.8	31.9 ± 9.3	14.5 ± 4.3
Induction [%]	102	82	144	175
ANOVA p-values	not significant	not significant	not significant	0.021

Induction is expressed as 75 mg/kg vs. control ratio

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 tested in microsomes isolated at necropsy from the livers in male and female Beagle dogs administered tapentadol by oral gavage at doses of 10-120 (highest dose reduced to 80 mg/kg/day on Day 22 of treatment) mg/kg/day for 13 weeks (Study TP2593) and 10-80 mg/kg/day by gavage for 52 weeks (Study TP2441 [PKN309]), and by intravenous injection at doses of 1-7.5 mg/kg/day for 4 weeks (Study TP1968). 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 under the conditions of this study. Therefore, there may be a potential for interactions with tapentadol by drugs metabolized by N-demethylase. Also, as demonstrated in the *in vitro* and clinical studies discussed above, tapentadol may potentially decrease plasma concentrations of drugs that undergo glucuronidation. Conversely, drugs that inhibit or activate these metabolic enzymes may increase or decrease, respectively, the plasma concentrations of tapentadol with co-administration. The findings are presented below (table provided from the original NDA submission):

The mean ( $\pm$  S.D.) P450 contents and enzyme activities in male dogs were:

Dose [mg/kg]	P450 [pmol/mg]	ECOD [pkat/mg]	AND [pkat/mg]	GT [pkat/mg]
Control	564 $\pm$ 39	32.4 $\pm$ 5.4	31.1 $\pm$ 1.5	17.5 $\pm$ 0.4
120	475 $\pm$ 87	25.0 $\pm$ 3.3	40.2 $\pm$ 1.4	13.2 $\pm$ 1.7
Induction [%]	84	77	129	75
ANOVA p-values	not significant	not significant	0.001	0.012

Induction is expressed as 120 mg/kg vs. control ratio

The mean P450 contents and enzyme activities in female dogs were:

Dose [mg/kg]	P450 [pmol/mg]	ECOD [pkat/mg]	AND [pkat/mg]	GT [pkat/mg]
Control	443 $\pm$ 47	32.8 $\pm$ 11.0	33.7 $\pm$ 6.9	15.7 $\pm$ 1.6
120	566 $\pm$ 81	51.8 $\pm$ 14.1	55.7 $\pm$ 10.9	14.9 $\pm$ 3.3
Induction [%]	128	158	165	95
ANOVA p-values	not significant	not significant	0.042	not significant

Induction is expressed as 120 mg/kg vs. control ratio

Oral gavage treatment for 52 weeks in the dogs produced the following results (table provided from the original NDA submission):

Table 3.1: Mean ( $\pm$  S.D.) P450 contents and enzyme activities in male dogs

Dose [mg/kg]	P450 [pmol/mg]	ECOD [pkat/mg]	AND [pkat/mg]	GT [pkat/mg]
Control	1008 $\pm$ 224	44.5 $\pm$ 7.8	55.9 $\pm$ 11.1	67.9 $\pm$ 14.5
30	994 $\pm$ 130	64.5 $\pm$ 4.5	76.9 $\pm$ 9.7	38.4 $\pm$ 6.0
80	1133 $\pm$ 188	65.4 $\pm$ 11.1	95.9 $\pm$ 21.3	55.6 $\pm$ 16.6
Induction [%] 30 mg/kg	99	145	138	58
Induction [%] 80 mg/kg	112	147	172	82
ANOVA 30 mg/kg	not sign.	0.02	not sign.	0.03
p-values 80 mg/kg	not sign.	not sign.	0.04	not sign.

Induction is expressed as 30 mg/kg/day or 80 mg/kg/day vs. control ratio

Table 3.2: Mean ( $\pm$  S.D.) P450 contents and enzyme activities in female dogs

Dose [mg/kg]	P450 [pmol/mg]	ECOD [pkat/mg]	AND [pkat/mg]	GT [pkat/mg]
Control	894 $\pm$ 87	56.7 $\pm$ 15.5	55.3 $\pm$ 3.6	64.1 $\pm$ 3.0
30	1108 $\pm$ 115	83.1 $\pm$ 25.9	86.9 $\pm$ 22.2	46.9 $\pm$ 4.6
80	913 $\pm$ 242	113 $\pm$ 13.4	107 $\pm$ 30	40.2 $\pm$ 5.0
Induction [%] 30 mg/kg	124	146	157	72
Induction [%] 80 mg/kg	102	200	193	63
ANOVA 30 mg/kg	not sign.	not sign.	not sign.	0.005
p-values 80 mg/kg	not sign.	0.009	0.04	0.002

Induction is expressed as 30 mg/kg/day or 80 mg/kg/day vs. control ratio

There were dose-related increases in ethoxycoumarin O-deethylase activity to 145%-200% control values and N-demethylase activity to 138%-193% control values at 30-80 mg/kg/day in the male and female dogs at the 52-week assessment. There were no changes in cytochrome P450 content, and glucuronyltransferase activity was decreased in the male and female dogs to 58%-82% of control values (decrease of 20%-40%) in the dose range studied.

Enzyme activity analysis in microsomes from the livers in dogs given IV tapentadol injections at doses of 1-7.5 mg/kg/day for 4 weeks (Study TP1968) showed no effects on cytochrome P450 content and N-dealkylation (aminopyrine N-demethylase), O-dealkylation (O-ethoxycoumarin O-deethylase), and aminophenol glucuronyltransferase activities. There was greater activity by tapentadol on glucuronyltransferase activity by the oral route in the previous study.

### 3.3.10 Clinical Exposure for Comparison in the Toxicology Studies

Clinical tapentadol pharmacokinetic assessments showed rapid and complete absorption by the oral route, with a mean bioavailability of approximately 32%. There were dose-proportional increases in systemic exposure (C<sub>max</sub> and AUC). Slight accumulation was found in the comparison of single and repeated dose exposure; AUC values for the parent drug were 1.6 times the single dose values, and for the main glucuronide metabolite of approximately 1.8 times the single dose values when given q.i.d. Steady state is observed after 3 or 4 doses. Peak plasma levels were found at approximately 1¼ hours after administration. The clinical half-life is 4 hours and clearance is approximately 1530 ml/min following oral treatment. In the clinical evaluation of food effect, exposure (AUC values) increased 25% and C<sub>max</sub> increased 16% in the fed state, compared to fasting exposure values. Tapentadol is approximately 20% protein bound in human plasma, and the high volume of distribution (540 L) indicates wide tissue distribution. Oral tapentadol is subject to rapid and extensive metabolism of nearly all of the parent drug dose. The main pathway of tapentadol metabolism in humans is by glucuronidation by uridine diphosphate glucuronyl transferase to tapentadol-O-glucuronide. Additionally, there is minor metabolism of approximately 3% by CYP2C9 and CYP2C19, and approximately 2% by CYP2D6 to metabolites that subsequently undergo conjugation. Excretion is predominantly by the urinary route, with approximately 3% of the dose excreted as parent drug, 55% excreted as the glucuronide metabolite, and approximately 15% excreted as a sulfate conjugated metabolite. The minor role of the cytochrome P450 metabolism of tapentadol in humans suggests a low risk of pharmacokinetic interactions with drugs that undergo Phase I oxidative metabolism, which is supported by the results of the clinical and *in vitro* drug interaction studies.

The cross study means for clinical PK parameters after single dose immediate-release tapentadol are provided in the following table provided by the Sponsor, for comparison with the nonclinical pharmacokinetics results:

Table 32: A cross Study Mean Pharmacokinetic Parameters After a Single Dose of Tapentadol IR, Dose –Normalized to 100 mg Tapentadol (Dataset for cross-study comparison)

Parameter	n	Mean ± SD	%CV
t <sub>max</sub> , h	631	1.25 (0.50-6.27) <sup>*</sup>	
C <sub>max</sub> , ng/mL	631	90.1 ± 36.2	39
AUC <sub>0-∞</sub> , ng·h/mL	576	417 ± 143	34
t <sub>1/2</sub> , h	576	4.3 ± 0.8	16
CL <sub>R</sub> , mL/min	78	99.0 ± 37.3	38

Data expressed as mean ± SD, except for t<sub>max</sub> where median (range) is provided; n: number of observations.

\* more than 90% of observations was below or equal to 3 hrs.

Cross-reference: post-hoc analysis, data on file

The results of pharmacokinetic assessments in several multiple dose studies in human volunteers, at doses relevant to the proposed indication are presented in the following table from the Sponsor, for comparison of exposure in the nonclinical studies:

**Table 34: Pharmacokinetic Parameters for Tapentadol at Steady State Following Q6h Dosing in Healthy Subjects (Study HP5503/13, HP5503/25)**

Dose (mg)	C <sup>1</sup> <sub>min,ss</sub> (ng/mL)	C <sub>max,ss</sub> (ng/mL)	PK Parameters				Acc Ratio	Fluct. index
			C <sub>avg,ss</sub> (ng/mL)	AUC <sub>0-6</sub> (ng·h/mL)	AUC <sub>τ</sub> (ng·h/mL)			
<b>HP5503/13</b>								
75 (n=10)	41.0±24.1 [59]	76.2±31.0 [41]	54.1±23.8 [44]	229±90.3 [39]	324±143 [44]	1.44±0.41 [28]	68.9±19.4 [28]	
100 (n=10)	62.7±19.7 [32]	118±33.1 [28]	82.3±20.5 [25]	299±87.5 [29]	494±123 [25]	1.73±0.54 [31]	66.7±29.8 [48]	
125 (n=10)	67.6±28.2 [42]	138±64.6 [47]	94.4±33.2 [35]	413±132 [32]	567±199 [35]	1.50±0.51 [34]	71.9±23.0 [32]	
150 (n=9)	86.6±41.1 [47]	160±61.0 [38]	113±37.5 [33]	439±121 [28]	675±225 [33]	1.70±0.62 [36]	63.5±20.7 [33]	
175 (n=9)	92.9±33.3 [36]	162±42.2 [26]	123±27.6 [23]	446±126 [28]	737±166 [23]	1.70±0.30 [18]	57.8±21.4 [37]	
<b>HP5503/25</b>								
100 (n=55/58)	55.2±25.2 [46]	129±42.0 [42]	78.4±24.3 [31]	ND	465±146 [31]	ND	96	
150 (n=55/58)	93.3±50.7 [54]	197±89.1 [45]	122±48.0 [39]	ND	729±282 [39]	ND	86	

Data expressed as mean ± SD [%CV]; ND: not determined; n: number of observations; <sup>1</sup> for study HP5503/25 C<sub>avg,ss</sub> was used to calculate FI

The findings in the clinical study comparing tapentadol pharmacokinetic parameters when administered with and without food are presented below (table provided from the original NDA submission):

**Table 6: Tapentadol Pharmacokinetic Parameters After Single-Dose Administration of Tapentadol IR Tablet With and Without Food (Study HP5503/34)**

	100 mg IR Tablet Fed (PD2213) (n=35)	100 mg IR Tablet Fasted (PD2213) (n=34)	Fed/Fasted Ratio, % (90% CI) (n=34)
C <sub>max</sub> , ng/mL	83.4 ± 28.1 [33.7]	72.8 ± 30.8 [42.4]	115.99 (107.65 - 124.99)
AUC <sub>0-6</sub> , ng·h/mL	525 ± 154 [29.2]	421 ± 151 [36.0]	125.18 (119.24 - 131.42)
AUC <sub>∞</sub> , ng·h/mL	536 ± 157 [29.3]	429 ± 154 [35.9]	125.18 (119.26 - 131.40)
t <sub>max</sub> , h	3.00 (1.02 - 6.00)	1.50 (1.00 - 4.00)	
t <sub>1/2</sub> , h	3.9 ± 0.4 [10.6]	4.2 ± 0.4 [10.2]	

Data expressed as mean ± SD [%CV], except for t<sub>max</sub> median(range).

Cross-reference: Mod5.3.1.1\HP5503\34\Attach1.7; Mod5.3.1.1\HP5503\Table10.

### 3.4 TOXICOLOGY

#### 3.4.2 Single-dose toxicity

**Summary:** Single oral (PO) and intravenous (IV) dose toxicology was evaluated in mice and rats (Study TP1992). The LD50 values in the mice were 47 mg/kg IV and 300-350 mg/kg PO (approximately 17 times the MRHD for a single 100 mg oral dose in a 70 kg patient, on a body surface area basis), and in the rats were 46 mg/kg IV and >1000 mg/kg PO (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. The deaths were probably caused by 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 toxicity in rodents, were reversible in the surviving animals, with resolution several hours after dosing. The histopathology examination showed increased incidence of respiratory tract hyperemia (discoloration). The following review was conducted for the original IND submission (Kathleen Haberny, Ph.D.).

***Investigation of Acute Toxicity After Single Oral or Intravenous Administration in Rats and Mice: Study TP 1992/95***

**Amendment # 000, Vol #2**

**Conducting laboratory and location:** Grunenthal GMBH - Research Centre, D-52078 Aachen/FRG

**Date of study initiation:** October 1995

**GLP compliance:** Signed and present

**QA- Report** Yes ( x ) No ( )

**Methods:** Sprague Dawley rats ( [ ] weights 119-180 g ) males and 108-158 g females, n=3/sex/dose) and NMRI mice ( [ ] weights 20-29 g males and 19-25 g females, n=3/sex/dose) were each administered a single dose of BN 200 (Gruenthal BmbH, Aachen, Germany, Batch No. 07, purity 98% by TLC) in physiological saline ( [ ] Batch No. BK3173) by oral gavage (1 ml/100 g body weight [rat] or 0.5 ml/20 g body weight [mouse]) or

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intravenous injection (1 ml/100 g body weight [rat] or 0.5 ml/20 g body weight [mouse], 3 minutes/injection) into the tail vein. The oral doses were 450, 550 and 750 mg/kg in the rat and 250, 300 and 350 mg/kg in the mouse. The intravenous doses were 20, 40 and 50 mg/kg in the rat and 30, 40 and 50 mg/kg in the mouse.

The study parameters were mortality (daily for 14 days), clinical signs (up to 6 hours after administration and once daily for 14 days), body weight (daily for 14 days), and post-mortem investigations (macroscopic and microscopic). The tissues examined were brain, lungs, heart, spleen, liver, kidney, stomach, small intestine and large intestine. The tissues were prepared by fixation in 10% buffered formaldehyde solution fixation, imbedding in paraffin and staining, and Hematoxylin-Eosin (H&E) staining.

**Results:** The approximate LD<sub>50</sub> values for BN200 were 47 mg/kg IV and 311 mg/kg PO in the mouse, and 46 mg/kg IV and 1230 mg/kg PO in the rat. The LD<sub>Lo</sub> values for BN200 were 40 mg/kg IV and 300 mg/kg PO in the mouse and 40 mg/kg IV and 450 mg/kg PO in the rat. The deaths were preceded by convulsions, exophthalmos, Straub tail, increased respiratory frequency, hyperactivity, abnormal gait, increased sensitivity to touch and noise, cyanosis, dyspnea, and increased escape response in the mice, and by convulsions, hunched posture, lateral recumbency and tremor in the rats. There were no treatment-related effects on body weights. Macroscopic and microscopic post mortem investigation showed treatment-related discoloration in the respiratory tract (congestive hyperemia).

**Key Study Findings:**

- IV LD50 47 mg/kg in mice, 46 mg/kg in rats
- Oral LD50 300-350 mg/kg in mice, 1230 mg/kg in rats
- IV LDLo 40 mg/kg in mice and rats
- Oral LDLo 300 mg/kg in mice, 450 mg/kg in rats
- Deaths within 15 minutes of IV and several hours of oral dosing
- Clinical signs irritability, hyperactivity, cyanosis, exophthalmos, Straub tail, increased sensitivity to touch and noise, increased escape response, irregular respiration, lateral recumbency, tremor, and convulsions, of several hours duration
- Histopathological examination showed congestive hyperemia of respiratory tract

### 3.4.3 Repeat-dose toxicity

**Summary:** Repeated dose nonclinical toxicology studies on tapentadol were conducted in CD-1 and NMRI mice, Wistar and Sprague Dawley rats, Beagle dogs, and Cynomolgus monkeys. The routes used in the studies included oral by dietary admixture and gavage, and intravenous (IV) and subcutaneous (SC) injections. The main repeated dose toxicology studies examined oral toxicity, because this is the intended clinical route. The results of the non-pivotal studies conducted for dose-selection in the main toxicology evaluations are summarized below to provide additional insight on timing and severity of emerging toxicity in the longer duration studies, and potential reversibility or tolerance development to adverse tapentadol effects. Brief overviews of the non-pivotal toxicology studies are followed by reviews of the main toxicology study reports.

Mouse:

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Two-week oral toxicity was examined in male (M) and female (F) CD-1 mice, given tapentadol at doses of 0, 50, 125, and 250 mg/kg/day by dietary admixture, and 50, 100, and 200 mg/kg/day by gavage (GLP Study TP2470), to determine appropriate dose levels for investigation in subsequent toxicity studies in mice. The observations revealed no treatment-related mortality, clinical signs, changes in food consumption and body weights, and microscopic findings. 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 M mice, and increased relative spleen, thyroid, parathyroid, and liver weights in the F mice in the gavage study, and increased relative pituitary weights in F mice in the dietary administration study. The toxicokinetic evaluation showed higher systemic exposure (C<sub>max</sub> 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. The doses chosen for the pivotal 13-week evaluation by oral gavage toxicity in mice were 50, 100, and 200 mg/kg/day based on the results of this experiment.

Tapentadol treatment-related hepatotoxicity was found in a dietary administration study in M and F NMRI mice at higher doses than in the previous 2-week studies, of 0, 50, 150, 250, 500, and 1000 mg/kg/day for 13-weeks (Dose Range-Finding Study TP2379, Study 800526). Clinical biochemical analyses showed reduced albumin and A/G ratios in the M at ≥150 mg/kg/day and in the high dose (HD, 1000 mg/kg/day) 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 500-1000 mg/kg/day, compared to controls. Bilirubin was reduced in both the M and F mice at doses of ≥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. Additionally, 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. The liver findings and low bioavailability found in the toxicokinetic analyses, may have resulted from adaptive changes in the liver due to rapid and extensive metabolism by Phase II glucuronidation and treatment-related changes in hepatic protein synthesis, fat metabolism, and hemoglobin product breakdown.

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A second 13-week toxicity study was conducted in M and F CD-1 mice using oral gavage administration at doses of 0, 10, 30, 100, and 200 mg/kg/day (Study TP2496). The doses were chosen based on preliminary testing that showed intolerability of a 300 mg/kg/day PO dose in the mice. The observations showed decreased body weights and food consumption compared to controls in the M mice given 10 and 200 mg/kg/day and in the F mice at all dose levels. No treatment-related clinical signs were revealed, but there were findings in the serum chemistry and organ weight evaluations. Alanine aminotransferase was increased in the M and F at the highest 2 doses (statistically significant in the HD M), and aspartate aminotransferase was increased in a dose-related manner in all treated mice (statistically significant at 100 mg/kg/day in the F mice). Also, there was a treatment-related decrease in bilirubin, although not statistically significant, at ≥100 mg/kg/day. There was a slight increase in liver weights in all groups, which was statistically significant in the HD F. The increased liver weights

were without microscopic correlates in the histopathology examination. These results are consistent with the findings in the previous studies in mice, showing hepatic effects by tapentadol that may be related to increased liver metabolic activity at the higher doses. There was a dose-proportional increase in systemic exposure ( $C_{max}$  and  $AUC_{0-t}$ ), with no meaningful differences as a function of gender. Exposure was lower at the end of the treatment period, compared to that on Day 1, and so there was no evidence of accumulation.

The main target organ of toxicity was the liver in the oral studies in mice. Increased liver weights were observed at dietary doses of  $\geq 50$  mg/kg/day (lower doses not studied) after 2 weeks, and  $\geq 250$  mg/kg/day after 13 weeks, and at oral gavage doses of 200 mg/kg/day (lower doses not studied) for 2 weeks and  $\geq 10$  mg/kg/day for 13 weeks. While no other signs of hepatotoxicity were found in the 2-week dietary study at  $\geq 50$  mg/kg/day and gavage study at 200 mg/kg/day, 13 weeks of dosing resulted in increased liver enzymes (alanine aminotransferase at  $\geq 100$  (M) and 200 (F) mg/kg/day, and aspartate aminotransferase at  $\geq 10$  (M) and  $\geq 100$  (F) mg/kg/day oral gavage tapentadol. Dietary treatment for 13 weeks at higher doses of 500 (M) and 1000 (F) mg/kg/day, but not gavage treatment at up to 200 mg/kg/day, resulted in findings of hepatocellular hypertrophy with group cell necrosis. The lower doses associated with liver weight effects in the 13-week gavage study compared to the associated doses in the dietary study reflect higher exposures achieved by gavage dosing. Likewise, the lower doses associated with hepatotoxicity in the M mice compared to those in the F mice in the 13-week dietary study are consistent with the higher exposure found in the M. The results of these studies show a relationship of higher exposure and longer duration of treatment with increased severity of hepatotoxic effects.

#### **Rat:**

Seven-, 10- and 14-day dose range-finding studies were conducted in rats to characterize maximum tolerated doses for the pivotal studies. Preliminary palatability testing in M and F Wistar rats given tapentadol at doses of 0, 250 and 1000 mg/kg/day by dietary admixture (Study TP2367) for 7 days showed no treatment-related clinical signs or mortality, and toxicity was observed only at the highest dose level. The HD M and F rats showed reduced food consumption and body weights compared to the control rats, that was more severe in the M than in the F, particularly at the end of the dosing period. Food consumption and body weights recovered to a greater extent in the F during the 7-day dosing period, possibly 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. No treatment-related effects suggestive of hepatotoxicity were reported in this study. Tapentadol exposure and toxicity were higher in the M than in the F rats, as found in the 13-week dietary study in mice. This finding is not clear in light of the observation that tolerance to potential palatability effects may have developed more quickly in the F, as suggested by 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.

