

toxicity was reversible at the doses, routes and durations studied, based on absence of hepatotoxic findings after treatment-free recovery periods.

Dog:

Repeated dose toxicology studies were conducted in Beagle dogs, with dosing durations of 10 and 28 days by the IV route, 9 days and 13-weeks by the SC route, and 2, 13, and 52 weeks by oral gavage. The main target organs of toxicity in the dog were the CNS, and gastrointestinal (GI) and cardiovascular (CV) systems.

Intravenous toxicity was assessed in M and F dogs given tapentadol for up to 10 consecutive days (1-7.5 mg/kg/day for 4 consecutive days with sacrifice 12 days after the last dose in the first dose-escalation phase dogs, and 7.5 mg/kg/day for 10 consecutive days with sacrifice 24 hours after the last dose in the maintenance treatment (second study phase) dogs. The results showed transient, treatment-related salivation (M), restlessness and whimpering (F, ≥ 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 after the injections. There were slight decreases in food consumption in the M and F at ≥ 5 mg/kg/day, and slight body weight loss in the HDF after treatment Day 2. Moderate body weight loss was found in most HD animals at the end of the 10-day treatment period. There were observations at the HD of increased glutamate dehydrogenase, total lipids, cholesterol, triglyceride, phospholipid, iron concentration and protein at the end of the study. Histopathology was not conducted in this study, but the macroscopic examination showed red foci at the injection sites in HD dogs.

A subsequent 4-week study on intravenous tapentadol toxicity in dogs (1-7.5 mg/kg/day) showed treatment-related clinical signs consistent with those observed in the 10-day study, with dose-related increases in incidence and severity of excessive salivation, decreased activity, hind-leg buckling uncoordinated movements, vomiting, urination, ventral recumbency and rhinorrhea beginning during or immediately after injection and lasting for 1-2 hours. Tachypnea, panting, retching, and defecation were seen with less frequency than were the other signs. Food consumption (F at ≥ 3 mg/kg/day and HD M) and body weights (HD F) were reduced in the F (≥ 3 mg/kg/day). There were no treatment-related effects on any other parameter, including ophthalmologic examinations, electrocardiograms (including QT interval), and inspection of the injection sites. Special assessments of microsomal drug metabolizing enzymes in the livers at necropsy showed no inhibition or induction of P450 enzymes, and no effects on phase II aminophenol glucuronyl transferase activity.

Subcutaneous tapentadol toxicity was tested in Beagle dogs dosed for a 7-9-day period (10 mg/kg b.i.d. followed by 7.5 mg/kg b.i.d.). The treatment-related findings were similar to those in the IV toxicology studies in dogs, and included CNS behavioral signs

known to be associated with opioid receptor agonist activity. The observations were dose-related increases in incidence and severity of decreased activity, recumbency, tremor, salivation, somnolence, forelimb and hindlimb buckling, uncoordinated movements and occasional whimpering. The male dogs also showed vomiting, and occasional pale or loose feces and fecal mucus. Several dogs showed injection site swelling. Food intake and body weights were reduced in the first dosing week in both treatment periods, and resolved after increasing the duration of the feeding period over the remainder of the study period. The reversibility of reduced food consumption and body weights, and observed decreases in the severity of the behavioral effects may have also been related to development of partial tolerance to tapentadol effects.

Two 3-month SC tapentadol toxicity studies were conducted in Beagle dogs (20-160 mg/kg/day dose escalation phase for 13 days followed by 40 mg/kg/day treatment phase on Days 14-91 in the first study, and 4-16 mg/kg/day in the second study). One F dog given 16 mg/kg/day died on dosing day 17 in the second study. The treatment-related clinical signs were similar to those observed in the IV toxicity studies in dogs, and included dose related restlessness, decreased activity, drowsiness, fearfulness, vocalization, unsteady gait, hindlimb weakness, ventral recumbency, spontaneous urination and defecation, vomiting and salivation, with increased respiratory frequency and forced respiration in several animals beginning around 30 minutes after dosing and lasting for up to 5 hours. The higher doses administered (≥ 40 mg/kg/day SC) produced defense behavior, tremor, twitches, and convulsions in 1 dog. A potential relationship of the tremors to possible seizure activity was not addressed. Acute tolerance was suggested by observed decreases in severity and duration of the behavioral signs after the second than after the first daily doses. Also, the clinical signs were progressively reduced in severity over the course of the treatment duration, further suggesting the development of partial tolerance to tapentadol CNS effects. Injection site inspections indicated that the dogs scratched the sites throughout the study. Reduced body weights, body temperature and heart rate observed early in the studies showed gradual, slight recovery throughout the dosing periods, but body weights remained below control levels at the end of the second study. Food consumption, greatly reduced during the first weeks of treatment, was also only partially reversed by extension of the feeding periods. The results of the ECG measurements showed treatment-related absolute and corrected QT prolongation at 30 minutes after dosing compared to baseline values in the dogs given ≥ 4 mg/kg b.i.d during Week 1, and trends toward increased absolute QT values in Weeks 4 and 13.