Continuous IV and SC tapentadol infusion toxicity was compared in a 14-day feasibility study in M and F Sprague-Dawley rats (Study TP2471). The IV groups received doses of 0, 15, 30, 45, 60, 90, and 120 mg/kg/day, and the SC groups received 30 and 45 mg/kg/day. The results showed treatment-related mortality, in 2 rats at 30 and 5 at 45 mg/kg SC (6 attributed to dorsal swellings), and in 4 rats administered 120 mg/kg IV (attributed to severe clinical signs). The treatment-related findings also included infusion site swellings by the SC route, and exophthalmus, subdued behavior, and reduced body weight gain in the IV treated rats. The toxicokinetic analyses showed similar exposure by both routes, and dose-related increases in exposure. Body weights and body weight gains were reduced in all treated rats in this study. The necropsy examinations showed enlarged and red lymph nodes at the higher IV doses, and subcutaneous dorsal cavity swelling with fluid exudate in most of the SC treated rats. The NOAEL was 90 mg/kg IV, but was not determined for the SC route. The results of this study supported the feasibility of using continuous intravenous tapentadol infusion in subsequent toxicology studies in rats.

IV tapentadol toxicity was examined in a 4-week study in rats (Study 602548, n=10/sex/dose) given once daily doses of 0, 3, 7, and 15 mg/kg/day (in physiological saline) at infusion rates of 0.3 ml/minute, for the original IND. The results showed deaths in 2 HD M (on Days 5 and 17) and in 1 HD F (on Day 19), and dose-related increases in the incidence and severity of fearfulness, sedation, excitability and hunched posture in the MD and HD rats. Food consumption was reduced in the HD F. Clinical biochemistry changes included increased alanine aminotransferase and alkaline phosphatase activity in the HD M, indicating increased hepatic activity. The target organs identified in the histopathology evaluation were the gastrointestinal tract, adrenal glands and liver. Red foci in the stomach and hyperemia in the lamina propria of the gastric mucosa were found in the HD M. Adrenal congestion was observed in the HD F rats. Single cell hepatocellular necrosis was also found in 1 HDM and 1 HDF. The rats that died showed congestive changes in the lungs, liver, thymus, mandibular lymph nodes and adrenals. The toxicokinetic assessments revealed a dose-proportional increase in exposure, with no differences in exposure after 4 weeks treatment compared to exposure on Day 1, and thus, no evidence of accumulation. The C<sub>max</sub> was observed at approximately 15 minutes after dosing. These results are consistent with the overall pattern of toxicity by tapentadol in the rats in previous studies, with similar treatment-related clinical signs, mortality rates, and hepatotoxicity effects.

M and F Wistar rats were treated for 10 consecutive days at tapentadol doses of 0, 100/400\*, and 300/600\* mg/kg/day (\*doses were increased on Day 6) in a range-finding oral gavage study (TP1969). There were no treatment-related effects on mortality, body weights, ophthalmoscopic examinations, organ weight measurements, and in the macroscopic examinations. Treatment-related clinical signs emerged during the dose escalations on Day 6 in the M and F, and included frightened appearance and sedation in both groups. The clinical signs are consistent with the known high dose opioid agonist effects as a class. Food consumption was reduced in the M at both doses. Toxicokinetic analyses were not conducted in this study; however, based on findings of increased

exposure in M than in F rodents in previous studies, higher exposure in the M in this study may explain the observed increases in treatment-related toxicity in the M.

Four-week oral gavage toxicity was also evaluated in M and F (n=10/sex/dose) Wistar rats for the original IND submission (Study TP1971 [ ] Study 602561), to determine the effects of tapentadol at once daily doses of 0, 300, 425, and 600 mg/kg/day (in physiological saline). Treatment-related deaths were observed in 1 MD M (Day 23), 1 MD F (Day 2), and 3 HD F (Days 9, 22, and 26), with microscopic findings of congestion in the lungs, liver, kidneys, lymph nodes and thymus in these animals. The surviving rats showed dose-related sedation, and decreased food consumption in the M in all groups and F at the MD and HD. Body weight gain was lower in the MD M and HD M. White blood cell counts were increased significantly in the MD F (+45%) and HD F (+66%), and lymphocytes were increased in the MD F (+68%) and HD F (+76%). There were multiple treatment-related changes in the clinical biochemistry parameters, predominantly at the HD, with increases in serum glucose (HD M), bilirubin (HD F), alanine aminotransferase (+29% in MD F and HD F), lactate dehydrogenase (+99% in HD F), and electrolytes calcium, phosphorus, and chloride in the MD F and HD F. Corneal opacities were observed in 1 HD M and 2 HD F, with persistent papillary membrane in 3 HD F. No treatment-related effects were observed in the necropsic examination. Brain was re-examined (Study TP2384, see under Special Toxicology Studies, Section 3.4.8 of this review) for histopathological evidence of morphological lesions in areas known to be sensitive to NMDA-receptor binding activity (CA1 region), indicative of neuronal injury and vacuolation, and found negative.

b(4)

Repeated oral dose toxicity was tested for a longer duration of 13-weeks in a non-GLP dose range finding study in M and F Wistar rats (n=10/sex/dose) given tapentadol by admixture in the diet at daily doses of 0, 250, 500, and 1000 mg/kg/day, in support of dose selection for a 2-year carcinogenicity study in Wistar rats. There were no deaths during the study. Observations for clinical signs found alopecia in the MD M and HD M and F, but no behavioral signs. Body weights were reduced in all treated groups; the reductions were statistically significant in the MD M (-13%) and in the HD M (-16%) and HD F (-8%). There was a dose-related reduction in body weight gain in the M at the LD (-14%), MD (-23%), and HD (-30%), and in the HD F (-15%).

Treatment-related hematology changes in the 13-week dietary toxicity study were observed in the F rats, and included increased hemoglobin (HD), HCT (HD), reticulocytes (LD, HD), HFR (retic. fluorescence ratio: high, all treated F groups), and MFR (retic. fluorescence ratio: middle, LD, HD), and decreased LFR (retic. fluorescence ratio: low, LD, HD). The serum chemistry analyses revealed treatment-related decreased triglycerides and ASAT in the MD (-14%) & HD (-18%) M, and bilirubin in the MD (-31%) & HD (-33%) F. Treatment-related effects in the liver were evident by increased GGT levels in the MD (+105%) & HD (+95%) M, LDH (+88%) in the HDF, and ALP in the MD (+84%) and HD (+116%) F. No ophthalmoscopy changes were found in any treated group.

The results of the necropsic examination in the 13-week dietary administration study showed significant treatment-related organ weight changes with increases in absolute brain (LD, MD, & HD M), relative (to BW) brain (all treated M & F groups), relative liver weights (all treated M groups, dose-related at +9% to +35%), kidney weights (all treated M & F), testes (MD, HD M), and ovaries (LD, HD F). Heart (HD M & F), thymus (all treated M groups), and spleen (HDM) weights were reduced at the end of the study. Dilation and discoloration in the duodenum was found in all HD F, and thymus size reduction in the treated males and hepatocellular hypertrophy in the MD (30% in M & F) and HD (100% in the M and 90% in the F) corresponded to the observed changes in the weights of those organs. Additionally, fatty change was increased in the rats at the MD (M) and HD (M & F) in a dose-related manner. Thus, the main target organ of toxicity in this study was the liver, as revealed in the shorter duration studies in rats. No activation of Kupffer cells, hepatocellular necrosis or liver fibrosis were found in this study. The presence of increased LDH levels in the HD female rats is suggestive of cellular damage during treatment. The toxicokinetic analyses demonstrated dose-related increases in systemic exposure to the parent drug and glucuronide metabolite, that was higher in the F than in the M at all doses for both components.

Evaluation of 13-week oral (gavage) toxicity was repeated in a second GLP study in Wistar rats (n=5/sex/dose) given twice daily (5-h interval) tapentadol doses of 0, 30, 100, and 200 mg/kg (0, 60, 200, and 400 mg/kg/day in 0.9% physiological saline) (Study TP2645). There were no treatment-related deaths. Tapentadol administration was associated with dose-related increased incidence, severity, and duration of exophthalmus in all treated groups, and mouthing bedding material, soft feces and hunched posture at the highest doses. The clinical signs found in this 13-week oral gavage study are noteworthy when compared to the absence of clinical signs in the previous 13-week dietary study in rats at considerably higher doses, and reflect the higher systemic tapentadol exposure (AUC<sub>0-24</sub>, Wk 13) in this study, of up to 4828 ng.h/ml in the M and 11829 ng.h/ml in the F at 400 mg/kg/day when given by gavage, compared to mean (all weeks combined) exposure (AUC) of 1891 ng.h/ml in the M and 2373 ng.h/ml in the F administered 1000 mg/kg/day by dietary admixture. Considerable exposure to the tapentadol O-glucuronide metabolite was demonstrated in the TK analyses in this study, but was not measured in the previous 13-week dietary study. Body weight gains were reduced in the M at all doses, with increased severity of up to -12% at the MD and HD.

Evaluation of hematology parameters in the 13-week gavage study in rats showed increased white blood cell counts, lymphocytes, and large unstained cell counts in the HD F. There were slight treatment-related increases in aspartate aminotransferase in the HD M (+23%) and F (+22%), and alanine aminotransferase in the HD M (+35%) and F (17%). Tapentadol treatment was associated with increased serum sodium and chloride in the treated M rats, and potassium, protein and globulin in the F, with decreased albumin and albumin/globulin ratio in the HD F. All treated M and F rats showed increased absolute and relative liver weights, which was statistically significant in the MD F (+14.3%). The necropsy examination revealed isolated dark red foci in the stomach fundus mucosa in 2 HD M, and minimal to mild hepatocellular centrilobular hypertrophy in 1 each in the LD M, MD M and MD F, and in all HD M and 3/5 HD F.

A chronic (26-week) toxicity study was conducted in M and F Wistar rats (initially n=10/sex/dose) given once daily oral gavage doses of 0, 75, 150, 300\*, and 450\* mg/kg/day (Study TP2397). The 450 mg/kg/day group was terminated after 13 weeks due to excessive mortality, and an additional 10 rats/sex initiated dosing of 300 mg/kg/day or vehicle to provide for a HD recovery evaluation, with matching control group. Also, a 75 mg/kg/day dose level was added to allow for a 3-dose study comparison (0, 75, 150, and 300 mg/kg/day). Satellite groups were evaluated for toxicokinetic parameters for tapentadol and the metabolite, tapentadol O-glucuronide (n=6/sex/dose). Additional evaluations were conducted on 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 mortality (found dead or sacrificed *in extremis*) during the dosing period, in 1/20, 0/20, 15/30, and 17/30 females at 75, 150, 300, and 450 mg/kg/day, respectively, and 0/20, 3/20, 11/30, and 16/30 males at 75, 150, 300, and 450 mg/kg/day, respectively vs. 0/30 each in the control M and F. The deaths were probably a result of respiratory depression, a known class effect of mu-opioid receptor agonist agents. The clinical signs were similar in the animals that died prematurely and those that survived to the end of the dosing periods. Treatment-related clinical signs were also characteristic of mu-opioid agonist effects, and included dose-related increases in the incidence and severity of excitability, recumbency, hunched posture, labored respiration, and general poor condition during the first 13 weeks of the dosing period at doses of  $\geq 150$  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 on in the study. There were no treatment-related clinical signs. Body weight gains were higher in the M and F compared to controls, during the recovery period.

The clinical laboratory tests in the 26-week oral toxicity study in rats found increased leukocyte counts, due to increased lymphocytes and segmented neutrophils in the F at 300 and 450 mg/kg/day, decreased PT and APTT in the M at 450 mg/kg/day, and increased fibrinogen in the M and F at 450 mg/kg/day, in Week 13. Additionally, at the end of the dosing period, there was a significant decrease in RBCs in the treated males (75-300 mg/kg/day). Liver enzymes ASAT (+660%) and ALAT (+149%) were increased in the M at the highest dose of 450 mg/kg/day in Week 13. There was a dose-related increase in ALP at 150 and 300 mg/kg/day in Weeks 13 and 26. Both M and F showed increased LDH at 300 and 450 mg/kg/day (Week 13 and end of study in the 300 mg/kg/day groups), and albumin in all treatment groups at the end of the dosing period.

In the necropsic examinations at the end of the dosing period, liver weights were increased in the M at 150 (+11%), 300 (+23%) and 450 (+45%) early termination group) mg/kg/day, and in the F at 300 (+15%) and 450 (+31%, early termination group) mg/kg/day compared to controls. Treatment-related microscopic findings were noted in the liver only, with dose-related increases in the incidence of centrilobular or diffuse hepatocellular hypertrophy in all M and F groups given  $\geq 150$  mg/kg/day tapentadol (up to 6/20 M and 2/20 F at 450 mg/kg/day), accompanied by fatty change in the groups that

received  $\geq 300$  mg/kg/day, perhaps reflecting increased transport of fatty acids from adipose tissue and use of lipids for energy due to decreased food consumption and body weight decreases. No evidence of necrosis was found in the liver at any dose level, and there were no observations of hypertrophy in the liver after the 8-week treatment-free recovery period, suggesting reversibility upon withdrawal of drug treatment. The microscopic changes in liver may result, at least in part to adaptations related to increased liver enzyme activity, supported by clinical laboratory findings of increased serum liver enzymes and increased glucuronyl transferase activity in the examination of liver microsomes. 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 than in the M rats. Also, there was increased exposure in Week 26 compared to exposure on Day 1, in contrast to the findings in previous shorter-duration studies suggestive of the absence of accumulation effect. Plasma exposure at the NOAEL in this study (75 mg/kg/day) represented approximately 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.

The results of the repeated dose studies in rats pointed to target organ toxicity by tapentadol primarily in the central nervous system (CNS) and liver in this species. These studies showed greater treatment-related toxicity by the subcutaneous (SC) than by the intravenous (IV) routes, suggested by the observations of treatment-related mortality, clinical signs and decreased body weights at lower SC than IV doses, although systemic exposures (AUC) were similar by these routes. Tapentadol toxicity also emerged at lower IV and SC than oral (dietary and gavage) doses, as would be expected due to rapid metabolic clearance by the oral route. In the oral toxicity studies in rats, 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. Treatment-related changes in liver function and hepatotoxicity were indicated by increased liver enzymes (e.g., ALAT, ASAT, ALP, etc.), liver weights and microscopic findings of hepatocellular hypertrophy, with increasing severity and at lower doses in the longer duration studies. Treatment-related fatty changes in liver were revealed after longer treatment durations of 13 and 26 weeks by dietary and gavage administration. Additionally, liver necrosis was observed in the 4-week IV study in single male and female rats at the highest dose administered only. However, no Kupffer cell activation was found in the rats administered tapentadol by any route or duration. The treatment-related effects in liver were reversible during the treatment-free recovery periods at the doses, routes (except for necrosis in the IV study) and durations evaluated.

Dog:

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Tapentadol toxicity was studied in Beagle dogs for 10 and 28 days by the intravenous (IV) route, for 9 days and 13-weeks by the subcutaneous (SC) route, and for 2, 13, and 52 weeks by oral (PO, gavage) administration.

In the 10-day dose-range finding IV toxicity study (TP1967, [ ] Study 602460), M and F Beagle dogs received tapentadol via 5-minute injection in the cephalic vein at doses from 1 to 7.5 mg/kg/day for 4 consecutive days, and were sacrificed 12 days after the last dose. A second group of dogs received 7.5 mg/kg/day tapentadol for 10 consecutive days and were sacrificed 24 hours after the last dose. The observations included clinical pathology and electrocardiogram parameters. There were transient treatment-related salivation (M), restlessness and whimpering (F) at doses of  $\geq 5$  mg/kg/day, and rhinorrhea, panting, labored breathing, decreased activity and uncoordinated movements at 7.5 mg/kg/day, with lateral recumbency in the HD M and limb buckling in the HD F. The clinical signs were evident during or immediately after injection, and lasted approximately 1-3 hours. There were slight decreases in food consumption in the M and F at 5 and 7.5 mg/kg/day, and slight body weight loss in the HD F after treatment Day 2. Moderate body weight loss was found in most animals given 7.5 mg/kg/day at the end of the 10-day treatment period. There were no treatment-related effects on ECG and hematology parameters. The clinical biochemistry examinations showed slight increases in glutamate dehydrogenase in 1 M, total lipids, cholesterol, triglyceride and phospholipid in 1F, and iron concentration and protein in 2 F at 7.5 mg/kg/day at the end of the study. Histopathology examinations were not performed in this study; the macroscopic examination showed red foci at the injection sites at the end of the 10-day dosing period at 7.5 mg/kg/day. These results were used to support dose selection for the 4-week IV toxicology study in dogs.

b(4)

IV tapentadol toxicity was studied in Beagle dogs (n=3/sex/dose) given consecutive daily 5-minute injections of 0, 1, 3, and 7.5 mg/kg/day for 4 weeks (GLP Study TP1968, [ ] Study 602526). The clinical signs, noted at doses of  $\geq 3$  mg/kg/day IV, were consistent with those observed in the 10-day study, and included dose-related increases in the incidence and severity of excessive salivation, decreased activity, hindleg bucking uncoordinated movements, vomiting, urination, ventral recumbency and rhinorrhea beginning during or immediately after injection and lasting for 1-2 hours. There was a lower incidence of tachypnea, panting, retching, and defecation. Food consumption was reduced in the F ( $\geq 3$  mg/kg/day) and M (7.5 mg/kg/day), with body weight loss in the HD F. No biologically relevant treatment-related effects were observed in the ophthalmoscopic examinations, electrocardiograms (including QT interval, recordings conducted immediately after and 1 hour after dosing at baseline, Day 2, and at 4 weeks), clinical laboratory assessments, organ weight measurements, and macroscopic and microscopic examinations, including inspection of the injection sites, compared to baseline and control values. Additional investigation of microsomal drug metabolizing enzymes in livers from the treated dogs showed no inhibition or induction of P450 enzymes, and no effects on phase II aminophenol glucuronyl transferase activity. The toxicokinetic analyses confirmed exposure to the test article in this study.

b(4)

A preliminary, non-GLP subcutaneous (SC) maximum tolerated dose (MTD) tapentadol toxicity study was conducted in M and F Beagle dogs, for a 7-9-day period (Study TP2564, [ ] study 852677). The first group of dogs received twice daily SC injections (in 0.9% physiological saline) at 10 mg/kg b.i.d. (20 mg/kg/day) for 7 days and were sacrificed 17 days after the last dose. The second group was administered 7.5 mg/kg b.i.d. (15 mg/kg/day) for 9 days and was sacrificed 6 days after the end of the dosing period. The parameters monitored in this study were viability, clinical signs, body weights, food consumption, and inspection of the injection sites after sacrifice. The findings were similar to those in the previous intravenous studies in dogs, and included CNS behavioral signs known to be associated with opioid receptor agonist activity. The observations were dose-related increases in the incidence and severity of decreased activity, recumbency, tremor, salivation, somnolence, forelimb and hindlimb buckling, uncoordinated movements and occasional whimpering. The M dogs also showed vomiting, and occasional pale or loose feces and fecal mucus. Injection site swellings were noted in several dogs. Food intake and body weights (-0.4 to -0.8 kg) were reduced in the first dosing week in both treatment periods (10 and 7.5 mg/kg/day), and resolved with increasing the duration of the daily food availability periods over the rest of the study. The reversibility of reduced food consumption and body weights may also be related to tolerance to the drug effects, although CNS signs persisted to the end of dosing with decreased severity.

b(4)

Two 3-month SC tapentadol toxicity studies were conducted in Beagle dogs. The first non-GLP pilot study (TP2455) in M dogs used twice daily (7-hour intervals) tapentadol injections at doses of 0, 10 (Day 1 and 2), 20 (Days 3-7), and 40 (Days 8-13) mg/kg b.i.d. (20, 40 and 80 mg/kg/day) in a dose-escalation phase, followed by a treatment phase at 20 mg/kg b.i.d. (40 mg/kg/day) on Days 14-91. The initial treatment-phase dose of 40 mg/kg b.i.d. (80 mg/kg/day) was found to be intolerable, and was subsequently reduced to 20 mg/kg b.i.d. from Day 16 to the end of the 91-day study. Standard toxicology parameters, except histopathology were assessed. There were no treatment-related deaths, and the clinical signs were similar to those observed in the previous IV toxicity studies in dogs. In the dose-escalation phase, there were dose-related increases in the incidence, severity and duration of restlessness, fearfulness, vocalization, drowsiness, unsteady gait, hindlimb weakness, ventral recumbency, spontaneous urination and defecation, vomiting and salivation, with increased respiratory frequency and forced respiration in several animals. Higher doses revealed defense behavior, mouth licking and stretched out legs, tremor, twitches, and convulsions at 40 and 80 mg/kg/day (1 dog on Day 4, 9, and 13, during dose escalation). The clinical signs described above were also observed during the treatment phase, but were progressively reduced in severity over the course of the study, suggesting partial development of tolerance to the CNS effects of tapentadol in this species. By Day 84, only sporadic hind-leg weakness, vocalization, restlessness, drowsiness and increased respiratory rate were observed. Clinical observations and local tissue inspections indicated that the dogs scratched the injection sites throughout the study. Body weights were reduced during the dose-escalation (Days 1-13, -0.1 to +2.3 kg); during the treatment phase, body weights gradually recovered from Day 14 to the end of the study in most dogs, again indicating the development of tolerance to the body weight effects. Slight, reversible reductions in body temperature

and heart rates were observed at the 40 mg/kg/day dose. There were no abnormal findings in the clinical laboratory tests. The necroscopy examination showed only subcutaneous ecchymoses at the injection sites, with hemorrhages, edema and gelatinous consistency of underlying tissues. Toxicokinetic analyses showed consistent exposure to the test article and main metabolite tapentadol-O-glucuronide, with dose-proportional increases in concentrations. The peak plasma concentrations were observed at 0.4-0.5 h. Exposure to the glucuronide metabolite was higher and the half-life was longer (approximately 4h) than for the parent drug (1.77 h). Exposure (AUC), was higher in the dog that convulsed than in the other dogs, but there were no differences in peak plasma concentration (C<sub>max</sub>). The doses tested in this study appeared to exceed the MTD, and failed to expose LOAEL and NOAEL values for SC tapentadol in the dogs.

A second, 3-month, GLP toxicity study (Study TP2559, [ ] Study 852678) was performed in M and F Beagle dogs (n=4/sex/dose, 2/sex/dose 4-week recovery animals) to determine the LOAEL, NOAEL, and MTD levels by the SC route in dogs, for possible suitability of SC dosing in the design of a chronic study in this species. The dogs were administered tapentadol at daily doses of 0, 2, 4, and 8 mg/kg SC b.i.d. (0, 4, 8, and 16 mg/kg/day, in 0.9% physiological saline, 5-hour inter-dose interval) in the scapula region or sub-lumbar fossa on the flank, with rotation of injection sites. Standard toxicology parameters and electrocardiography were evaluated at baseline, and in Weeks 1, 4, and 13. The results showed death in one HD F on Day 17, for which a cause was not determined, and therefore a possible relationship to treatment could not be excluded. CNS-related clinical signs were observed at all doses and included dose-related increases in incidence and severity of decreased activity, recumbency, salivation, whimpering, vomiting and tremor, beginning around 30 minutes after dosing, and lasting for up to 5 hours. No convulsions, which were observed at the 40 and 80 mg/kg/day doses in the previous study, were seen in this study. It is unknown if the treatment-related tremor in this study was related to seizure activity. The severity of the clinical signs was higher after the first, than after the second daily dose, indicating possible short-term tolerance. Food consumption was reduced at 8 (-18% to -29%) and 16 (-19% to -32%) mg/kg/day, with corresponding decreases in body weights of -0.2 to -0.5 kg during the first dosing week. Extension of the feeding period had little effect on food consumption, which remained below the levels of control dogs by 20% to 27%, nor on body weights at the high dose, although slight recovery was observed suggesting development of tolerance to drug effects on these parameters.

b(4)

The clinical laboratory assessments in the second 3-month SC toxicity study in dogs showed no effects on hematology parameters, but there were treatment-related increased serum protein and albumin levels, and increased relative urine density in Week 4 at the high dose, that persisted to the end of the recovery period in the high dose female dogs. These effects may have been due to slight dehydration that resulted from treatment-related vomiting in the dogs affected. There were no findings in the ophthalmologic examination. The results of the electrocardiogram measurements showed prolongation of the absolute and corrected QT intervals at 30 minutes after dosing compared to baseline values in the dogs given 8 and 16 mg/kg/day SC tapentadol during Week 1. There were trends toward increased absolute QT values in Weeks 4 and 13, although these did not

reach statistical significance, and there were no differences from controls when corrected for heart rate (Van de Water's and Fridericia's).

The necropsy showed no treatment-related changes in organ weights. Macroscopic examination at necropsy showed drug treatment-related hemorrhage in the mesentery, dark red discolorations in the lungs, stomach, small and large intestines, uterus and vagina. In the surviving dogs, there were treatment-related, but not dose-related increases in incidence and severity of dark red discoloration in the injection sites (6, 7, and 6 dogs at 4, 8, and 16 mg/kg/day). The injection site inspections found hemorrhages, inflammatory infiltrates that were both acute and subacute, fibrosis, phlebitis and thrombophlebitis. The local toxicity was only partially reversible during the 4-week recovery period; fibrosis and chronic focal or multifocal perivasculitis were also found in animals given the highest tapentadol dose. The toxicokinetics assessments showed dose-related increases in systemic exposure to the parent drug and the main metabolite tapentadol O-glucuronide, with slightly increased exposure (AUC) after 13 weeks compared to the exposure on Day 1 at the mid-dose and high-dose. There were no consistent differences in exposure to the parent drug between the M and F dogs. A NOAEL was not established in this study, based on the clinical signs, the clinical laboratory findings probably related to vomiting-induced dehydration in all groups, and to injection site toxicity. There was evidence of partial tolerance development to the adverse treatment-related effects, and reversibility of most signs during the recovery period. However, these results suggested that the SC route is probably not suitable for chronic tapentadol toxicity testing in dogs.

Two 2-week, oral (gavage) dose range-finding toxicity studies were performed in Beagle dogs. In the first experiment (Study TP1993), M and F dogs (n=2/sex/dose) were administered tapentadol (referred to as BN 200 in this study, in physiological saline) once daily by oral gavage (1 ml/kg) at doses of 0, 50 (LD) and 150 (HD) mg/kg/day. The measures included electrocardiographic examinations at baseline and at the end of the dosing period, and standard necropsy with organ weights and macroscopic and microscopic examinations. There were no deaths during the study, but convulsions were observed in 1 LD M (on Day 9), and in 1 HD M (Day 3) and 1 HD F (Days 7 and 10). Additionally, vomiting occurred after dosing in one LD F on Day 9, and in most of the HD dogs on some days after dosing during the first week and on 3 days during the second treatment week, suggesting partial development of tolerance to tapentadol emetic effect. Irregular respiration was observed in 1 LD M and in all HD dogs after dosing, periodically throughout the study. The treated dogs generally showed abdominal and lateral recumbency during the dosing period. Food consumption and body weights were reduced compared to controls at the HD throughout dosing. There were no findings in the hearing test using reaction to high frequency tone, and in the ECG and urinalysis assessments. Hematology showed treatment-related decreased hemoglobin and erythrocytes, but not exceeding historical control range, and there were no findings in the evaluation of clinical chemistry. The macroscopic examinations were also negative. The results of the microscopic examinations showed activation of the enteric lymphatic system (Peyer's patches) in the small and large intestines, and activated lymphoid follicles in the gastric mucosa and proximal small intestine at both doses, suggesting

reactive hyperplasia in the germinal centers indicative of gastrointestinal immune response. Plasma test article sampling showed dose-related increases in plasma concentrations.

A second 2-week 2-Phase dose range-finding oral (gavage) study was conducted in Beagle dogs (Study TP2406, [ ] Study 800166, n=2/sex/dose with replacement of 1 HDF following a premature death). The dogs in Phase I were given tapentadol at sequentially increasing doses of 10-350 mg/kg/day (in tap water) for 13 consecutive days, with dose increases at 1-3 day intervals. In Phase II, the dogs were administered 320 mg/kg/day initially, followed by dose reduction (deescalating dose paradigm) to 280 mg/kg/day for 1 administration in a replacement F after death in 1 F at 320 mg/kg/day, and then reduction to a maintenance dose of 200 mg/kg/day for 14 consecutive days in all dogs. Histopathology evaluation was not conducted. The results of this study showed one death (F at 320 mg/kg/day). Treatment-related clinical signs noted at doses of 80 mg/kg/day and above were dose-related decreased activity, recumbency, and salivation. Whimpering ( $\geq 220$  mg/kg/day), somnolence ( $\geq 280$  mg/kg/day), dyspnea, tachypnea or panting ( $\geq 80$  mg/kg/day), and tremors ( $\geq 160$  mg/kg/day) were observed following dosing. The clinical signs were evident at approximately 15 minutes after dosing and persisted for up to 8 hours after dosing. Convulsions were seen in the F at the highest dose of 350 mg/kg/day in Phase I and at 320 mg/kg/day and again at 280 mg/kg/day, with occasional convulsions at the 200 mg/kg/day dose in Phase II. The ECG assessments revealed no test article-related effects.

b(4)

Food consumption was reduced in all treated dogs at 80 mg/kg/day and above in the dose-escalation phase of the study, and at all doses throughout the treatment period in the second, dose-de-escalation and maintenance dose phase (320-200 mg/kg/day). Body weight loss was observed in all dogs during dose de-escalation at 320 and 280 mg/kg/day, and in 1 M at 200 mg/kg/day during the remainder of the 2-week maintenance dose period. In contrast to the results of the first 2-week oral toxicity study, liver weights were increased in all tapentadol-treated dogs at the end of this study, but were without macroscopic and clinical laboratory correlates. Based on the results of this study, the maximum tolerated dose (MTD) was considered to be in the range of 180-200 mg/kg/day by oral gavage, and the dose of 180 mg/kg/day was selected for further investigation in a 13-week oral gavage toxicity study in dogs.

Evaluation of tapentadol toxicity by the oral gavage route for 13 weeks was carried out in Beagle dogs (n=4/sex/dose) given daily doses of 0, 10, 35, and 80 mg/kg/day (in tap water, GLP Study TP2415, [ ] Study 813655). The study included investigation of reversibility in a 4-week recovery phase after the end of the 13-week treatment period in control and high dose auxiliary groups (n=2/sex/group). The high dose was originally 120 mg/kg/day, but was reduced to 80 mg/kg/day on Day 22 through the end of the dosing period due to intolerability, indicated by severe central nervous system (CNS) toxicity. The dogs at the 120 mg/kg/day dose showed extreme decreased activity, recumbency, whimpering, fearfulness, panting, tachypnea, apathy and convulsions (total of 2M and 2F at this dose) with paddling movements, twitching and tremors in 4 dogs. One M dog that showed severe clinical signs including convulsions was found dead on

b(4)

Day 21, and another M that convulsed during treatment at the 120 mg/kg/day dose was sacrificed in extremis on Day 23, after dose reduction to 80 mg/kg/day. The convulsions began 30-60 minutes after tapentadol administration, except for a convulsion immediately after dosing in one of the F. The clinical signs observed at 120 mg/kg/day were also observed at the 80 and 35 mg/kg/day dose levels. Most signs were of lower incidence and severity at the mid-dose and adjusted high-dose, except that decreased activity, salivation and recumbency were similar at 120 and 80 mg/kg/day. Additionally, there was a dose-related increase in emesis and mucus in the feces in the treated groups. The clinical signs were observed beginning 15-30 minutes after dosing, and lasted for up to 5 hours. Food consumption was reduced in at 120/80 mg/kg/day in the males and at 35 and 120/80 mg/kg/day in the females, predominantly during the first several weeks of dosing. Body weight gain was reduced 0.2-1.2 kg in most dogs given 120 mg/kg/day in Weeks 1-2, at slightly reduced the end of the study in the females given 80 mg/kg/day for the remainder of the treatment period. There were no treatment-related differences from controls in the clinical signs and body weights after the 4-week recovery period.

The results of the ECG measurements in the 13-week oral toxicity study in dogs showed QT prolongation, with similar results after correction for heart rate (QTc), at 35 mg/kg/day in Week 13, and in Weeks 1 (120 mg/kg/day) and 13 (80 mg/kg/day) in the high dose groups. There were no treatment-related findings in the ophthalmologic examination and organ weight measurements. Treatment-related decreased gamma glutamyltransferase and increased serum sodium were found. Macroscopic and microscopic examinations during necropsy showed thymic atrophy and prostate gland inflammation at 35 and 120/80 mg/kg/day. There was a possible treatment relationship to increased adrenal cortical hypertrophy in the males given 35 and 120/80 mg/kg/day. Evaluation of hepatic microsomal enzyme activity in liver samples recovered during the necropsy in the high-dose and control dogs showed a statistically significant treatment-related induction of aminopyrine N-demethylase activity in the M and F dogs, and inhibition of glucuronyltransferase activity in the M dogs. The NOAEL in this study was 10 mg/kg/day, representing systemic exposure of approximately 0.04 times the clinical exposure at the MRHD of 600 mg/kg/day on an AUC basis and on the basis of peak plasma exposure (C<sub>max</sub>), possibly more relevant to the CNS and cardiovascular effects (convulsions, QT interval prolongation) noted during the study.

The results of a chronic, 52-week oral toxicity study in male (M) and female (F) Beagle dogs (Study TP2441) were submitted to this application in support of marketing safety, although chronic nonclinical studies are not required for an acute indication. The dogs (n=4/sex/dose) were administered 0 (tap water vehicle), 10 (LD), 30 (MD), and 80 (HD) mg/kg/day tapentadol once daily by oral gavage. Satellite groups (2/sex/dose) were given vehicle control and 80 mg/kg/day test article, for assessment of reversibility after a 4-week recovery period at the conclusion of the 52-week dosing period. Toxicokinetics were assessed in all main treatment animals. In addition to the standard necroscopic evaluations, liver samples were collected from all dogs for analysis of enzyme activity. The target organs of toxicity were the central nervous system (CNS) and cardiovascular system. One HD F was euthanized *in extremis* on dosing Day 12 due to convulsions on Days 4 and 12, which occurred within 30 minutes after dosing. Dosing was suspended in

this dog on Days 5-7, and then resumed on Day 8. No necroscopic abnormalities were found in this dog. Convulsions were also seen in another HD F on Days 178, 308, 316, 344, 345, and 358, starting at 20-30 minutes after dosing and lasting for up to 5 hours. The convulsions were associated with paddling movements, muscle twitching, recumbency, tremor, labored breathing, and decreased activity, and were reversed with naloxone. No convulsions were observed in the treated dogs during the recovery period. There were also treatment-related clinical signs of salivation at all doses, decreased activity, recumbency, vomiting, tremor, and occasional whimpering at the MD and HD, and fearfulness at the HD, beginning 15-30 minutes after drug administration, and lasting for up to 5 hours. Reduced food consumption (-11% to -23% in the M and -9% to -11% in the F) and body weights (-0.2 to -0.8 kg) were observed at the HD during the first several weeks of dosing.

The results of the ECG in the 52-week study in dogs revealed slight but statistically significant prolongation of the QT and corrected QT (Van de Water's and Fridericia's corrections) intervals in the 1 hour post-dose recordings in most of the HD dogs compared to baseline and control values throughout the treatment period. There were no other treatment-related ECG effects during the dosing period, and no ECG findings during the recovery period. Slight, minimal treatment-related decreases in partial thromboplastin time (PTT) values were found in the HD dogs, which was not reversible during recovery after termination of dosing. Plasma sodium was increased at the HD compared to controls but not to baseline values, and there were no related changes in other electrolytes. The necropsy results showed no treatment-related effects on organ weights, and macroscopic and microscopic findings. Minimal to slight focal gliosis with perivascular mononuclear cell infiltration in the medulla oblongata and/or pons in 2 MD M and 1 MD F, and in 1 HD F, found with no relationship to observed seizures, are considered to be spontaneous in agreement with the Sponsor. In the liver enzyme activity analysis, there were no tapentadol effects on cytochrome P450 content; however, there were dose-related increases in O-deethylase activity in the F, and dose-related increases in N-demethylase activity in the M and F. 2-aminophenol glucuronyltransferase activity was decreased in the M and F dogs. The NOAEL in the 52-week toxicity study in dogs was 10 mg/kg/day, representing systemic exposure to the parent drug that is approximately 0.05 times the exposure at the clinical maximum recommended human dose (MRHD) of 600 mg/day in a 70 kg patient, on an AUC basis. Peak plasma tapentadol concentrations (C<sub>max</sub>) at the NOAEL, which may be relevant to the tapentadol CNS (particularly convulsions) and cardiovascular (QT prolongation) observations, represented approximately 0.06 times the peak plasma concentration at the MRHD.

The main target organs of tapentadol toxicity in the repeated dose studies in dogs were the central nervous system (CNS), cardiovascular system (CV), gastrointestinal system (GI) and local toxicity in the intravenous and subcutaneous toxicity studies. The CNS clinical signs observed across dose ranges, routes and durations, included salivation, restlessness, recumbency, decreased activity, rhinorrhea, panting, labored breathing, and tachypnea. In the 3-month b.i.d. SC study in the dogs, the signs were more severe after the first than after the second daily dose, suggesting development of short term tolerance,

a known characteristic of mu-opioid receptor agonist treatment. Also, the severity of the clinical signs decreased with increasing duration of treatment within several of the studies, indicating longer-term tolerance development to the opioid-induced behavioral effects. Most notable of the clinical signs were opioid-characteristic (in dogs) convulsions, observed in males and/or females treated by SC injection for 3 months at doses of 40 and 80 mg/kg/day (NOEL = 20 mg/kg/day SC), and by oral gavage at 50 and 150 mg/kg/day (NOEL = not determined) and 200-350 mg/kg/day (NOEL = 160 mg/kg single dose during dose escalation phase) for 2 weeks, at 120 mg/kg/day for 13 weeks (NOEL = 80 mg/kg/day), and at 80 mg/kg/day for 52 weeks (NOEL = 30 mg/kg/day). No convulsions were observed in IV treated dogs at up to 7.5 mg/kg/day for 4 weeks duration. The convulsions were accompanied by paddling movements, tremors, and twitching. A possible relationship to seizure activity of the observed tremors in several of the studies in dogs without reports of convulsions was not further investigated. There was no evidence of tolerance development to tapentadol convulsant effect in the dogs. Although most of the dogs that convulsed were either sacrificed in extremis or received dose reductions following the seizures, a female given 80 mg/kg/day by oral gavage for 52 weeks presented with convulsions on multiple days up to day 358 of dosing.

Tapentadol-related cardiovascular toxicity was manifest by QT prolongation in the electrocardiogram measurements (ECG) in the dog. QT prolongation was found at 8 and 16 mg/kg/day SC for 3 months (NOEL = 4 mg/kg/day) particularly during the first week of treatment, and at 35 and 80 mg/kg/day (Week 13) and 120 mg/kg/day (Week 1) in the 13-week gavage study (NOEL = 10 mg/kg/day) and 80 mg/kg/day in the 52-week oral gavage study (NOEL 30 mg/kg/day). No other ECG effects were found in these studies. QT prolongation may be predicted due norepinephrine reuptake inhibition by tapentadol.

Treatment-related GI toxicity was detected in dogs given tapentadol using several routes. Dogs administered SC tapentadol injections in the 3-month study showed reversible hemorrhage in the mesentery, and dark red discolorations in the stomach, small and large intestines at all doses from 4-16 mg/kg/day. The results of several oral gavage studies found enteric lymphatic system activation (Peyers patches) in the intestines and activated lymphoid follicles in the gastric mucosa and small intestines in the 2-week oral gavage study at both doses tested (50 and 150 mg/kg/day), that might reflect GI immune response, according to the examining pathologist. Local tissue toxicity was found to a greater degree of severity in dogs given SC than IV injections. Red foci were found at the highest dose of 7.5 mg/kg/day in the 4-week IV study, and injection site hemorrhage and fibrosis, with scratching of the site by the dogs was observed throughout the dosing periods in both 3-month SC studies at all doses from 20-80 mg/kg/day in one study and from 4-16 mg/kg/day in the second study.

#### Monkey:

A 2-week pilot study was conducted in *Cynomolgus* monkeys (n=3 males, Study TP2316), to examine the toxicokinetics and toxicity of tapentadol (in 0.9% physiological saline) given by repeated IV (5 mg/kg/day, 1 ml/kg, 2 minute infusion) and oral (15

agonists and agents that inhibit norepinephrine reuptake. The main treatment-related target organs of toxicity, including the liver in rat, CNS in rat and dog, and cardiovascular system (QT prolongation) in dog, underscore the importance of screening and monitoring patients during treatment. Most of the toxic effects associated with tapentadol administration, such as CNS depression and hepatic changes in the nonclinical studies were reversible after withdrawal of treatment. These adverse effects are also monitorable for the most part, such as by periodic clinical laboratory assessments during clinical treatment. However, severe CNS toxicity (particularly convulsions), and possible adverse cardiovascular effects (such as arrhythmias) may not be easily monitorable in an outpatient setting. Therefore,

discussed with the patients during treatment.

b(5)

**4 Week Intravenous Toxicity Study with BN 200 in the Rat: Study 602548**

Amendment # 000, Vol #6, Page #1

Conducting laboratory and location: [

[

]

b(4)

Date of study initiation: August 24, 1995

GLP compliance: Signed and present

QA- Report Yes (x) No ( )

**APPEARS THIS WAY  
ON ORIGINAL**

**Methods:** Male and female Wistar rats, 7 weeks, weights 162-209 g males and 120-150 g females, n=10/sex/dose main study and 3/sex/dose/timepoint for the PK analysis) were administered BN-200 (presently known as CG 5503, Batch #06, in physiological saline, purity 99%) at 0, 3, 7 and 15 mg/kg/day daily for 28-29 days. The dose volume was 8 ml/kg and dosing speed was 0.3 ml/minute.

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The observations were mortality (twice daily), clinical signs (once daily with weekly palpation for tissue masses), food consumption and body weights (weekly), ophthalmoscopic examinations (prefest and week 4), clinical laboratory investigations (hematology, coagulation, clinical biochemistry, urinalysis, at 4 weeks), and necropsy (organ weights, gross and histopathology examinations, at 4 weeks). The following organs were weighed: adrenal glands, brain, heart, kidneys, liver, spleen and testes. The following organs and tissues were examined microscopically: adrenal glands, aorta, auricles, brain (including medulla/pons, cerebral and cerebellar cortex), cecum, colon, duodenum, epididymides, esophagus, extra-orbital lacrimal gland, eyes with optic nerve and Harderian gland, femur with bone marrow, heart, ileum, jejunum, kidneys, larynx, liver, lungs (infused with formalin), lymph nodes (mandibular, mesenteric), mammary gland, nasal cavity, ovaries, pancreas, pituitary gland, prostate gland, rectum, salivary glands (mandibular, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, midthoracic, lumbar), spleen, sternum with bone marrow, stomach, testes, thymus (if present), thyroid gland/parathyroid gland, tongue, trachea, urinary bladder (infused with formalin), uterus, all gross lesions, and injection sites. Blood was drawn from the satellite animals on day 1 and on day 29 for determination of plasma drug levels.

**Results:** There were 3 deaths during the study, in 2 high-dose (15 mg/kg/d) males (day 5 and 17) and 1 high-dose female (day 19). Dose-related (incidence and severity) clinical signs were observed at the mid-dose (7 mg/kg/d) and high-dose, and included fearfulness, sedation, excitability and hunched posture. Food consumption was slightly decreased (-8%) in the female rats at 15 mg/kg/d. There were no treatment-related effects on body weights, ophthalmoscopic examination, hematology and urinalysis parameters, and organ weights.

The clinical biochemistry analysis showed decreased glucose at the high dose, increased alanine aminotransferase and alkaline phosphatase activity in the high-dose males, increased sodium in the high-dose females, altered protein electrophoretic pattern (decreased albumin fraction, increased alpha-1 and alpha-2 globulin fraction, increased beta-globulin fraction, increased gamma globulin fraction, and decreased albumin to globulin ratio in the mid- and high-dose females. These findings were within, or only slightly outside the range of the historical data.

In the macroscopic examination, red foci were observed in the stomachs of the high-dose male rats. Histopathologic evaluation showed hyperemia in the lamina propria of the gastric mucosa and congestion in the mandibular lymph nodes in the high-dose male rats and adrenal congestion in the high-dose female rats. Congestive changes in the lungs, liver, thymus, mandibular lymph nodes and adrenals were observed in the rats that died. Liver necrosis (single cell) was observed in 1 high-dose male and 1 high-

dose female rat. These observations were attributed to spontaneous tissue alterations in rats by the pathologist. There were no local irritation effects at the injection sites (vascular endothelium) except mechanical irritation in all groups including the controls.

In the analysis of BN 200 plasma levels, there were no significant differences in plasma concentrations within dosing groups between days 1 and 28, and between male and female rats. The plasma BN 200 concentrations increased proportionally with dose. The peak plasma concentrations, measured at 15 minutes after administration of BN 200 at doses of 0, 3, 7, and 15 mg/kg, were 0, 195, 323, and 2209 ng/ml respectively in the male rats and 0, 257, 106, and 1204 ng/ml respectively in the female rats on Day 1. The peak plasma concentration, measured at 15 minutes after administration of BN 200 at doses of 0, 3, 7, and 15 mg/kg, were 0, 295, 725, and 1835 ng/ml respectively in the male rats, and 0, 195, 638, and 1801 ng/ml respectively in the female rats on Day 28. The analysis confirmed systemic exposure of all rats to BN 200.

**Key Study Findings:**

- IV BN 200 given daily for 4 weeks lethal in 3/20 high-dose (15 mg/kg/d) rats
- Dose-related clinical signs: fearfulness, sedation, excitability and hunched posture
- Food consumption decreased in female rats at 15 mg/kg/d
- Clinical biochemistry: decreased glucose at the high dose, increased alanine aminotransferase and alkaline phosphatase activity in the high-dose males, increased sodium in the high-dose females, altered protein electrophoretic pattern in mid-dose (7 mg/kg/d) and high-dose females
- Necroscopy: hyperemia in the gastric mucosa, congestion in the mandibular lymph nodes in high-dose male rats and adrenal congestion in the high-dose female rats,
- Congestive changes in rats that died: in lungs, liver, thymus, mandibular lymph nodes and adrenals
- Target organs of toxicity: stomach and intestines, mandibular lymph nodes and adrenals
- NOAEL 3 mg/kg/d IV BN 200

**4 Week Oral Toxicity (Gavage) Study with BN 200 in the Rat: study 602561 Amendment # 000, Vol #7, Page #1**

Conducting laboratory and location:

[Redacted area]

b(4)

Date of study initiation: October 23, 1995

GLP compliance: Signed and present

QA- Report Yes (x) No ( )

Methods: Male and female Wistar rats

[Redacted area], ages 4 weeks, weights 54-77 g males and 55-73 g females, n=10/sex/dose in the main study and 3/sex/dose/timepoint in the satellite animals) were used in this study. The rats were administered BN-200 (presently known

b(4)

as CG 5503, Batch #06, in physiological saline, purity 99%) by oral gavage, at doses of 0, 300, 425 and 600 mg/kg once daily for 4 weeks. The dose volume was 10 ml/kg.

The observations were mortality (twice daily), clinical signs (once daily with weekly palpation for tissue masses), food consumption and body weights (weekly), ophthalmoscopic examinations (pretest and week 4), clinical laboratory investigations (hematology, coagulation, clinical biochemistry, urinalysis, at 4 weeks), and necropsy (organ weights, gross and histopathology examinations, at 4 weeks). The following organs were weighed: adrenal glands, brain, heart, kidneys, liver, spleen and testes. The following organs and tissues were examined microscopically: adrenal glands, aorta, auricles, brain (including medulla/pons, cerebral and cerebellar cortex), cecum, colon, duodenum, epididymides, esophagus, extra-orbital lacrimal gland, eyes with optic nerve and Harderian gland, femur with bone marrow, heart, ileum, jejunum, kidneys, larynx, liver, lungs (infused with formalin), lymph nodes (mandibular, mesenteric), mammary gland, nasal cavity, ovaries, pancreas, pituitary gland, prostate gland, rectum, salivary glands (mandibular, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, midthoracic, lumbar), spleen sternum with bone marrow, stomach, testes, thymus (if present), thyroid gland/parathyroid gland, tongue, trachea, urinary bladder (infused with formalin), uterus, and all gross lesions. Blood was drawn from the satellite animals on day 1 and on day 29 for determination of plasma drug levels.

**Results:** There were 5 treatment-related deaths during the study; 1 male (Day 23) and 1 female (day 2) rat given 425 mg/kg/d PO (mid-dose), and 3 female (Days 9, 22, and 26) rats given 600 mg/kg/d (high-dose) PO BN 200. Additional deaths in the low (300 mg/kg/d) and high dose groups were due to trauma related to dosing, anesthesia, and blood sampling procedures. Dose-related sedation was observed in all groups given BN 200. Food consumption was decreased slightly (-3% to -14%) in the male rats at all doses, and in the female rats at 425 and 600 mg/kg/d PO (weeks 2-4), compared to controls. Body weight gain was lower (-2% to -4%) in the mid-dose and high-dose male rats. There were no treatment-related effects on ophthalmoscopic parameters.

In the hematology evaluation, total leukocyte and absolute lymphocyte counts were higher in the mid-dose and high-dose female rats compared to controls. Clinical biochemistry showed increased potassium and chloride in males and females at all doses, and increased total bilirubin (high-dose), alanine aminotransferase (mid- and high-dose), lactate dehydrogenase (high-dose), creatine kinase (high-dose), calcium (mid- and high-dose) and phosphorus (low-, mid- and high-dose), and decreased albumin and albumin to globulin ratio in the female rats. Glucose and urea were increased in the high-dose male rats. Protein electrophoretic fractions were altered slightly by decreased alpha 2-globulin in the male rats (all doses), increased sum of beta globulins in the female rats (all doses) and male rats (high-dose), and increased alpha 1-globulin in males and females (high-doses). Urine output was increased in the male rats at all doses and in the high-dose female rats. Specific gravity and osmolality of the urine was lower in the high-dose females.

In the necroscopic examination, there were no treatment-related effects on organ weights, macroscopic observations, and microscopic findings in the animals that

survived to necropsy. The animals that died showed congestive changes in the lungs, liver, kidneys, lymph nodes and thymus.

In the toxicokinetic analysis, BN 200 plasma levels were higher in the female rats than in the male rats at most timepoints, and were higher overall when measured after daily oral dosing for 28 days than after a single oral dose (Day 1), although considerable interindividual variability was observed. The plasma BN 200 concentrations increased with increasing dose. The peak plasma concentrations, measured at 15 minutes after administration of BN 200 at doses of 0, 300, 425, and 600 mg/kg, were 0, 598, 249, and 635 ng/ml respectively in the male rats and 0, 492, 767, and 2016 ng/ml respectively in the female rats on Day 1. On Day 28, the peak plasma concentration, measured at 15 minutes after administration of BN 200 at doses of 0, 300, 425, and 600 mg/kg, were 0, 669, 697, and 2967 ng/ml respectively in the male rats, and 0, 2139, 2442, and 6446 ng/ml respectively in the female rats. The analysis confirmed systemic exposure of all rats to BN 200.

**Key Study Findings:**

- PO BN 200 given daily for 4 weeks in rats lethal in 2/20 rats at 425 mg/kg/d and 3/20 rats at the 600 mg/kg/d
- Rats that died showed congestive changes in the lungs, liver, kidneys, lymph nodes and thymus at necropsy
- Sedation observed in all groups (300-600 mg/kg/d)
- Food consumption and body weight gain slightly lower in the male rats at 450 and 600 mg/kg/d
- Leukocyte and lymphocyte counts increased in the females (at 425 and 600 mg/kg)
- Clinical biochemistry: changes predominantly in female rats (425 and 600 mg/kg), included increased potassium, chloride, total bilirubin, alanine aminotransferase, lactate dehydrogenase, creatine kinase, calcium and phosphorus, and decreased albumin and albumin to globulin ratio
- Urinalysis: increased glucose and urea in the males (600 mg/kg), and altered protein electrophoretic fractions (decreased alpha 2-globulin in the male rats, increased sum of beta globulins and alpha 1-globulin in male and female rats)
- NOAEL and LOAEL not identified
- Systemic exposure confirmed by TK analysis

**Study title:** BN200 (GRT CG5503): 13-Week Dose Range Finding Oral (Feeding) Toxicity Study in the Mouse (From review dated February 4, 2002, IND 61,345, SN013 submitted December 10, 2001, Kathleen Haberny, Ph.D.)

**APPEARS THIS WAY  
ON ORIGINAL**

**Study title: BN200 (GRT CG5503): 13-Week Dose Range Finding Oral (Feeding) Toxicity Study in the Mouse**

**Key study findings:**

- Decreased body weights compared to controls in males (-8% at both 500 and 1000 mg/kg/d)
- Decreased body weight gains in males (-21%, -30%, -32% at 250, 500 and 1000 mg/kg/d respectively) and females (-24%, -20%, -45% at 250, 500, and 1000 mg/kg/d respectively)
- Increased reticulocytes and LFR and decreased HFR and MFR in males (1000 mg/kg/d)
- Increased urea (males at 1000 mg/kg/d), bilirubin (males at 250-1000 mg/kg/d and females at 500-1000 mg/kg/d)
- Decreased cholesterol (males at 500 and 1000 mg/kg/d)
- Increased liver weights in males (500-1000 mg/kg/d) and females (1000 mg/kg/d), increased relative liver weights and spleen weights in males (250-1000 mg/kg/d), increased relative liver weights in females (500-1000 mg/kg/d)
- Decreased kidney weights in males (500-1000 mg/kg/d)
- Accentuated lobular pattern in liver in males (500-1000 mg/kg/d), hepatocellular hypertrophy in males and females (500-1000 mg/kg/d), group cell necrosis in liver in males (250-1000 mg/kg/d) and females (1000 mg/kg/d)
- MTD not established for the carcinogenicity study in male mice due to decreased body weight gains of >20% and group cell necrosis in liver at the low dose (250 mg/kg/d); thus the MTD is < 250 mg/kg/d
- MTD 500 mg/kg/day in female mice due to decreased body weight gains at 20% and absence of target organ toxicity at that dose

Study no: 800526

Volume # 4, and page # 1

Conducting laboratory and location:

Date of study initiation: February 20, 2001

GLP compliance: No

QA report: yes ( ) no (x)

Drug: BN200 (GRT CG5503), lot # CEWS113, radiolabel: None, and % purity: 97.7%

Formulation/vehicle: Drug substance in feed

Methods (unique aspects):

Dosing:

Species/strain: Mouse  NMRI(SPF)

#/sex/group or time point (main study): 10

Satellite groups used for toxicokinetics: 9/sex/dose

Age: 7 weeks

Weight: 26-36 g males, 22-29 g females

Doses in administered units: 0, 250, 500, 1000 mg/kg/day, adjusted for body weight throughout study

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Route, form, volume, and infusion rate: Oral by admixture in diet, daily for 91 days

**Observations and times:**

Mortality/Viability: Twice daily  
 General Cageside Observations: Once daily  
 Body weights: Pretest and Weekly  
 Food consumption: Pretest and Weekly  
 Ophthalmoscopy: Not conducted  
 EKG: Not conducted  
 Hematology: Pretest and day of termination (Week 13)  
 Clinical chemistry: Pretest and day of termination (Week 13)  
 Urinalysis: Not conducted  
 Gross pathology: At 13 weeks  
 Organs weighed: See Histopathology Inventory below, at 13 weeks  
 Histopathology: See Histopathology Inventory below, at 13 weeks  
 Toxicokinetics: Day 3, Weeks 6 and 13 at 3 time points 6 hours apart in 3 different mice/timepoint  
 Other: None

**Results:**

Mortality: No deaths  
 Clinical signs: No treatment-related signs. No palpable tissue masses

**Body weights:**

**Body Weights: Mean ± SD (% decrease from control value)**

		0 mg/kg/d n=10	250 mg/kg/d n=19	500 mg/kg/d n=19	1000 mg/kg/d n=19
Males:	Day 1	35.3 ± 1.9	35.1 ± 2.5	35.1 ± 2.5	35.5 ± 2.8
	Day 91	47.2 ± 3.8	44.4 ± 4.0 (-6%)	43.7 ± 4.1* (-8%)	43.7 ± 2.8* (-8%)
Females	Day 1	27.6 ± 2.2	27.6 ± 2.3	27.1 ± 2.0	28.2 ± 2.8
	Day 91	35.4 ± 3.1	33.8 ± 4.7 (-5%)	33.2 ± 2.6 (-6%)	32.5 ± 2.8 (-8%)

\*p<0.05

**Percent Body Weight Gain From Baseline on Day 91: Mean ± SD (%change compared to controls)**

		0 mg/kg/d n=10	250 mg/kg/d n=19	500 mg/kg/d n=19	1000 mg/kg/d n=19
Males:	Day 91	34 ± 10.2	27 ± 8.6* (-21%)	24 ± 4.6** (-30%)	23 ± 5.3** (-32%)
Females	Day 91	29 ± 14.0	22 ± 11.2 (-24%)	23 ± 7.8 (-20%)	16 ± 9.6** (-45%)

\*p<0.05; \*\*p<0.01

The percent body weight gains in the males administered CG5503 at 250, 500, and 1000 mg/kg/day were 79%, 70%, and 68% of the percent weight gain in the controls, respectively. The percent body weight gains in the female mice administered CG5503 at 250, 500, and 1000 mg/kg/day were 76%, 79%, and 55% of the percent gains in the female controls, respectively.

Food consumption: No treatment-related effects  
 Ophthalmoscopy: Not done  
 Electrocardiography: Not done

Hematology: See under Results of the Clinical Laboratory Investigations below  
 Clinical chemistry: See under Results of the Clinical Laboratory Investigations below

**Results of the Clinical Laboratory Investigations# :Week 13 (% change from control)**

		0 mg/kg/d n=10	250 mg/kg/d n=10	500 mg/kg/d n=10	1000 mg/kg/d n=10
<b>Hematology</b>					
Retic. (Reticulocyte, %)	Males	1.93	2.10	2.32	2.76** (+43%)
Retic. Count (1/L)	Males	0.1947	0.2015	0.2282	0.2696** (+38%)
Retic. fluorescence ratio: high (HFR, %)	Males	19.9	20.3	17.0	12.6** (-37%)
Retic. fluorescence ratio: middle (MFR, %)	Males	22.6	23.5	21.1	17.2** (-24%)
Retic. Fluorescence ratio: low (LFR, %)	Males	57.4	56.3	61.9	70.2** (+23%)
<b>Clinical Biochemistry</b>					
Urea (mmol/L)	Males	7.80	8.69	9.02	10.59* (+36%)
Bilirubin, total (mmol/L)	Males	2.64	1.39** (-48%)	1.63** (-38%)	1.16** (-57%)
	Females	2.12	1.56	1.35* (-36%)	1.20** (-43%)
Cholesterol, total (mmol/L)	Males	4.84	4.58	3.57* (-26%)	3.14** (-35%)
Alkaline phosphatase (mckat/L)	Males	2.83	2.62	3.05	3.74* (+32%)
Potassium (mmol/L)	Males	4.10	4.06	3.89	3.71* (-10%)
Albumin (l)	Males	0.609	0.546** (-10%)	0.559* (-8%)	0.546** (-10%)
	Females	0.627	0.622	0.616	0.596* (-5%)
Alpha1-globulin (g/l)	Males	2.75	3.15	3.27* (+19%)	3.21* (+16%)
Alpha2-globulin (g/l)	Males	10.14	11.54* (+14%)	10.94	11.52* (+14%)
Beta-globulin (l)	Males	0.143	0.175* (+22%)	0.170	0.181* (+25%)
	Females	0.145	0.151	0.152	0.160* (+10%)
Albumin/globulin Ratio	Males	1.56	1.21** (-22%)	1.27** (-18%)	1.20** (-24%)
	Females	1.68	1.65	1.60	1.48* (-12%)
Beta-globulin (g/L)	Males	8.14	10.14	9.86	10.77* (+32%)
	Females	8.27	8.55	8.70	9.22** (+11%)
Chloride (mmol/L)	Females	110.7	111.6	112.6	113.4* (+3%)

#Only parameters with significant treatment-related changes compared to controls are presented; there were no significant findings in the remaining clinical laboratory parameters or in sex not represented; values represent group means; percent change from controls in parentheses  
 \*p<0.05; \*\*p<0.01

Urinalysis: Not done

Organ weights: See under Results of the Necropsic Evaluation below

Gross pathology: See under Results of the Necropsic Evaluation below

Histopathology: See under Results of the Necropsic Evaluation below

**Results of the Necropsic Evaluation #**

		0 mg/kg/d n=10	250 mg/kg/d n=10	500 mg/kg/d n=10	1000 mg/kg/d n=10
Liver Weights (g)	Males	1.76	1.95	2.19** (+25%)	2.54** (+44%)
	Females	1.41	1.53	1.53	1.78** (+26%)
Relative Liver Weights (g/g body wt)	Males	4.12	5.01** (+22%)	5.60** (+36%)	6.60** (+60%)
	Females	4.64	5.10	5.34** (+15%)	6.12** (+32%)
Kidney Weights (g)	Males	0.595	0.550	0.507** (-15%)	0.487** (-19%)
Relative Spleen Weights	Males	0.237	0.296** (+25%)	0.293** (+24%)	0.308** (+30%)
Accentuated Lobular Pattern in Liver (% treated animals)	Males	0%	30%	70%**	90%**
Liver Group Cell Necrosis (% treated animals, mean grade)	Males	0%	20%(1.0)	10%(1.0)	30%(1.5)
	Females	0%	0%	0%	30%(1.0)
Hepatocellular Hypertrophy (% treated animals, mean grade)	Males	20%(1.5)	0%	90%(2.2)	100%(3.7)
	Females	0%	0%	10%(1.0)	90%(1.9)

#Only organs with significant changes compared to controls are presented; there were no significant findings in the remaining organs or tissues or in the sex not represented; values represent group means unless otherwise defined: percent change vs. controls in parentheses

\*p<0.05; \*\*p<0.01

#Grade scale: 1=minimal; 2=slight; 3=moderate; 4=marked

#### Toxicokinetics:

The following toxicokinetic data in mice was given:

Species*	Dose	Route	CG5503		CG5503 Glucuronide	
			Cmax (ng/ml)	AUC <sub>0-24h</sub> (ng.h/ml)	Cmax (ng/ml)	AUC <sub>0-24h</sub> (ng.h/ml)
Mice	500 mg/kg/d	Dietary	2-30	220-460	900-5,000	40,000- 90,000
Human (male-female)	100 mg	Oral	96	373-374	3700	19000
	200 mg	Oral	210-215	814-943	-	-

\*Mouse data from the 90-day toxicity study, human data from Phase I PK study

Toxicokinetic data were not provided for the 250 and 1000 mg/kg/day doses.

Summary of individual study findings: Oral CG5503 administered daily for 13-weeks in mice, produced treatment-related decreases in body weights in males (-8% and -8% at 500 and 1000 mg/kg/d PO, respectively). Body weight gains were significantly reduced in males at all doses (-21%, -30%, -32% at 250, 500 and 1000 mg/kg/d, respectively) and females at 1000 mg/kg/d PO (-45%).

Target organs of toxicity included the liver with increased organ weight (+25% and +44% at 500 and 1000 mg/kg/day in males and +26% at 1000 mg/kg/day in females), accentuated lobular pattern (in 30%, 70% and 90% males at 250, 500 and 1000 mg/kg/day, respectively), hypertrophy (in 90% and 100% males at 500 and 1000 mg/kg/day and 90% females at 500 and 1000 mg/kg/day) and necrosis (20%, 10% and 30% males at 250, 500 and 1000 mg/kg/day respectively, and 30% females at 1000 mg/kg/day). Other organ weight changes included increased spleen weights in males at all doses, and decreased kidney weights in the males at 500 and 1000 mg/kg/d.

Increased reticulocyte count in males at 1000 mg/kg/d (+38%), and clinical biochemistry changes (decreased bilirubin, increased urea, decreased cholesterol) in males at all doses (250-1000 mg/kg/d) and females at 500 and 1000 mg/kg/d were observed.

The MTD in the 13-week study in mice is 500 mg/kg in females due to a 20% decrease in body weight gain at this dose and significant liver toxicity at 1000 mg/kg/day. An MTD was not identified in males due to liver toxicity, including group cell necrosis at all doses. This is not in agreement with the sponsor's conclusion. The sponsor concluded that the MTD for both sexes was 250 mg/kg/day.

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**4 Week Intravenous Toxicity Study with BN 200 in the Dog: Study 602526**

Amendment # 000, Vol #5, Page #1

Conducting laboratory and location:

Date of study initiation: September 28, 1995

GLP compliance: Signed and present

QA- Report Yes (x) No ( )

Methods: Male and female pure-bred beagle dogs   
ages 9-10 months, weights 8.3-9.5 kg males and 7.3-9.7 kg females, n=3/sex/dose

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were used for this study. The dogs were administered BN-200 (presently known as CG 5503, Batch #06, in physiological saline, purity 99%) by intravenous injection using an infusion pump  at doses of 0, 1, 3 and 7.5 mg/kg once daily for 4 weeks. The doses were selected based on the results of a preliminary study  Project No. 602460). The dose volume was 1 ml/kg and the infusion rate was 0.2 ml/kg/minute.

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The observations were mortality (twice daily), clinical signs (once daily with weekly palpation for tissue masses), food consumption (daily), body weights (weekly), ophthalmoscopic examinations (pretest and week 4), electrocardiogram (at baseline and at just before and one hour after injection on days 2 and 28), clinical laboratory investigations (hematology, coagulation, clinical biochemistry, urinalysis, at 4 weeks), and necropsy (organ weights, gross and histopathology examinations, at 4 weeks). The following organs were weighed: adrenal glands, brain (including brainstem), heart, kidneys, liver, pituitary gland, prostate gland, salivary glands (mandibular with sublingual and parotid), spleen, testes with epididymides, and thyroid gland with parathyroid. The following organs and tissues were examined microscopically: adrenal glands, aorta, bone (femur including articular surface), bone marrow (sternum), brain (including medulla/pons, cerebral and cerebellar cortex), epididymides, esophagus, eyes with optic nerve and Harderian gland, eyes with optic nerve, female mammary gland area, male mammary gland area, gallbladder, heart, injection sites, kidneys, large intestine (cecum, colon and rectum), larynx, liver, lungs (infused with formalin), lymph nodes (retropharyngeal, mesenteric), ovaries, pancreas, pituitary gland, prostate gland, salivary glands (mandibular, parotid, sublingual), sciatic nerve, skeletal muscle, skin, small intestine (duodenum, jejunum, ileum), spinal cord (cervical, midthoracic, lumbar), spleen, stomach, testes, thymus, thyroid gland/parathyroid gland, tongue, trachea, urinary bladder, uterus with vagina, and all gross lesions. Blood was drawn from each dog on days 1 (pre-dose and 15min, 1h, 2h, and 4h after dosing) and 28 (pre-dose and 15 min, 1h, 2h, 4h, and 24h after dosing) for determination of plasma drug concentrations.

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**Results:** There were no deaths in the dogs during the study. Rhinorrhea was observed in one low-dose (1 mg/kg/d IV) female on the first day of treatment. Treatment-related clinical signs, observed in the dogs administered BN 200 at 3 and 7.5 mg/kg IV, were excessive salivation, decreased activity, sporadic buckling of the hindlegs, uncoordinated movements, vomiting of mucus, urination, ventral recumbency and serous rhinorrhea. Tachypnea, panting, vomiting, retching, lateral recumbency and defecation were observed infrequently in these groups. The onset of the clinical signs occurred during or immediately after dosing, and the effects lasted for 1-2 hours after dosing.

Food consumption decreased in the female dogs at 3 mg/kg/d IV, and in 1/3 male dogs and 2/3 female dogs given 7.5 mg/kg/d IV BN 200. Body weights were reduced in the female dogs given 3 mg/kg/d IV, and in 2/3 female dogs given 7.5 mg/kg/d IV BN 200.

There were no treatment-related effects on ophthalmoscopic parameters, electrocardiographic parameters, and in the clinical laboratory parameters of hematology, clinical biochemistry and urinalysis. The P-Q interval was slightly

increased in the high dose males and females. A slight increase in mean erythrocyte count and hemoglobin concentration in the high-dose (7.5 mg/kg/d) females was within historical control range. Mean cholesterol, phospholipid and protein levels were increased in the mid-dose (3 mg/kg/d) and high-dose female dogs were slightly, but not significantly increased over the baseline values. These changes were not dose-related and were within historical control range.

In the necroscopic examination, there were no treatment-related effects on organ weights, macroscopic findings, and histopathology findings, and no local toxicity at the injection site. Grade 1 hepatic vacuolation, probably of spontaneous origin, was observed in one high-dose male. In that animal, there were no corresponding alterations in liver enzymes except for increased gamma glutamyl transferase (GGT), and no significant difference in liver weight compared to the liver weights of other dogs in Group 4, and compared to the mean liver weights in all groups. Red discoloration in the gastrointestinal tract, and phlebitis and subcutis inflammation and at the injection site were observed in all groups including the controls.

In the toxicokinetic analysis, there were no significant differences in plasma concentrations within dosing groups between days 1 and 28, and between male and female dogs. The plasma BN 200 concentrations increased proportionally with dose. The peak plasma concentrations, measured at 15 minutes after administration of BN 200 at doses of 0, 1, 3 and 7.5 mg/kg, were 0, 83, 259, and 704 ng/ml respectively in the male dogs and 0, 83, 268, and 902 ng/ml respectively in the female dogs on Day 1. The peak plasma concentration, measured at 15 minutes after administration of BN 200 at doses of 0, 1, 3, and 7.5 mg/kg, were 0, 79, 353, and 736 ng/ml respectively in the male dogs, and 0, 78, 257, and 818 ng/ml respectively in the female dogs on Day 28. The analysis confirmed systemic exposure of all dogs to BN 200.

**Key Study Findings:**

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**Key Study Findings:**

- IV toxicity in dogs at 3 and 7.5 mg/kg/d for 4 weeks shown by clinical signs of excessive salivation, decreased activity, sporadic buckling of the hindlegs, uncoordinated movements, vomiting of mucus, urination, ventral recumbency, serous rhinorrhea, tachypnea, panting, and defecation, decreased food consumption and body weights
- Hepatic vacuolation in one high-dose male dog, without concurrent changes in liver enzymes except for an increase in GGT, and no significant alteration in liver weight
- Liver identified as a potential target organ of toxicity
- NOAEL 1 mg/kg/d IV

***BN 193 / BN 200 / BN 210 Two Week Toxicity Study in Beagle Dogs After Daily Oral Administration: Study TP 1993/95***

**Amendment #000, Vol #5, Page #259**

**Conducting laboratory and location:** Department of Toxicology and Pathology of the Grunenthal Research Centre, Aachen

**Date of study initiation:** October 1995

**GLP compliance:** Signed and present

**QA- Report Yes (x) No ( )**

**Methods:** Male and female beagle dogs (ages 5-6 months, weights 8.4-10.4 kg males and 7.7-9.0 kg females, n=2/sex/treatment/dose) were used in this study. The dogs were administered one of three tramadol analogues: BN 193 (Batch #06, purity 99.7%), BN 200 (Batch #07, purity 98%) or BN 210 (Batch #05, purity 99%) orally by stomach tube, at 50 and 150 mg/kg once daily for 2 weeks. The doses were selected based on the results of a dose-range finding study showing that doses of 200 mg/kg or greater induced severe clinical symptoms including lateral recumbency, increased respiratory frequency, tremor, vomitus, and diarrhea. The test articles were dissolved in 0.9% physiological saline (Batch #BK 3173) and the dose volume was 10 ml/10 kg.

The observations were mortality, general behavior and food consumption (daily), body weight (weekly), hearing test (high frequency tone, pre-test and at the end of treatment), electrocardiographic examinations (pre-test and at the end of treatment), clinical pathology (urinalysis, hematology, blood coagulation, and clinical chemistry, at the end of treatment), and necropsy (organ weights, and macroscopic and microscopic examination). The following organs were weighed: liver, lungs, spleen, adrenal glands, brain, thyroids (2), heart, testes (2), kidneys (2), pituitary gland, pancreas, ovaries (2), and prostate. The following organs and tissues were examined microscopically: heart, lungs, liver, gall bladder, kidneys, adrenal glands, uterus, vagina, ovaries, urinary bladder, testes/epididymides, prostate, tongue, spleen, thymus, salivary gland, lymph nodes (mandibular and mesenteric), stomach, pancreas, small intestine (duodenum, jejunum, ileum), large intestine (colon, caecum), brain (cerebrum, brain stem, cerebellum, medulla, oblongata), spinal cord, peripheral nerve (N. ischiadicus), pituitary, thyroids, aorta, skeletal muscle, trachea, esophagus, skin/mammary gland, eyes, bone (femur including knee joint), bone marrow (sternum), and altered organs. Blood was drawn at 15 minutes and 1, 2, 4 and 24 hours after dosing on days 1 and 14 for determination of plasma drug concentrations.

**Results:** The results are here described for the test article BN 200, unless otherwise stated. There were no deaths in any treatment group. The clinical signs in the low-dose (LD) dogs (50 mg/kg PO) were periodic salivation (4/4 dogs), irregular respiration (3/4 dogs), vomiting (1/4 dogs), and convulsions (1/4 dogs). In the high-dose (HD) dogs (150 mg/kg PO), salivation (4/4 dogs), jerking (3/4 dogs, sporadic), tremor (3/4 dogs, sporadic), irregular respiration (4/4 dogs, throughout the study), convulsions (2/4 dogs), abdominal and lateral recumbency (4/4 dogs, throughout study), vomiting (4/4 dogs, first 10 days of dosing), and diarrhea (1/4 dogs on dosing Day 1). Similar clinical signs and additionally, hemorrhagic diarrhea were observed in the dogs given BN 193 and BN 210.

Food consumption was reduced in the high-dose groups, to the greatest extent in the dogs given BN 200 at the dose of 150 mg/kg/d PO. Body weight was reduced overall in 1/4 dogs given 150 mg/kg/d BN 200 and 1/4 dogs given 150 mg/kg/d BN 193. There were no treatment-related effects on hearing, heart rate, ECG measures, and on clinical chemistry and urinalysis parameters. In the hematology analysis, hemoglobin and erythrocytes were decreased slightly in the dogs given BN 200 and BN 210, but the

values were within historical range. Hematocrit values were also decreased in the dogs administered BN 210. Partial thromboplastin time increased in one low-dose dog administered BN 200 and in one high-dose dog given BN 193.

In the necropsy, there were no treatment-related effects on terminal organ weights according to the sponsor. However, the organs of the control dogs were not weighed, and no historical data was provided for comparison; these data will be requested from the sponsor. The macroscopic examination showed activated Peyer's patches (hyperplasia) in the gastric mucosa and in the small and large intestine with discoloration of the mesenteric lymph nodes in all test-article treated dogs. These observations were accompanied by histopathological changes indicating reactive hyperplasia of the germinal centers in lymphatic tissue of the distal jejunum and ileum. Hemorrhages were also observed in the germinal centers of the lymphoid follicles in the cecum. Additional microscopic findings were fibrosis in the mandibular lymphoid node (all HD dogs), thyroid intrafollicular cell detritus (3/4 at LD, all HD), spleen congestion (all treated dogs), thymus congestion (2/2 HDF), juvenile testes and epididymis (2/2 LDM, 2/2 HDM), and immature prostate (2/2 HDM).

The plasma BN 200 concentrations increased proportionally with dose. The peak plasma concentrations, measured at 15 minutes after administration of BN 200 at doses of 50 and 150 mg/kg PO, were 40 and 180 ng/ml respectively in the male dogs and 12 and 60 ng/ml respectively in the female dogs on Day 1. The peak plasma concentration, measured at 15 minutes after administration of BN 200 at doses of 50 and 150 mg/kg PO, were 22 and 67 ng/ml respectively in the male dogs, and 24 and 49 ng/ml respectively in the female dogs on Day 28. The analysis confirmed systemic exposure of all dogs to orally administered BN 200.

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ON ORIGINAL**

**Key Study Findings:**

- PO oxicity in dogs at 50 and 150 mg/kg/d for 2 weeks: clinical signs of salivation, irregular respiration, vomiting, and convulsions (1/4 dogs LD, 2/4 HD dogs), jerking (3/4 HD dogs), abdominal and lateral recumbency
- Food consumption and body weights reduced (150 mg/kg/d)
- Gross necropsy: activated Peyer's patches (hyperplasia) in the gastric mucosa and in the small and large intestine with discoloration of the mesenteric lymph nodes in all treated dogs
- Microscopic changes indicating reactive hyperplasia of the germinal centers in lymphatic tissue of the distal jejunum and ileum, hemorrhages in the germinal centers of the lymphoid follicles in the cecum, fibrosis in the mandibular lymphoid node, thyroid intrafollicular cell detritus, spleen congestion, thymus congestion, juvenile testes and epididymis, and immature prostate
- Exposure to the test article confirmed by TK analysis
- Target organs of toxicity: lymphatic tissues of the stomach and small and large intestine
- The NOAEL and LOAEL not identified
- Little or no differences in toxicity of the three tramadol analogues BN 193, BN 200 and BN 210

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ON ORIGINAL**

**Study title:** BN200 (GRT CG5503): 13-Week Dose Range Finding Oral (Feeding) Toxicity Study in the Mouse (From review dated February 4, 2002, IND 61,345, SN013 submitted December 10, 2001, Kathleen Haberny, Ph.D.)

**APPEARS THIS WAY  
ON ORIGINAL**

**Study title: BN200 (GRT CG5503): 13-Week Dose Range Finding Oral (Feeding) Toxicity Study in the Mouse**

**Key study findings:**

- Decreased body weights compared to controls in males (-8% at both 500 and 1000 mg/kg/d)
- Decreased body weight gains in males (-21%, -30%, -32% at 250, 500 and 1000 mg/kg/d respectively) and females (-24%, -20%, -45% at 250, 500, and 1000 mg/kg/d respectively)
- Increased reticulocytes and LFR and decreased HFR and MFR in males (1000 mg/kg/d)
- Increased urea (males at 1000 mg/kg/d), bilirubin (males at 250-1000 mg/kg/d and females at 500-1000 mg/kg/d)
- Decreased cholesterol (males at 500 and 1000 mg/kg/d)
- Increased liver weights in males (500-1000 mg/kg/d) and females (1000 mg/kg/d), increased relative liver weights and spleen weights in males (250-1000 mg/kg/d), increased relative liver weights in females (500-1000 mg/kg/d)
- Decreased kidney weights in males (500-1000 mg/kg/d)
- Accentuated lobular pattern in liver in males (500-1000 mg/kg/d), hepatocellular hypertrophy in males and females (500-1000 mg/kg/d), group cell necrosis in liver in males (250-1000 mg/kg/d) and females (1000 mg/kg/d)
- MTD not established for the carcinogenicity study in male mice due to decreased body weight gains of >20% and group cell necrosis in liver at the low dose (250 mg/kg/d); thus the MTD is < 250 mg/kg/d
- MTD 500 mg/kg/day in female mice due to decreased body weight gains at 20% and absence of target organ toxicity at that dose

Study no: 800526

Volume # 4, and page # 1

Conducting laboratory and location:

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Date of study initiation: February 20, 2001

GLP compliance: No

QA report: yes ( ) no (x)

Drug: BN200 (GRT CG5503), lot # CEWS113, radiolabel: None, and % purity: 97.7%

Formulation/vehicle: Drug substance in feed

**Methods (unique aspects):**

**Dosing:**

Species/strain: Mouse,   NMRI(SPF)

#/sex/group or time point (main study): 10

Satellite groups used for toxicokinetics: 9/sex/dose

Age: 7 weeks

Weight: 26-36 g males, 22-29 g females

Doses in administered units: 0, 250, 500, 1000 mg/kg/day, adjusted for body weight throughout study

b(4)

Route, form, volume, and infusion rate: Oral by admixture in diet, daily for 91 days

**Observations and times:**

- Mortality/Viability: Twice daily
- General Cageside Observations: Once daily
- Body weights: Pretest and Weekly
- Food consumption: Pretest and Weekly
- Ophthalmoscopy: Not conducted
- EKG: Not conducted
- Hematology: Pretest and day of termination (Week 13)
- Clinical chemistry: Pretest and day of termination (Week 13)
- Urinalysis: Not conducted
- Gross pathology: At 13 weeks
- Organs weighed: See Histopathology Inventory below, at 13 weeks
- Histopathology: See Histopathology Inventory below, at 13 weeks
- Toxicokinetics: Day 3, Weeks 6 and 13 at 3 time points 6 hours apart in 3 different mice/timepoint
- Other: None

**Results:**

- Mortality: No deaths
- Clinical signs: No treatment-related signs. No palpable tissue masses

**Body weights:**

**Body Weights: Mean ± SD (% decrease from control value)**

		0 mg/kg/d n=10	250 mg/kg/d n=19	500 mg/kg/d n=19	1000 mg/kg/d n=19
Males:	Day 1	35.3 ± 1.9	35.1 ± 2.5	35.1 ± 2.5	35.5 ± 2.8
	Day 91	47.2 ± 3.8	44.4 ± 4.0 (-6%)	43.7 ± 4.1* (-8%)	43.7 ± 2.8* (-8%)
Females	Day 1	27.6 ± 2.2	27.6 ± 2.3	27.1 ± 2.0	28.2 ± 2.8
	Day 91	35.4 ± 3.1	33.8 ± 4.7 (-5%)	33.2 ± 2.6 (-6%)	32.5 ± 2.8 (-8%)

\*p<0.05

**Percent Body Weight Gain From Baseline on Day 91: Mean ± SD (%change compared to controls)**

		0 mg/kg/d n=10	250 mg/kg/d n=19	500 mg/kg/d n=19	1000 mg/kg/d n=19
Males:	Day 91	34 ± 10.2	27 ± 8.6* (-21%)	24 ± 4.6** (-30%)	23 ± 5.3** (-32%)
Females	Day 91	29 ± 14.0	22 ± 11.2 (-24%)	23 ± 7.8 (-20%)	16 ± 9.6** (-45%)

\*p<0.05; \*\*p<0.01

The percent body weight gains in the males administered CG5503 at 250, 500, and 1000 mg/kg/day were 79%, 70%, and 68% of the percent weight gain in the controls, respectively. The percent body weight gains in the female mice administered CG5503 at 250, 500, and 1000 mg/kg/day were 76%, 79%, and 55% of the percent gains in the female controls, respectively.

- Food consumption: No treatment-related effects
- Ophthalmoscopy: Not done
- Electrocardiography: Not done

Hematology: See under Results of the Clinical Laboratory Investigations below  
 Clinical chemistry: See under Results of the Clinical Laboratory Investigations below

**Results of the Clinical Laboratory Investigations# :Week 13 (% change from control)**

		0 mg/kg/d n=10	250 mg/kg/d n=10	500 mg/kg/d n=10	1000 mg/kg/d n=10
<b>Hematology</b>					
Retic. (Reticulocyte, %)	Males	1.95	2.10	2.32	2.76** (+43%)
Retic. Count (T/L)	Males	0.1947	0.2015	0.2282	0.2696** (+38%)
Retic. fluorescence ratio: high (HFR, %)	Males	19.9	20.3	17.0	12.6** (-37%)
Retic. fluorescence ratio: middle (MFR, %)	Males	22.6	23.5	21.1	17.2** (-24%)
Retic. Fluorescence ratio: low (LFR, %)	Males	57.4	56.3	61.9	70.2** (+23%)
<b>Clinical Biochemistry</b>					
Urea (mmol/L)	Males	7.80	8.69	9.02	10.59* (+36%)
Bilirubin, total (mmol/L)	Males	2.64	1.39** (-48%)	1.63** (-38%)	1.16** (-57%)
	Females	2.12	1.56	1.35* (-36%)	1.20** (-43%)
Cholesterol, total (mmol/L)	Males	4.84	4.58	3.57* (-26%)	3.14** (-35%)
Alkaline phosphatase (mckat/L)	Males	2.83	2.62	3.05	3.74* (+32%)
Potassium (mmol/L)	Males	4.10	4.06	3.89	3.71* (-10%)
Albumin (l)	Males	0.609	0.546** (-10%)	0.559* (-8%)	0.546** (-10%)
	Females	0.627	0.622	0.616	0.596* (-5%)
Alpha1-globulin (g/l)	Males	2.75	3.15	3.27* (+19%)	3.21* (+16%)
Alpha2-globulin (g/l)	Males	10.14	11.54* (+14%)	10.94	11.52* (+14%)
Beta-globulin (l)	Males	0.143	0.175* (+22%)	0.170	0.181* (+25%)
	Females	0.145	0.151	0.152	0.160* (+10%)
Albumin/globulin Ratio	Males	1.56	1.21** (-22%)	1.27** (-18%)	1.20** (-24%)
	Females	1.68	1.65	1.60	1.48* (-12%)
Beta-globulin (g/L)	Males	8.14	10.14	9.86	10.77* (+32%)
	Females	8.27	8.55	8.70	9.22** (+11%)
Chloride (mmol/L)	Females	110.7	111.6	112.6	113.4* (+3%)

#Only parameters with significant treatment-related changes compared to controls are presented; there were no significant findings in the remaining clinical laboratory parameters or in sex not represented; values represent group means; percent change from controls in parentheses  
 \*p<0.05; \*\*p<0.01

Urinalysis: Not done

Organ weights: See under Results of the Necropsic Evaluation below

Gross pathology: See under Results of the Necropsic Evaluation below

Histopathology: See under Results of the Necropsic Evaluation below

**Results of the Necropsic Evaluation#**

		0 mg/kg/d n=10	250 mg/kg/d n=10	500 mg/kg/d n=10	1000 mg/kg/d n=10
Liver Weights (g)	Males	1.76	1.95	2.19** (+25%)	2.54** (+44%)
	Females	1.41	1.53	1.53	1.78** (+26%)
Relative Liver Weights (g/g body wt)	Males	4.12	5.01** (+22%)	5.60** (+36%)	6.60** (+60%)
	Females	4.64	5.10	5.34** (+15%)	6.12** (+32%)
Kidney Weights (g)	Males	0.595	0.550	0.507** (-15%)	0.487** (-19%)
Relative Spleen Weights	Males	0.237	0.296** (+25%)	0.293** (+24%)	0.308** (+30%)
Accentuated Lobular Pattern in Liver (% treated animals)	Males	0%	30%	70%**	90%**
Liver Group Cell Necrosis (% treated animals, mean grade)†	Males	0%	20%(1.0)	10%(1.0)	30%(1.3)
	Females	0%	0%	0%	30%(1.0)
Hepatocellular Hypertrophy (% treated animals, mean grade)‡	Males	20%(1.5)	0%	90%(2.2)	100%(3.7)
	Females	0%	0%	10%(1.0)	90%(1.9)

#Only organs with significant changes compared to controls are presented; there were no significant findings in the remaining organs or tissues or in the sex not represented; values represent group means unless otherwise defined: percent change vs. controls in parentheses

\*p<0.05; \*\*p<0.01

#Grade scale: 1=minimal; 2=slight; 3=moderate; 4=marked

#### Toxicokinetics:

The following toxicokinetic data in mice was given:

Species*	Dose	Route	CG5503		CG5503 Glucuronide	
			Cmax (ng/ml)	AUC <sub>0-24h</sub> (ng h/ml)	Cmax (ng/ml)	AUC <sub>0-24h</sub> (ng h/ml)
Mice	500 mg/kg/d	Dietary	2-30	220-460	900-5,000	40,000-90,000
Human (male-female)	100 mg	Oral	96	373-374	3700	19000
	200 mg	Oral	210-215	814-943	-	-

\*Mouse data from the 90-day toxicity study, human data from Phase I PK study

Toxicokinetic data were not provided for the 250 and 1000 mg/kg/day doses.

Summary of individual study findings: Oral CG5503 administered daily for 13-weeks in mice, produced treatment-related decreases in body weights in males (-8% and -8% at 500 and 1000 mg/kg/d PO, respectively). Body weight gains were significantly reduced in males at all doses (-21%, -30%, -32% at 250, 500 and 1000 mg/kg/d, respectively) and females at 1000 mg/kg/d PO (-45%).

Target organs of toxicity included the liver with increased organ weight (+25% and +44% at 500 and 1000 mg/kg/day in males and +26% at 1000 mg/kg/day in females), accentuated lobular pattern (in 30%, 70% and 90% males at 250, 500 and 1000 mg/kg/day, respectively), hypertrophy (in 90% and 100% males at 500 and 1000 mg/kg/day and 90% females at 500 and 1000 mg/kg/day) and necrosis (20%, 10% and 30% males at 250, 500 and 1000 mg/kg/day respectively, and 30% females at 1000 mg/kg/day). Other organ weight changes included increased spleen weights in males at all doses, and decreased kidney weights in the males at 500 and 1000 mg/kg/d.

Increased reticulocyte count in males at 1000 mg/kg/d (+38%), and clinical biochemistry changes (decreased bilirubin, increased urea, decreased cholesterol) in males at all doses (250-1000 mg/kg/d) and females at 500 and 1000 mg/kg/d were observed.

The MTD in the 13-week study in mice is 500 mg/kg in females due to a 20% decrease in body weight gain at this dose and significant liver toxicity at 1000 mg/kg/day. An MTD was not identified in males due to liver toxicity, including group cell necrosis at all doses. This is not in agreement with the sponsor's conclusion. The sponsor concluded that the MTD for both sexes was 250 mg/kg/day.

Study Title: CG5503: 13 Week Oral (Gavage) Administration Toxicity Study in the Mouse (From review dated August 2, 200, IND 61,345, SN029SX, Adam Wasserman, Ph.D.)

**Key study findings:** CG5503 was administered by oral gavage to CD-1 mice (12/sex/group) in doses of 0 (saline vehicle), 10, 30, 100 or 200 mg/kg for a total of 13 weeks for the purposes of determining appropriate dosing for a 2 year carcinogenicity bioassay. Key study findings were as follows:

1. There were no clear drug-related mortalities under the conditions of the assay. The noted mortalities were attributed to either dosing error or were not dose-related.
2. There were no changes in body weight, food consumption or hematology under the conditions tested.
3. There were mild alterations in clinical chemistry (increased AST, ALT, globulin) that reached statistical significance in the high dose animals, but were not considered biologically relevant due to the generally small changes observed (i.e. < 2-fold changes in all cases).
4. High dose animals demonstrated a slight increase in liver weights compared to controls (7.4% in males and 11.4% in female).
5. The liver was the only tissue examined histopathologically. There was little pathology in females, primarily consisting of congestion in 1/11 MHD and HD females with no evidence in males and hepatocyte vacuolation, considered minimal in nature, in 1/11 MHD females and 2/11 HD females. Hepatocyte vacuolation was observed in 1/12 MHD males but was absent from HD males and thus does not appear to be an important result of drug treatment. Other liver findings, such as inflammatory cell foci, were observed at an equal incidence in control animals as well as in lower dosed animals and were not considered a treatment-related effect. Focal cell necrosis was observed in male and female mice but was never greater than 1/12 in male animals and was not observed in the HD group, HD females had evidence of focal cell necrosis in 2/11 animals but 2/11 female control animals also were reported to demonstrate this finding. Taken together, focal cell necrosis does not appear to be a treatment-related effect in CD-1 mice at doses used.
6. Overall, the study indicated that 200 mg/kg/day CG5503 was well tolerated and therefore the maximum tolerated dose appears to be greater than 200 mg/kg/day.

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7. The CD-1 mouse appears to be less sensitive to CG5503 than the NMRI mouse.

Study no: TP 2496/PK N293

Volume #, and page #: 1/1

Conducting laboratory and location [ ]

Date of study initiation: April 8, 2003

GLP compliance: Yes

QA report: yes ( ) no (X) To be included with Final Report

Drug, lot #, radiolabel, and % purity: CG5503, Batch # CEWS146, 98% purity

Formulation/vehicle: Sterile Saline (0.9% w/v NaCl)

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Methods (unique aspects): In a 2-week prior dose-justification study performed at [ ] 200 mg/kg/day was well tolerated by CD-1 mice receiving the drug via an oral (gavage) route. Prior studies in NMRI mice used drug administered through diet. A preliminary oral (gavage) dose of 300 mg/kg/day x 5 days was given to 5/sex/group animals to determine overt toxicity and overall tolerance. On Day 1 of dosing, 1 female died within one minute of dosing and 2 females and 1 male were sacrificed after severe convulsions began within ten minutes of administration. Thus, mortality was observed in 3/5 females and 1/5 males on Day 1 of an intended five-day dosing strategy. The surviving animals demonstrated hyperactivity and the study was prematurely stopped on Day 1. This dose of 300 mg/kg/day was considered too high for use in the 13-week study and thus 200 mg/kg/day was selected to be the top dose for this study. In main study, all animals were sham dosed for up to 7 days prior to the start of treatment and were identified with subcutaneous electronic transponder. At the conclusion of the study, animals were anesthetized with i.p. sodium pentobarbital prior to exsanguination.

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Dosing:

Species/strain: [ ] CD-1(ICR)BR mice obtained from [ ]  
#/sex/group or time point (preliminary study): 5/sex/group  
#/sex/group or time point (main study): 12/sex/group  
Satellite groups used for toxicokinetics or recovery: 18/sex/group  
Age: 6 - 8 weeks old at initiation of study  
Weight: 27 - 42.1 g (♂); 20.8 - 31.2 g (♀)  
Doses in administered units:  
Preliminary Study: 300 mg/kg/day  
Main Study: 0, 10, 30, 100, or 200 mg/kg/day  
Route, form, volume, and infusion rate:  
Preliminary and Main Studies: Oral gavage, 10 mL/kg volume

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Observations and times:

Clinical signs: Twice daily, am/pm for routine health checks and daily for signs of ill health or overt signs of toxicity  
Body weights: Prior to treatment on first day of dosing and thereafter at weekly intervals prior to sacrifice and necropsy  
Food consumption: Weekly. Calculated as grams/animal/week  
Ophthalmoscopy: Not done  
EKG: Not done

**Hematology:** Week 13 taken from the first 6 male and 6 female animals in each group (where possible). Blood samples will be taken from main study decedents as per the necropsy schedule.

Hematology parameters measured		
Hemoglobin concentration	Mean cell volume	Platelet count
RBC count	Mean cell hemoglobin conc.	Platelet crit
Packed cell volume	Hemoglobin distribution width	Mean platelet volume
Reticulocytes	Red cell distribution width	Mean platelet volume
		Tot & Diff WBC count

**Clinical chemistry:** Week 13 taken from the last 6 male and 6 female animals in each group (where possible)

Clinical Chemistry parameters measured		
AST	Potassium	Total protein
ALT	Calcium	Albumin
ALP	Inorganic phosphorous	Globulin
Sodium	Chloride	Albumin/globulin ratio
Glucose	Total bilirubin	

**Urinalysis:** Not done

**Gross pathology:** All main study animals and decedents

**Organs weighed:** Brain, heart, liver, kidney, spleen, testes and epididymides (See table below)

**Histopathology:** Liver only (See table below)

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**Histopathology Inventory for IND # 61,345**

Study	13-Week Oral Toxicity (Gavage) Study in CD-1 mice (TP 2496/PK N293)			
Species	CD-1 mice			
*, organ wt obtained; X, histopathology performed; + tissues preserved				
Adrenals			+	
Aorta			+	
Bone Marrow smear			+	
Bone (femur)			+	
Brain	*		+	
Cecum			+	
Cervix			+	
Colon			+	
Duodenum			+	
Epididymis	*		+	
Esophagus			+	
Eye			+	
Fallopian tube			+	
Gall bladder			+	
Gross lesions			+	
Harderian gland			+	
Heart	*		+	
Ileum			+	
Injection site			+	
Jejunum			+	
Kidneys	*		+	
Lachrymal gland			+	
Larynx			+	
Liver	*	X	+	
Lungs			+	
Lymph nodes, cervical			+	
Lymph nodes mandibular			+	
Lymph nodes, mesenteric			+	
Mammary Gland			+	
Nasal cavity			+	
Optic nerves			+	

Ovaries			+	
Pancreas			+	
Parathyroid			+	
Peripheral nerve			+	
Pharynx			+	
Pituitary			+	
Prostate			+	
Rectum			+	
Salivary gland			+	
Sciatic nerve			+	
Seminal vesicles			+	
Skeletal muscle			+	
Skin			+	
Spinal cord			+	
Spleen	*		+	
Sternum			+	
Stomach			+	
Testes	*		+	
Thymus			+	
Thyroid			+	
Tongue			+	
Trachea			+	
Urinary bladder			+	
Uterus			+	
Vagina			+	
Zymbal gland			+	
Standard List				

**Toxicokinetics:** Blood samples were taken on Day 1 and Day 13 from the 18/sex/group satellite animals and sent to Sponsor for analysis. Report is contained in Attachment IIb of this submission (N 029-SX). Whole blood samples, approximately 0.5 ml (to allow for at least 0.2 ml plasma), were drawn from the orbital sinus of mice under halothane anesthesia and collected into tubes containing EDTA anticoagulant. Blood samples were drawn at pre-dose, 0.25, 0.5, 1, 2 and 5 hr post-dose using 3 animals/time-point/group. Animals were discarded after final blood collection on Week 13.

**Results:**

**Mortality:** Three animals died during the study, all females. Two animals (67F [control], 113F [200 mg/kg/day]) were believed to have died due to "dosing procedures" according to Sponsor whereas one animal (107F [100 mg/kg/day]) the cause of death was not determined. None of these deaths were considered by the Sponsor to be related to CG5503, though with one animal having an uncertain cause of death I believe this cannot be entirely ruled

out; nevertheless, there were 60 animals that received CG5503 with only one death undetermined.

**Clinical signs:** Primarily consisted of dose-related hair loss/thinning fur which occurred in a minority (33 → 8%) ♂HD between weeks 1-8. Females showed a similar profile of thinning fur with resolution by week 9, though a number of female control animals also demonstrated hair loss during this period.

**Body weights:** There was no significant treatment-related effects observed on body weight or body weight gain during the course of the study in either males or females.

Assessment: Body wt gain in ♂		CG5503 dose (mg/kg/day)				
		0	10	30	100	200
Start → 4 wks	Abs	2.7 (7.7)	2.5 (7.5)	3.2 (9.0)	2.6 (7.2)	3.0 (9.0)
Start → 13 wks	(% Δ)	6.1 (17.3)	6.1 (18.2)	7.2 (20.2)	6.2 (17.2)	6.1 (18.0)

Assessment: Body wt gain in ♀		CG5503 dose (mg/kg/day)				
		0	10	30	100	200
Start → 4 wks	Abs	3.5 (13.7)	2.5 (9.7)	3.4 (12.8)	2.4 (9.1)	3.5 (13.8)
Start → 13 wks	(% Δ)	6.6 (25.9)	5.5 (21.3)	6.5 (24.5)	5.6 (21.3)	6.5 (25.6)

**Food consumption:** No significant changes in food consumption were apparent over the duration of the study.

Food consumption (% change compared with vehicle control; weeks 1-13)					
	0	10	30	100	200
♂	-	-9.7	2.6	-1.8	-6.2
♀	-	-3.8	0	-5.1	2.5

**Ophthalmoscopy:** Not done

**Electrocardiography:** Not done

**Hematology:** No treatment related effects were observed and the several statistically significant findings were not dose-dependent and thought due to individual animal variation.

**Clinical chemistry:**

	% Change from Vehicle Controls										
	Males						Females				
	0	10	30	100	200	0	10	30	100	200	
AST	71 ± 11 g/L	1.4	12.7	25.4	26.8	79 ± 20 g/L	6.3	16.5	59.5*	22.8	
ALT	41 ± 5 g/L	12.2	14.6	34.1	68.3*	50 ± 11 g/L	-16.0	-14	34	24	DR*
Globulin	22 ± 1 g/L	-4.5	0	4.5	9.1	DR**					

Two way ANOVA, regression and Dunnett's test  
\* p< 0.05, \*\* p<0.01; DR = significant dose response test

Treated groups demonstrated dose-related alterations in AST, ALT and Globulin parameters. As can be seen in the table above, treated groups showed elevations in AST which were generally dose-dependent but only achieved statistical significance in females at the 100 mg/kg/day dose. ALT elevations in males were dose-dependent and reached significance at the high dose (+68.3% above vehicle control). ALT in females showed a significant dose-response relationship, although no single treated group was different from control. Similarly, globulin levels also demonstrated a significant dose-response relationship in males, whereas no such response was observed in female mice.

Urinalysis: Not done

Organ weights:

	% Change from Vehicle Controls									
	Males					Females				
	0	10	30	100	200	0	10	30	100	200
Kidney	-	1.0	4.4	0	0	-	-3.8	-2.7	-8.9	-7.6
Spleen	-	-7.9	-2.3	-7.9	7.4	-	2.8	-2.8	7.0	-0.7
Liver	-	3.9	6.4	2.2	7.4	-	2.8	3.0	7.7	11.4*
Heart	-	-6.7	-1.3	-5.4	-6.7	-	-5.3	-4.1	-1.2	-1.8
Brain	-	0	0	-1.8	-0.8	-	2.0	1.6	1.4	0.6
Test/Epid	-	6.1	-4.8	0.2	-3.7	N/A				

\* p< 0.05

Treated females demonstrated increased liver weight that appeared dose-related and reached statistical significance in the HD females (11.4% above controls, p< 0.05). Kidney weight was generally decreased in females as was heart weight in both sexes but these changes were mild (< 10%) and did not reach statistical significance.

Gross pathology: Contained in histopathology assessment.

Histopathology:

No clear treatment-related gross or microscopic pathology was observed on necropsy examination. Liver demonstrated a dark focus in 1/11 HD females examined. Microscopic examination of the liver noted congestion in 1/11 UMD (100 mg/kg/day) and 1/11 HD females, but was absent in males. Hepatocyte vacuolation was observed in 1/11 UMD and 2/11 HD females and 1/12 UMD males, but HD males did not demonstrate this finding. Controls and lower doses in both sexes did not show evidence of vacuolation or congestion. Lungs of 1/12 HD males had a red area which was related to congestion/hemorrhage on microscopic analysis, a finding which was also observed in 1/12 male controls.

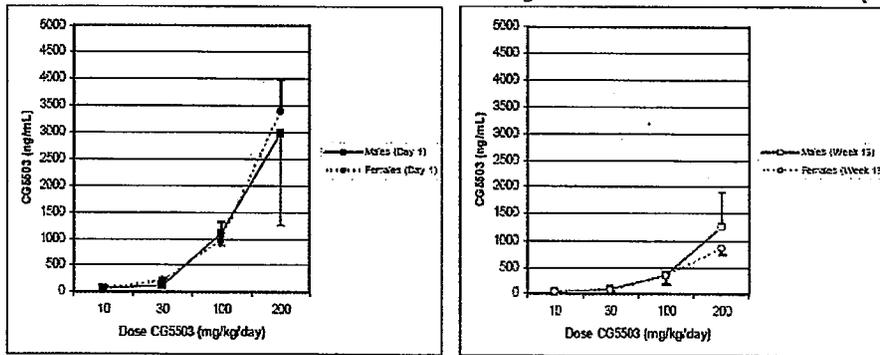
	Males					Females					Notes
	0	10	30	100	200	0	10	30	100	200	
Liver											
Congestion	0	0	0	0	0	0	0	0	1/11	1/11	
Hepatocyte vacuolation	0	0	0	1/12	0	0	0	0	1/11	2/11	Minimal

Toxicokinetics: Preliminary toxicokinetic data was submitted as an attachment (Iib) to this volume and was directly from Sponsor rather than [ ]

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	Dose	Day 1		Week 13		% Change C <sub>max</sub> Day 1 → Week 13
		T <sub>max</sub>	C <sub>max</sub>	T <sub>max</sub>	C <sub>max</sub>	
♂	10	0.25	74 ± 25	0.25	32 ± 9	-57%
	30	0.5	129 ± 5	0.5	87 ± 26	-32%
	100	0.25	1096 ± 230	0.5	347 ± 54	-68%
	200	0.25	2996 ± 1724	0.25	1256 ± 648	-42%
♀	10	0.25	57 ± 33	0.25	27 ± 10	-53%
	30	0.25	208 ± 53	0.25	74 ± 28	-64%
	100	0.5	968 ± 353	0.25	337 ± 153	-65%
	200	0.25	3394 ± 593	0.25	855 ± 116	-75%

Plasma Concentrations of CG5503 after Oral Gavage to Male Mice in a 13-Week Study



Summary of individual study findings: CG5503 was administered by oral gavage to CD-1 mice (12/sex/group) in doses of 0 (saline vehicle), 10, 30, 100 or 200 mg/kg for a total of 13 weeks for the purposes of determining appropriate dosing for a 2 year carcinogenicity bioassay. Satellite TK groups (18/sex/group) received the same dosing, the results of which were sent and analyzed directly by Sponsor.

Mortality was observed in 3 females during the course of the study, two of which were attributable to dosing error and one of which occurred in the MHD (100 mg/kg) group. No mortality was observed in the HD groups of either sex so this was not considered treatment-related in nature.

Relatively little toxicity was observed even at the HD in males and females. Body weight and food consumption were unaffected, hematology was unchanged, mild alterations in clinical chemistry (AST, ALT, globulin) were observed and even reached statistical significance but were not considered biologically relevant due to the generally small changes observed (i.e. < 2-fold changes in all cases).

Organ weights changed only slightly and non-significantly in males, with the liver showing some dose-dependent increase in weight (7.4% vs. controls), with significant, though mild increases observed in females (11.4% at HD vs. controls). Histopathologic assessment of the liver, which

was the only tissue examined in this study, revealed little pathology in females, primarily consisting of congestion in 1/11 MHD and HD animals with no evidence in males and hepatocyte vacuolation, considered minimal in nature, in 1/11 MHD females and 2/11 HD females. Hepatocyte vacuolation was observed in 1/12 MHD males but was absent from HD males and thus does not appear to be an important result of drug treatment. Other liver findings, such as inflammatory cell foci were observed at a high incidence in control animals as well as in lower dosed animals and was not considered a treatment-related effect. Focal cell necrosis was observed in male and female mice but was never greater than 1/12 animals in males and was not observed in the HD group males, HD females had evidence of focal cell necrosis in 2/11 animals but 2/11 control animals also were reported to demonstrate this finding. Taken together, focal cell necrosis does not appear to be a treatment-related effect in CD-1 mice at doses used.

**Toxicology conclusions:**

Little toxicity was observed with repeated dosing of CG5503 up to 200 mg/kg/day by oral gavage in CD-1 mice. Thinning of fur in the high dose animals, mild changes in liver enzymes correlating with mild changes in liver weight which were not significant in males but significant in females were apparent alongside large reductions in exposure to parent drug over the course of the study. Tissues other than liver were not assessed by microscopy for histopathology as this was the target organ in the previous 13-week study in NMRI mice. Nevertheless, no obvious changes in hematology or clinical chemistry were observed indicating another (i.e. new) potential target organ for CG5503 in this strain of mouse.

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**Study title:** 13-Week Dose Range Finding Oral (Feeding) Toxicity Study in the Wistar Rat (From review dated February 4, 2002, IND 61,345, SN013 submitted December 10, 2001, Kathleen Haberny, Ph.D.)

**Key study findings:**

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- Decreased body weights in males (-13% and -16% at 500 and 1000 mg/kg/d respectively), decreased body weight gains in males (-14%, -23%, -30% at 250, 500 and 1000 mg/kg/d) and females (-15% at 1000 mg/kg/d)
- Increased hemoglobin (1000 mg/kg/d), HCT (1000 mg/kg/d), reticulocytes (250 and 1000 mg/kg/d), HFR (250-1000 mg/kg/d), MFR (250 and 1000 mg/kg/d), and decreased LFR (250 and 1000 mg/kg/d) in females
- Decreased triglycerides (males at 250 and 1000 mg/kg/d), ASAT (males 500-1000 mg/kg/d) bilirubin (females at 500 and 1000 mg/kg/d)
- Increased GGT (males at 500 and 1000 mg/kg/d), LDH (females at 1000 mg/kg/d), ALP (females at 500 and 1000 mg/kg/d)
- Treatment-related increase in relative liver weights (males), relative kidney weights (males and females), relative testes weights, relative brain weights (males and females), and relative ovary weights, treatment-related decrease in heart weights (males and females), thymus weights (males), spleen weights (males)
- Duodenum dilation/discoloration in females at 500 and 1000 mg/kg/d
- Decreased thymus size in males at 250-1000 mg/kg/d, females at 1000 mg/kg/d
- Hepatocellular hypertrophy in 30% males and females at 500 mg/kg/d, and 90%-100% male and females at 1000 mg/kg/d
- MTD for the carcinogenicity study in female rats was 250 mg/kg/d due to 30% incidence of hepatocellular hypertrophy at 500 mg/kg/d. In males, the MTD was identified as 250 mg/kg/day due to a 14% decreased in body weight gain and 30% incidence of hepatocellular hypertrophy at 500 mg/kg/day

Study no: 800515

Volume # 5, and page # 1

Conducting laboratory and location: [ ]

Date of study initiation: February 12, 2001

GLP compliance: No

QA report: yes ( ) no (x)

Drug BN200 (GRT CG5503), lot # CEWS113, radiolabel: None, and % purity: 97.7%

Formulation/vehicle: Drug substance in feed

Methods (unique aspects):

Dosing:

Species/strain: Rat, [ ]: WIST (SPF)

#/sex/group or time point (main study): 10

Satellite groups used for toxicokinetics or recovery: TK: 6

Age: 5 weeks

Weight: 87-111 g males, 75-100 g females

Doses in administered units: 0, 250, 500, 1000 mg/kg/day

Route, form, volume, and infusion rate: Oral, admixture in feed, daily for 91 days

Observations and times:

Clinical signs: Twice daily

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Body weights: Pretest and daily  
 Food consumption: Pretest and weekly  
 Ophthalmoscopy: Pretest and week 13  
 EKG: Not done  
 Hematology: Pretest and week 13  
 Clinical chemistry: Pretest and week 13  
 Urinalysis: Not done  
 Gross pathology: Week 13  
 Organs weighed: See Histopathology Inventory below, at 13 weeks  
 Histopathology: See Histopathology Inventory below, at 13 weeks  
 Toxicokinetics: Day 3, Weeks 6 and 13, at 3 time points 6 hours apart  
 Other: Not done

**Results:**

Mortality: No deaths  
 Clinical signs: Alopecia with dose-related increase in incidence and severity, no palpable tissue masses  
 Body weights:

**Body Weights (Mean ± SD)**

		0 mg/kg/d n=10	250 mg/kg/d n=16	500 mg/kg/d n=16	1000 mg/kg/d n=16
Males:	Day 1	145 ± 6.1	151 ± 8.0	147 ± 7.8	150 ± 8.3
	Day 91	408 ± 42.9	384 ± 29.2	353 ± 32.7** (-13%)	341 ± 26.0** (-16%)
Females:	Day 1	122 ± 10.4	120 ± 9.7	121 ± 7.8	121 ± 7.8
	Day 91	238 ± 25.7	232 ± 18.5	225 ± 16.6	218 ± 17.1(-8%)

\*p<0.05; \*\*p<0.01

**Percent Body Weight Gain Day 91 (Mean ± SD)**

		0 mg/kg/d n=10	250 mg/kg/d n=19	500 mg/kg/d n=19	1000 mg/kg/d n=19
Males:	Day 91	180.7 ± 26.7	154.4 ± 19.5** (-14%)	140.0 ± 15.3** (-23%)	127.2 ± 13.0**(-30%)
Females:	Day 91	95.8 ± 11.9	93.8 ± 11.9(-2%)	85.3 ± 11.2 (-10%)	81.3 ± 14.2*(-15%)

\*p<0.05; \*\*p<0.01

The percent body weight gains in the males administered CG5503 at 250, 500, and 1000 mg/kg/day were 85%, 77%, and 70% the percent weight gain in the controls, respectively. The percent body weight gains in the female rats administered CG5503 at 250, 500, and 1000 mg/kg/day were 98%, 89%, and 85% the percent weight gain in the controls, respectively.

Food consumption: No treatment-related effects  
 Ophthalmoscopy: No treatment-related effects  
 EKG: Not done  
 Hematology: See under Results of the Clinical Laboratory Investigations below  
 Clinical chemistry: See under Results of the Clinical Laboratory Investigations below

**Results of the Clinical Laboratory Investigations# (Week 13)**

	0 mg/kg/d n=10	250 mg/kg/d n=10	500 mg/kg/d n=10	1000 mg/kg/d n=10
Hematology				

Reticulocytes (Retic., T/L)	Females	0.2148	0.2675* (+25%)	0.2454 (+14%)	0.2638* (+23%)
Retic. fluorescence ratio: high (HFR, %)	Females	4.6	11.5** (+150%)	10.0** (+117%)	11.3** (+146%)
Retic. fluorescence ratio: middle (MFR, %)	Females	30.8	38.1** (+24%)	32.0 (+4%)	35.6* (+16%)
Retic. Fluorescence ratio: low (LFR, %)	Females	64.7	50.4** (-22%)	58.0 (-10%)	53.2** (-18%)
<b>Clinical Biochemistry</b>					
Triglycerides (mmol/L)	Males	1.15	0.78* (-32%)	0.90 (-22%)	0.74** (-36%)
Aspartate aminotransferase (mckat/L)	Males	1.18	1.17	1.02* (-15%)	0.97** (-18%)
Gamma-Glutamyl transferase (ukat/L)	Males	15.49	12.77	31.81** (+105%)	30.17** (+98%)
Urea (mmol/L)	Females	6.84	7.50	8.26** (+21%)	9.23** (+35%)
Total bilirubin (mcmol/L)	Females	2.80	2.68	1.94** (-31%)	1.88** (-33%)
Lactate dehydrogenase (mckat/L)	Females	2.28	3.25	2.79	4.28** (+88%)
Alkaline Phosphatase (mckat/L)	Females	1.24	1.45	2.28** (+84%)	2.68** (+116%)
Alpha2-Globulin (l)	Females	0.057	0.059	0.064** (+12%)	0.068** (+19%)
Beta-Globulin (l)	Females	0.145	0.146	0.160** (+10%)	0.167** (+9%)
Albumin/globulin Ratio	Females	1.52	1.50	1.39* (-9%)	1.28** (-16%)
Albumin (g/L)	Females	42.76	44.50	39.68* (-7%)	38.15** (-10%)
Alpha2-Globulin (g/L)	Females	4.03	4.37	4.36	4.60** (+15%)
Beta-Globulin (g/L)	Females	10.28	10.80	10.88	11.35** (+10%)

##Only parameters with treatment-related changes significantly different from controls are presented; there were no significant findings in the remaining clinical laboratory parameters; values represent group means  
\*p<0.05; \*\*p<0.01

Urinalysis: Not done

Organ weights: See under Results of the Necropsic Evaluation below

Gross pathology: See under Results of the Necropsic Evaluation below

Histopathology: See under Results of the Necropsic Evaluation below

**Results of the Necropsic Evaluation # (% difference from controls in parentheses)**

		0 mg/kg/d n=10	250 mg/kg/d n=10	500 mg/kg/d n=10	1000 mg/kg/d n=10
Brain Weights (g)	Males	1.95 ± 0.05	2.05 ± 0.05** (+10%)	2.04 ± 0.05** (+10%)	2.01 ± 0.08* (+3%)
Relative Brain Weights (g/g body wt)	Males	0.51 ± 0.05	0.56 ± 0.03* (+10%)	0.64 ± 0.06** (+25%)	0.66 ± 0.04** (+30%)
	Females	0.79 ± 0.07	0.85 ± 0.06	0.91 ± 0.06** (+15%)	0.92 ± 0.07** (+16%)
Heart Weights (g)	Males	1.061 ± 0.104	1.021 ± 0.120	0.935 ± 0.133	0.905 ± 0.100* (-15%)
	Females	0.769 ± 0.095	0.762 ± 0.069	0.719 ± 0.056	0.676 ± 0.069* (-13%)
Thymus Weights (g)	Males	0.36 ± 0.08	0.26 ± 0.03** (-28%)	0.22 ± 0.07** (-39%)	0.25 ± 0.05** (-30%)
Relative Thymus Weights (g/g body wt)	Males	0.09 ± 0.01	0.07 ± 0.01* (-22%)	0.07 ± 0.02** (-22%)	0.08 ± 0.02 (-12%)
Spleen Weights (g)	Males	0.799 ± 0.106	0.814 ± 0.129	0.685 ± 0.122	0.656 ± 0.042* (-18%)
Relative Liver Weights (g/g body wt)	Males	2.34 ± 0.11	2.56 ± 0.09* (+9%)	2.86 ± 0.25** (+22%)	3.16 ± 0.25** (+33%)
Relative Kidney Weights (g/g body wt)	Males	0.50 ± 0.05	0.57 ± 0.02* (+14%)	0.59 ± 0.07** (+18%)	0.62 ± 0.03** (+24%)
	Females	0.58 ± 0.04	0.64 ± 0.04** (+10%)	0.66 ± 0.05** (+14%)	0.63 ± 0.02* (+9%)
Relative Testes Weights (g/g body wt)	Males	0.94 ± 0.09	1.07 ± 0.14	1.13 ± 0.14** (+20%)	1.23 ± 0.13** (+30%)
Relative Ovaries Weights (g/g body wt)	Females	0.042 ± 0.005	0.052 ± 0.012* (+24%)	0.051 ± 0.009	0.053 ± 0.007* (+26%)
Duodenum dilation/discoloration (% treated animals)	Females	0%/0%	0%/0%	10%/20%	100%/100%
Thymus reduced in size (% treated animals)	Males	0%	90%	80%	90%
Hepatocellular Hypertrophy (% treated animals, mean grade+)	Males	0%	0%	30% (1.0)	100% (1.9)
	Females	0%	0%	30% (1.0)	90% (1.8)

#Only organs and tissues with significant differences compared to or changes from controls are presented; there were no significant findings in the remaining organs or tissues or in the sex not represented; values represent group means ( $\pm$  SD) unless otherwise defined

\* $p < 0.05$ ; \*\* $p < 0.01$

$\beta$ Grade scale: 1=minimal; 2=slight; 3=moderate; 4=marked

### Toxicokinetics:

The following toxicokinetic data was given:

Species*	Dose	Route	CG5503		CG5503 Glucuronide	
			C <sub>max</sub> (ng/ml)	AUC <sub>0-24h</sub> (ng.h/ml)	C <sub>max</sub> (ng/ml)	AUC <sub>0-24h</sub> (ng.h/ml)
Rat	500 mg/kg/d	Dietary	10-100	400-630	25,000- 60,000	100000- 200000
Human (male-female)	100 mg	Oral	96	373-374	3700	19000
	200 mg	Oral	210-215	814-943	-	-

\*Rat data from the 90-day toxicity study, human data from Phase I PK study

Toxicokinetic data was not available for the other dose groups.

**Summary of individual study findings:** CG5503 administered to male and female Wistar rats at doses of 250-1000 mg/kg/day for 13 weeks resulted in alopecia with dose-related increases in incidence and severity. Body weights were significantly decreased in the male rats at 500 (-13%) and 1000 (-16%) mg/kg/day and body weight gains were reduced in the males by -14%, -23%, and -30% at 250-1000 mg/kg/d, respectively, and in females by -15% at 1000 mg/kg/d.

The major target organ of toxicity was the liver. Relative liver weights were increased +9%, +22% and +33% at 250, 500 and 1000 mg/kg/day, respectively in the males. The histopathology examination showed hepatocellular hypertrophy in the liver of 30% male and female rats given 500 mg/kg/day and 90%(female)-100% (male) rats given 1000 mg/kg/day CG5503. The histopathology findings in the liver were accompanied by several changes in the clinical chemistry including increased G-GT in the male rats (+105% and +98% at 500 and 1000 mg/kg/day respectively) indicating increased metabolic rate, and increased LDH in the female rats by +88% at 1000 mg/kg/day, indicating potential cellular damage.

The MTD in the 13-week study in Wistar rats was 250 mg/kg/day in females and 250 mg/kg/day in the males. This determinant was based on a decrease in body weight gains of 23% at 500 mg/kg/day in the males, and hepatocellular hypertrophy in both sexes at 500 mg/kg/day. This is in agreement with the sponsor's conclusion that the MTD for both sexes was 250 mg/kg/day.

**Study title:** CG5503: 26-Week Oral Toxicity (Gavage) Study in the Rat

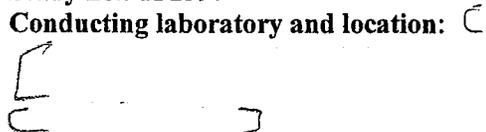
### Key study findings:

- Target organs were the CNS and liver:
  - Treatment-related effects by CG5503 (tapentadol) were characteristic of class effects by  $\mu$ -opioid receptor agonist agents in rats, with hepatic effects suggestive of adaptive liver metabolism
  - The effects were non-lethal, with the exception of respiratory depression possibly responsible for observed mortality

- Treatment-related effects were reversible during an 8-week recovery period
- Dose-related **↑ in deaths** without clear cause, resulting in early termination (Week 13) of the surviving rats at the 450 mg/kg/day dose, and addition of 10 rats/sex at 300 mg/kg/day with extension of the dosing period for those rats to 26 weeks to allow for adequate evaluation of reversibility
  - Deaths probably result of **respiratory depression**, a well-known class effect by  $\mu$ -opioid receptor agonist drugs
  - HD terminated early (Week 13) due to excessive mortality
- Clinical signs observed at  $\geq 150$  mg/kg/day
  - Dose-related **↑ excited behavior, recumbency, hunched posture, labored respiration, general poor condition**
  - Clinical signs consistent with known effects by  $\mu$ -opioid receptor agonists in rats
- BW & food consumption
  - **Reduced BW** in the males (M) (-6% to -7% compared to controls) and (F) (-4% to -7%) at 450 mg/kg/day and in the M (-6% to -7%) at 300 mg/kg/day
  - **Increased BWG** in the M (14.1%) and F (12.4%) compared to controls during the recovery period
  - **↓ food consumption** at 300 and 450 mg/kg/d in M and F and at 150 mg/kg/day in F
- Hematology
  - **↑ leukocyte count** (due to **↑ lymphocytes and segmented neutrophils**) in the F at 300 and 450 mg/kg/day
  - **↓ PT and APTT** in the M at 450 mg/kg/day in Week 13
  - **↑ fibrinogen** in the M and F at 450 mg/kg/day in Week 13
- Clinical chemistry
  - **↑ liver enzymes (ASAT, ALAT)** at 450 mg/kg/day in Week 13
  - Dose-related **↑ ALP and LDH** at 150 and 300 mg/kg/day in Weeks 13 and 26
- Urinalysis
  - **↑ urine volume** at all doses
  - **↑ specific gravity and osmolality** in F at 450 mg/kg/d
  - Effects reversed during recovery
- Organ weights: **↑ liver weights** at 300 and 450 mg/kg/day in M & F, and in M at 150 mg/kg/day, reversed during recovery period
- Gross pathology: **enlarged liver** at 150 (2 M), 300 (3 M) and 450 (6 M and 1 F) mg/kg/day
- Histopathology:
  - Centrilobular or diffuse **hepatocellular hypertrophy** at  $\geq 150$  mg/kg/day, possibly adaptive in response to increased liver metabolic activity
    - Found in absence of necrosis
    - Possible association with increases in liver enzyme activity, as increased glucuronyl transferase activity found in the evaluation of microsomes

- Reversible after 8-week recovery period
  - ↑ **fatty change** in liver at 300 mg/kg/day compared to controls
    - Possible relationship to altered lipid handling as a result of decreased food consumption and body weights
    - Reversible after 8-week recovery period
- Dose-related ↑ in exposure to test article in all treated groups
- NOAEL = 75 mg/kg/day
- Systemic exposure at the NOAEL represented approximately **0.8X** in the M (AUC = 391 ng.h/ml) and **1.7X** in the F (AUC = 857 ng.h/ml) the clinical exposure at the MRHD of 600 mg/day in a 70-kg patient on an AUC basis
- Systemic exposure at the NOAEL on a Cmax basis, possibly relevant to the CNS effects observed in the rats, represented approximately 2X in the males and 4.4X in the females the clinical Cmax at the MRHD
- Exposure to the glucuronide metabolite at the NOEL of 75 mg/kg/day represented approximately 9X the clinical exposure to the metabolite at the MRHD, AUC basis

Study no.: TP2397

Conducting laboratory and location: 

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Date of study initiation: May 28, 2001

GLP compliance: Yes

QA report: yes ( x ) no ( )

Drug CG5503 (BN200, tapentadol), lot # (Batch) CEWS112 and CEWS113, and % purity: 97.9% and 97.7%, respectively

**Methods**

**Doses:** 0 (saline vehicle control), 75, 150, 300, and 450 mg/kg/day

**Species/strain:** Wistar rats

**Number/sex/group or time point (main study):** The numbers of rats evaluated are presented in the following table (provided from the original NDA submission):

Group	No of animals	Dose [mg/kg]	Duration of treatment [weeks]	No of rats for treatment-free period	No of TK rats
1	30 M, 30 F	0	26	10 M, 10 F	None*
2	20 M, 20 F	150	26	None	6 M, 6 F
3	20 M, 20 F	300	26	10 M, 10 F	6 M, 6 F
4	30 M, 30 F	450	13	10 M, 10 F	None
5	20 M, 20 F	75	26	None	6 M, 6 F

\*Samples from control rats were collected from selected rats on day 1

The animal assignments are presented in the following table (provided from the original NDA submission):

mg/kg body weight	Group 1*	Group 2	Group 3	Group 4	Group 5 <sup>+</sup>	Group 6 <sup>++</sup>
Males A	1 - 20	31 - 50	51 - 70	71 - 100	237 - 256	
Males B	21 - 30					257 - 266
Males C1		201 - 203	207 - 209	213 - 215	297 - 299	
Males C2		204 - 206	210 - 212	216 - 218	300 - 302	
Females A	101 - 120	131 - 150	151 - 170	171 - 200	267 - 286	
Females B	121 - 130					287 - 296
Females C1		219 - 221	225 - 227	231 - 233	303 - 305	
Females C2		222 - 224	228 - 230	234 - 236	306 - 308	

A Toxicity testing (termination after 26 treatment weeks, except group 4)

B Recovery testing (termination after an additional 8-week recovery period, except group 4)

C Satellite animals for toxicokinetics

\* Control animals were treated with the vehicle only

<sup>+</sup> Additional group, treatment started at week 15.

<sup>++</sup> Supplemental recovery group, as all group 4 animals were sacrificed after week 13. Treatment of group 6 started at week 15.

**Route, formulation, volume, and infusion rate:** Test article dissolved in physiological saline solution (0.9% NaCl, administered by oral by gavage at 10 ml/kg; dose formulations analyzed for content, stability, and homogeneity at baseline, and in Weeks 13 and 26

**Satellite groups used for toxicokinetics or recovery:** 6/sex/dose at 75, 150 and 300 mg/kg/day for the TK evaluation, and 10/sex/dose at 0 and 300 mg/kg/day for recovery evaluation

**Age:** 6 weeks

**Weight:** 102-131 g males (M) and 85-109 g females (F)

**Unique study design or methodology:** The surviving HD (450 mg/kg/day) rats were sacrificed at the end of Week 13, after deaths were observed in 16/30 M and 17/30 F. Group 5 (75 mg/kg/day) M and F rats were added to the study in Week 14, and treated for 26 consecutive weeks, with an 8-week recovery period. Also, 10 additional M and 10 F were added to the study in Week 14 for a 26-week treatment period with 8-week recovery period, to replace animals that died or were sacrificed prematurely in the 300 mg/kg/day groups for adequate representation to assess recovery period reversibility. The rats were provided pelleted standard [ ] rat maintenance diet and community tap-water *ad libitum*.

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**Observation times:**

**Mortality:** Once daily

**Clinical signs:** Once daily during acclimatization, treatment and recovery periods

**Body weights:** Baseline and weekly throughout dosing period

**Food consumption:** Baseline and weekly throughout dosing period

**Ophthalmoscopy:** Baseline, Week 26 (end of dosing period) and Week 34 (after recovery period)

**EKG:** Not done

**Hematology:** Blood samples in fasted rats (18 hours) from the retro-orbital plexus, at 13, 26 and 34 weeks (end of recovery period); all standard parameters evaluated, including coagulation

**Clinical chemistry:** Blood samples in fasted rats (18 hours) from the retro-orbital plexus, at 13, 26 and 34 weeks (end of recovery period); all standard parameters evaluated

**Urinalysis:** Urine samples collected during 18-hour fast using metabolism cage, at 13, 26 and 34 weeks (end of recovery period); all standard parameters evaluated

**Gross pathology:** at 13 Weeks (Group 4), 26 Weeks (remaining main study groups) and 34 Weeks (recovery groups); the following were examined: adrenal glands, aorta, auricles, brain (including medulla/pons, cerebral and cerebellar cortex), cecum, colon, duodenum, epididymides, esophagus, extra-orbital lacrimal gland, eyes (with optic nerve and Harderian gland), femur (with bone marrow), heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (mesenteric, mandibular), mammary gland area, nasal cavity, ovaries, pancreas, pituitary gland, prostate gland, rectum, salivary glands (mandibular, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, mid-thoracic, lumbar), spleen, sternum (with bone marrow), stomach, testes, thymus, thyroid gland (with parathyroid gland), tongue, trachea, urinary bladder, uterus and all gross lesions

**Organ weights:** The following organs were weighed at necropsy: adrenal glands, brain, heart, kidneys, liver, spleen, and testes

**Histopathology:** Adequate Battery: yes ( x ), no ( )  
Peer review: yes ( ), no ( x )  
Toxicologic Pathologist:  }

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The following organs and tissues were examined (Groups 1, 3, and 6): adrenal glands, aorta, brain (including medulla/pons, cerebral and cerebellar cortex), cecum, colon, duodenum, esophagus, eyes (with optic nerve and Harderian gland), femur (with bone marrow), heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric, mandibular), mammary gland area, ovaries, pancreas, pituitary gland, prostate gland, rectum, salivary glands (mandibular, sublingual), sciatic nerve, skeletal muscle, skin, spinal cord (cervical, mid-thoracic, lumbar), spleen, sternum (with bone marrow), stomach, testes, thymus, thyroid gland (with parathyroid gland), trachea, urinary bladder, uterus, and all gross lesions. Additionally, the following organs were examined in Groups 2, 4, and 5: lungs, livers and gross lesions. The following were examined from

group 4 rats: liver, kidney, spleen, heart, brain (transverse sections of retrosplenial cortex, cingulum, and hippocampus), and gross lesions.

**Toxicokinetics:** Blood samples (0.5 ml) from the retro-orbital plexus on Dosing Day 1 and at Weeks 4, 13, and 26, at pre-dose, and at 0.25, 1, 2, and 3 hours after dosing, and in Week 26 at 5 hours after dosing, by cardiac puncture (1 ml); AUC<sub>0-8</sub> evaluated presumably due to the short tapentadol t<sub>1/2</sub>

**Other:** P450 content, N-dealkylation activity, O-dealkylation activity and glucuronyl transferase activity evaluated in microsomes from the livers of 5 M and 5 F each given 0 and 75 mg/kg/day, at necropsy (after dosing Week 26)

**Results:**

**Mortality:** Dose-related increase in spontaneous deaths; 1/20, 0/20, 15/30, and 17/30 females at 75, 150, 300, and 450 mg/kg/day, respectively, and 0/20, 3/20, 11/30, and 16/30 males at 75, 150, 300, and 450 mg/kg/day, respectively (note that the 450 mg/kg/day groups were terminated early in Week 13). The rats that died showed excited behavior, bedding in the mouth, lateral/ventral recumbency, hunched posture, labored breathing and generally poor condition, with decreased food consumption and body weights, increased leukocytes, reduced PT and APTT with increased fibrinogen. Additionally, increased ALAT and ASAT (males), urine volume, specific gravity and osmolality (females), and increased liver weights with liver enlargement and centrilobular or diffuse hepatocellular hypertrophy were observed in these animals. The incidences of mortality observed in this study are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	75		150		300		450			
Number of animals	30M	30F	20M	20F	20M	20F	20M	20F		
Noteworthy findings										
Died or sacrificed moribund	0	0	0	1	3	0	7	8	16	17

**Clinical signs:** Dose-related clinical signs were observed at doses of 150 mg/kg/day and above during Weeks 1-13 of dosing:

- Excited behavior at ≥ 150 mg/kg/day
- Bedding material in mouth at ≥ 150 mg/kg/day
- Ventral/lateral recumbency, hunched posture, labored breathing, poor condition observed at ≥ 300 mg/kg/day
- Signs observed are characteristic of μ-opioid CNS effects in rodents
- All signs reversible during 8-week recovery period

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

Daily dose (mg/kg)	Control		75		150		300		450	
	30M	30F	20M	20F	20M	20F	20M	20F	30M	30F
Clinical observations										
Excitement	-	-	-	-	+	+	+	+	+	+
Hair loss	-	-	-	-	+	+	-	++	-	+
Labored respiration	-	-	-	-	+	-	++	+	++	++
Hunched posture	-	-	yes	no	no	no	yes	yes	yes	yes
Poor condition	-	-	-	-	+	-	++	++	++	+++
Tremor	-	-	-	-	-	-	-	-	+	++

- = No noteworthy findings, + = mild, ++ = moderate, +++ = marked

**Body weights:**

- Reduced BW in the M at 300 (-6% to -7% in Weeks 19-26) and 450 (-6% to -7% until Week 11) mg/kg/day and in the F at 450 mg/kg/day (-4% to -7% in Weeks 2-7)
- Reduced BWG in the M at 300 mg/kg/day (-11% o -23% compared to controls), and 75 mg/kg/day (-13% compared to controls), but not at 150 mg/kg/day
- Increased BWG in the M (+14.1%) and F (+12.4%) at 300 mg/kg/day compared to 6.8% in control M and 7.2% in control F during the recovery period, demonstrating reversibility of the treatment-related effect on BW

The results (means shown for controls, percent differences from controls for the treated groups, statistical significance based on actual data and not on percent differences) of the body weight evaluation at the end of the study are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	Control		75		150		300		450	
	30M	30F	20M	20F	20M	20F	20M	20F	30M	30F
Body weight (g)	518	173	-7.7*	-6.6*	-1.8	-1.3	-6.8	-1.8	n.a.	n.a.

Dunnett's Test: \* = p<0.05, \*\* = p<0.01

**Food consumption:** Transient reduction early in dosing period at 150 (-5% to -12% in F, Weeks 1-9), 300 (-7% in M in Week 1, and -4% to -9% in F in Weeks 1-7) and 450 (-4% to -12% in M in Weeks 1-2, and -4% to -11% in F in Weeks 1-5) mg/kg/day, without dose-relationship

**Ophthalmoscopic examination:** No treatment-related effects

**Hematology:**

- Increased total leukocytes in F given 300 and 450 mg/kg/day in Weeks 13 and 26, due to increased lymphocytes in Week 13 and increased segmented neutrophils in Week 26
- Slight reduction in thromboplastin time (PT) and activated partial thromboplastin time (APTT) in M at 450 mg/kg/day in Week 13; reduced APTT in M at ≥150 mg/kg/day in Week 26

- Increased **fibrinogen** in the M and F at 450 mg/kg/day in Week 13, and in F at  $\geq 150$  mg/kg/day in Week 26

The notable hematology findings at the end of Week 13 are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg) Number of animals	Control		75		150		300		450	
	30M	30F	20M	20F	20M	20F	20M	20F	30M	30F
Red blood cells (T/L)	9.22	8.40	8.73**	8.37	8.86**	8.53	8.87**	8.41	9.00	8.46
Hematocrit (a)	0.49	0.48	0.47*	0.47	0.48	0.49**	0.49	0.50**	0.50	0.50
Lymphocytes (G/L)	3.88	2.62	4.14	3.18*	3.81	3.45**	3.69	3.83**	4.44	3.93
PT (sec)	12.4	11.5	12.2	12.5**	12.6	11.4	12.1*	11.5	12.2	12.1
APTT (sec)	24.7	20.7	22.4**	19.5*	22.4**	21.1	23.9**	21.2	24.0	22.7
Fibrinogen (mg/dL)	371	200	298**	179	354	277**	349	260	358	231

Dunnett's Test: \* =  $p < 0.05$ , \*\* =  $p < 0.01$

APTT = Activated partial thromboplastin time, n.a. = not applicable, PT = Thromboplastin time (=Prothrombin time)

**Clinical chemistry:**

- Slight increases in **ALT** at 450 mg/kg/day in M and F, Week 13, only
- Increased **AST** at 450 mg/kg/day in M and F, Week 13, only
- Slight increases in **ALP** and **LDH** at  $\geq 150$  mg/kg/day in Weeks 13 and 26, within historical range, no dose-dependence, in 1 sex only, not considered toxicologically relevant in agreement with the Sponsor

The notable serum chemistry findings at the end of the study are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg) Number of Animals	Control		75		150		300		450	
	20M	20F	20M	20F	20M	20F	20M	20F	20M	20F
Serum chemistry										
Glucose (mmol/L)	4.39	4.13	4.27	4.13	4.85	4.07	4.59	4.08	2.82	2.93
Aspartate Aminotransferase ( $\mu$ kat/L)	1.14	1.45	1.15	1.30	1.17	2.14	1.25	1.77	8.66	1.96
Alanine Aminotransferase ( $\mu$ kat/L)	0.52	0.58	0.47	0.42	0.52	0.56	0.46	0.49	1.29	0.47
Lactate Dehydrogenase ( $\mu$ kat/L)	2.20	3.04	2.08	3.25	2.19	2.84	3.02**	4.22*	4.53	5.87
Albumin (g/L)	34.38	42.24	32.80**	40.64	33.18**	37.95**	31.62**	36.64**	31.70	36.15

Dunnett's Test: \* =  $p < 0.05$ , \*\* =  $p < 0.01$

**Urinalysis:**

- Increased urine volume at  $\geq 75$  mg/kg/day (Week 13), and 300 mg/kg/day (Week 26)
- Increased specific gravity and osmolality in F at 450 mg/kg/day in Week 13

**Gross pathology:**

- **Liver enlargement** at 150 (2 M) and 300 (3 M) mg/kg/day, reversible during the recovery period

**Organ weights:**

- Increased **liver weights** in M (+16%) and F (+12%) at 300 mg/kg in Week 26, and at 450 mg/kg/day in Week 13 compared to Week 26 controls, and in the M at 150 mg/kg/day at 26 weeks

- No differences from controls liver weights in the treated rats following 8 weeks drug-free recovery period

**Histopathology:**

- **Centrilobular or diffuse hepatocellular hypertrophy** at 150-450 mg/kg/day
- **Fatty change in liver** at all doses including controls, without dose-relationship but with higher incidence at 300 mg/kg/day, reversible after 8-week recovery period

The results of the histopathology examination at the end of the study are presented in the following table (provided from the original NDA submission):

Daily dose (mg/kg)	Control		75		150		300		450	
	20M	20F	20M	20F	20M	20F	20M	20F	20M	20F
Number of Animals										
Liver (total affected)	8/20	9/20	18/20	11/20	15/20	11/20	18/20	17/20	15/20	9/20
Fatty change										
Centrilobular hypertrophy	0	0	0	0	4/20	1/20	5/20	0/20	6/20	2/20

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

Dose [mg/kg]	Males				Females				
	75	150	300	450†	75	150	300	450†	
<b>On day 1</b>									
$C_{max}$	[µg/L] ±	65	250	623	453	45	167	166	789
	Stand Dev	± 55	± 155	± 447	± 350	± 9	± 59	± 34	± 322
$AUC_{0-24}$	[µg·h/L] ±	77	255	445	679	79	249	302	1067
	Stand Dev	± 19	± 91	± 161	± 291	± 13	± 41	± 53	± 520
<b>Week 4</b>									
$C_{max}$	[µg/L] ±	117	311	961	411	237	295	507	2934
	Stand Dev	± 32	± 374	± 1308	± 132	± 24	± 56	± 118	± 1632
$AUC_{0-24}$	[µg·h/L] ±	176	233	434	541	407	398	1075	1986
	Stand Dev	± 52	± 147	± 408	± 182	± 71	± 75	± 201	± 660
<b>Week 13</b>									
$C_{max}$	[µg/L] ±	314	429	1312	1250	558	656	695	848
	Stand Dev	± 18	± 59	± 398	± 1216	± 492	± 204	± 128	± 302
$AUC_{0-24}$	[µg·h/L] ±	396	375	874	1236	542	844	1032	1530
	Stand Dev	± 223	± 26	± 150	± 764	± 295	± 370	± 268	± 660
<b>Week 26</b>									
$C_{max}$	[µg/L] ±	252	507	1451	nd	520	451	912	nd
	Stand Dev	± 113	± 173	± 8		± 422	± 129	± 1072	
$AUC_{0-24}$	[µg·h/L] ±	391	1060	1987	nd	857	1461	3088	nd
	Stand Dev	± 229	± 366	± 779		± 480	± 432	± 1482	

† = All surviving animals were sacrificed after week 13 due to high mortality.  
 nd = No data.

The results of the toxicokinetic assessments showed generally dose-linear increases in exposure in the M and F, with higher AUC values in the F than in the M, although the mean group values revealed high within-group variability, particularly at the 450

mg/kg/day dose. Metabolite (tapentadol O-glucuronide) exposure ( $AUC_{0-8h}$ ) tended to increase over the 26-week period: values at Week 26 were 148250, 270707, and 541414 ng.h/ml in the M, and 153093, 287857, and 542690 ng.h/ml in the F at 75, 150, and 300 mg/kg/day, respectively.

**Other:**

- Significant **induction of glucuronyl transferase activity** found at the end of the study in the microsomes from F but not M, given 75 mg/kg/day, compared to controls (higher dose groups not evaluated)

**Study title:** CG5503 (BN200): 13-Week Oral (Gavage) Toxicity Study in the Dog

**Key study findings:**

- 2 deaths at the HD (120/80 mg/kg/d)
- BWG reduced at the end of the study in the MD F (35 mg/kg/d) and HD F
- Food consumption reduced in the MDF and HDM and F
- Treatment-related clinical signs were **convulsions** at 120 mg/kg/day in the M, and dose-related increased severity and incidence of decreased activity, salivation, recumbency, and vomiting
- Treatment-related **QT prolongation** at the MD in Week 13, and HD in Weeks 1 and 13
- Treatment-related **decreased gamma glutamyltransferase** (not associated with hepatotoxicity) and increased serum sodium
- **Thymic atrophy** at the MD and HD, and **prostate gland inflammation** in the MD and HD M at the end of the dosing period
- Evaluation of hepatic microsomal enzyme activity showed **significant induction of aminopyrine N-demethylase activity** in M and F, and **inhibition of glucuronyltransferase activity** in M
- The NOAEL in this study was 10 mg/kg/day
- The systemic exposure ( $AUC_{0-t} = 18.9-17.1$  ng.h/ml, Day 91) at the NOAEL represented approximately **0.04 times** the clinical exposure ( $AUC = 500$  ng.h/ml) at the MRHD of 600 mg/day on an AUC basis.
- The  $C_{max}$  at the NOAEL, relevant to the CNS and QT prolongation effects of CG5503 in the Beagle dogs (4.2-4.4 ng/ml) represented approximately 0.04 times the  $C_{max}$  (118 ng/ml) at the MRHD.
- Plasma O-glucuronide metabolite not evaluated in the TK assessments

**Study no.:** TP2415

**Conducting laboratory and location:** [redacted]

**Date of study initiation:** August 20, 2001

**GLP compliance:** Yes

**QA report:** yes (x) no ( )

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**Drug Tapentadol (referred to as CG5503 and BN200), lot # (Batch) CEWS112, and % purity: 97.9%**

**Methods**

**Doses:** 0 (vehicle control), 10, 35, and 80 mg/kg/day

The animal assignments are presented in the following table (provided from the original NDA submission):

<b>Group Allocation</b>				
	<b>Group 1</b>	<b>Group 2</b>	<b>Group 3</b>	<b>Group 4</b>
	<b>0</b>	<b>10</b>	<b>35</b>	<b>120*</b>
	<b>mg/kg/day</b>	<b>mg/kg/day</b>	<b>mg/kg/day</b>	<b>mg/kg/day</b>
<b>Males</b>	1 - 6	7 - 10	11 - 14	15 - 20
<b>Females</b>	21 - 26	27 - 30	31 - 34	35 - 40

\*From treatment day 22 the dose level of group 4 was reduced to 80 mg/kg/day due to severe clinical signs and one death.

The last two males and females in group 1 and 4 were allocated to a 4-week recovery period after the treatment period.

The animals received the test item once daily by gavage for 13 weeks. The control animals of group 1 received only the vehicle.

**Species/strain:** Beagle dogs (pure-bred, de-wormed, vaccinated against distemper, leptospirosis, parainfluenza, contagious hepatitis, rabies, and parvovirus)

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

**Route, formulation, volume, and infusion rate:** Test article dissolved in tap water and administered by oral gavage at 2 ml/kg, once daily

**Satellite groups used for toxicokinetics or recovery:** 2/sex/dose at 0 and 80 mg/kg/day for evaluation of reversibility following a 4-week recovery period.

**Age:** 7-9 months

**Weight:** 6.6-10.8 kg

**Unique study design or methodology:** High dose groups originally received 120 mg/kg/day, with dose reduction to 80 mg/kg/day on Day 22 following mortality in 1 male dog on Day 21. Food (350 g pelleted standard dog maintenance diet) was provided 1h after dosing, and uneaten food removed from the cage after 3h. Tap water was provided *ad libitum*.

**Observation times and results**

**Mortality:** Twice daily

**Clinical signs:** Twice daily

**Body weights:** Once weekly from pretest and until necropsy

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**Food consumption:** Daily from pretest through end of study, reported weekly

**Ophthalmoscopy:** Baseline, Week 13 and after 4-week recovery period

**EKG:** Baseline, Weeks 1 and 13, and after 4-week recovery period

**Hematology:** Blood samples collected from overnight-fasted dogs (jugular vein) at baseline, Weeks 6 and 13, and after the 4-week recovery period; standard hematology parameters were evaluated.

**Clinical chemistry:** Blood samples collected from overnight-fasted dogs (jugular vein) at baseline, Weeks 6 and 13, and after the 4-week recovery period; standard clinical chemistry parameters were evaluated.

**Urinalysis:** Urine collected at baseline, Weeks 6 and 13, and after the 4-week recovery period from overnight-fasted dogs using a catheter; standard urinalysis parameters were evaluated.

**Gross pathology:** All dogs, including decedents were evaluated. Necropsy was performed in the surviving dogs after 13 weeks treatment and after the 4-week drug-free recovery period. The following

**Organ weights:** All dogs, including decedents at necropsy. The following organs were weighed: adrenal glands, brain (including brainstem), heart, kidneys, liver, pituitary gland, prostate gland, spleen, testes with epididymides, and thyroid gland with parathyroid.

**Histopathology:** Adequate Battery: yes ( x ), no ( )

Peer review: yes ( x ), no ( x )

Veterinary Pathologist:

Target organs were cross-checked by

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The tissues and organs from all dogs were examined, including the animals that died during the study. The following tissues and organs were examined microscopically: adrenal glands, aorta, bone (femur including articular surface), bone marrow (sternum), brain (including medulla/pons, cerebral and cerebellar cortex), epididymides, esophagus, eyes with optic nerve, female mammary gland area, gallbladder, heart, kidneys, large intestine (cecum, colon, rectum), larynx, liver, lungs, lymph nodes (retropharyngeal, mesenteric), ovaries, pancreas, pituitary gland, prostate, gland, salivary glands (mandibular, parotid), sciatic nerve, skeletal muscle, skin, small intestine (duodenum, jejunum, ileum), spinal cord (cervical, mid-thoracic, lumbar), spleen, stomach, testes, thymus, thyroid gland (including parathyroid gland), tongue, trachea, urinary bladder, uterus, and all gross lesions.

**Toxicokinetics:** Blood samples (4 ml drawn from jugular vein) collected from all dogs on Dosing Day 1 and during Week 13, at pre-dose, and 0.5, 1, 3, 6, and 12 hours after drug administration.

**Other:** Hepatic microsomal enzymes were evaluated in liver samples (left lobe, 20 g) recovered during necropsy in the HD and control dogs. P450 content, N-dealkylation activity, O-dealkylation activity and glucuronyltransferase activity were tested

**Results:**

**Mortality:** Spontaneous death in 1 HD M (No. 20) at 120 mg/kg/day on Day 21, and one HD M sacrificed *in extremis* (No. 18) on Day 23 after first 80 mg/kg dose following Day 22 dose reduction from 120 mg/kg/day. The cause of death could not be determined in either case; however, the observations in these dogs prior to death were decreased activity, whimpering, apathy, recumbency, fearfulness, panting, tachypnea, and convulsions. The necropsy examination of dog No. 18 showed alveolar macrophages with hemosiderin in the lung (phagocytic cells), characteristic of lung congestion, without evidence of chronic cardiac failure in this animal. Dog No. 20 showed slight interstitial pneumonia.

**Clinical signs:**

- Observed at  $\geq 35$  mg/kg/day with slight dose-relationship: decreased activity, salivation, recumbency, and occasional tremors, vomiting, whimpering and twitching, beginning at 15-30 minutes after dosing and lasting for approximately 5 hours after dosing, throughout the study
- Convulsions at the HD:
  - indicated by paddling movements, tremors and twitching
  - 3 occasions in Weeks 2 and 3 in HD M no. 18, 1 occasion each in HD M No. 20, HD F No. 37, and ND F No. 38 (at 120 mg/kg/day)
  - Observed between 0.5 and 1 hour after dosing, except for immediate effect in HD F No. 38.

The notable clinical observations are presented in the following table (provided from the original NDA submission):

Daily Dose (mg/kg)	0 (Control)		10		35		120:80	
	M	F	M	F	M	F	M	F
Number of Animals								
Clinical Observations								
Convulsions	-	-	-	-	-	-	++	-
Decreased activity	-	-	-	-	-	+	++	++
Salivation	-	-	+	++	++	++	++	-
Recumbency	-	-	-	-	Yes	Yes	Yes	Yes
Vomiting	-	-	Yes	-	Yes	Yes	Yes	Yes
Feces containing mucus	-	-	Yes	-	Yes	-	Yes	Yes

- = No noteworthy findings, + = Mild, ++ = Moderate, +++ = Marked

**Body weights:** Reduced at 120 mg/kg/day (- 0.2 to -1.2 kg) in Weeks 1 and 2 in the M and F, persisting in 2 HD F into Week 3, and reversed during the recovery period. The

body weights at the end of the dosing period are summarized in the following table (provided from the original NDA submission):

Daily Dose (mg/kg)	0 (Control)		10		35		120/80	
	4M	4F	4M	4F	4M	4F	4M	4F
Number of Animals								
Body Weight (% <sup>a</sup> )	10.4	8.8	+4.8	+5.7	+1.0	0	+5.8	0

<sup>a</sup> For controls, means are shown. For treated groups, percentage differences from controls are shown.

**Food consumption:** Slight reduction in the dogs given 120 mg/kg/day in Weeks 1-2, compared to baseline and control consumption. The food consumption is summarized in the following table (provided from the original NDA submission):

Daily Dose (mg/kg)	0 (Control)		10		35		120/80	
	4M	4F	4M	4F	4M	4F	4M	4F
Number of Animals								
Food Consumption (g/animal/day) <sup>a</sup>	322	292	+5.6	-0.7	+2.2	-19.9	-8.4	0

<sup>a</sup> For controls, means are shown. For treated groups, percentage differences from controls are shown.

**Ophthalmoscopy:** No treatment-related effects.

**EKG:**

- ↑QT interval at 120 mg/kg/day in Week 1, and after dose reduction in the HD groups to 80 mg/kg/day in Week 13.
- Slight ↑QT interval in several F at 35 mg/kg/day in Week 13.
- Corrected QT intervals (Van de Water's and Fridericia's) showed slight (but not statistically significant in the F) increases in these animals; therefore not entirely due to heart rate changes
- No other EKG findings including arrhythmias, except for one ventricular premature complex in 1 MD F in Week 1, considered to be spontaneous.
- No treatment-related effects during the recovery period.

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

Daily Dose (mg/kg)	0 (Control)		10		35		120/80	
	4M	4F	4M	4F	4M	4F	4M	4F
Number of Animals								
Electrocardiography								
QTc (rdW <sup>a</sup> msec) 0.5h after dosing	237	231	239	239	248	249	249	248
Arrhythmias								

<sup>a</sup> QTc = corrected QT interval.

**APPEARS THIS WAY  
ON ORIGINAL**