The necropsic examination in the 3-month SC toxicity studies in dogs showed local toxicity at the injection sites in both studies. The main injection site findings were subcutaneous ecchymoses with hemorrhages, edema and gelatinous consistency of underlying tissues, that indicated scratching by the dogs throughout the first study. There was considerable local injection site toxicity in the second study that was qualitatively similar to the findings in the first SC study, with dark red discoloration, hemorrhages, acute and subacute inflammatory infiltrates, fibrosis, phlebitis and thrombophlebitis, and at the HD (16 mg/kg/day) chronic focal or multifocal perivasculitis. The injection site effects were only partially reversible during the 4-week

recovery period, and suggested that the SC route is likely not suitable for evaluation of chronic tapentadol toxicity in dogs. Tapentadol-related GI toxicity was evident in the necropsy in the second study, by findings of hemorrhage in the mesentery, and dark red discolorations in the stomach, and small and large intestines. Toxicokinetic analyses showed consistent exposure to the test article and the metabolite tapentadol-O-glucuronide, with dose-proportional increases in concentrations, peak plasma concentrations at approximately 0.5 h, and considerably higher exposure to the metabolite ($t_{1/2} = 4\text{h}$) than to the parent drug ($t_{1/2} = 1.77\text{ h}$). Parent drug exposure (AUC) was higher in the dog that convulsed than in the other dogs, but there were no differences in peak plasma tapentadol concentration (C_{max}).

Oral (gavage) tapentadol toxicity was tested in two 2-week, and in 13- and 52-week studies in dog. The 2-week studies evaluated doses of 50 and 150 mg/kg/day in the first, and in the second study there was a 13-day dose escalation period (10-350 mg/kg/day) followed by a dose de-escalation period from 320 down to 200 mg/kg/day for 14 days. The clinical signs of tapentadol toxicity were generally similar to those observed in the IV and SC assays in the dogs (salivation, vomiting, irregular respiration, and recumbency), with observations of whimpering ($\geq 220\text{ mg/kg/day}$), somnolence ($\geq 280\text{ mg/kg/day}$), dyspnea, tachypnea or panting ($\geq 80\text{ mg/kg/day}$) and tremors ($\geq 160\text{ mg/kg/day}$) beginning approximately 15 minutes after oral dosing and persisting for up to 8 hours at the higher doses in the second assay. Convulsions were observed in several dogs in the first (1 M and 1 F at 50 mg/kg/day, and 1 M and 1 F at 150 mg/kg/day) and second (1F at 350, 320, 280, and occasionally at 200 mg/kg/day) study. ECG and hearing measurements were normal in both studies. Liver weights were increased in all treated dogs in the second but not in the first study, in the dogs given the dose escalations up to 350 mg/kg/day and then de-escalation from 350 to 200 mg/kg/day. Observed treatment-related increases in liver weights were without clinical laboratory, and macroscopic and microscopic correlates. Treatment-related GI toxicity revealed in the necropsies was manifest by activation of the enteric lymphatic system (Peyer's patches) in the small and large intestines with activated lymphoid follicles in the gastric mucosa and proximal small intestine suggesting hyperplasia in the germinal centers characteristic of gastrointestinal immune response.

The results of 13-week oral tapentadol toxicity observations in Beagle dogs (10-80 mg/kg/day) were comparable to those in the shorter term (2-week) oral studies and to the toxicity found by the IV and SC routes. Initial HD administration at 120 mg/kg/day produced severe CNS toxicity, beginning 15-30 minutes after dosing and lasting for up to 5 hours. The signs included tachypnea, apathy and convulsions with paddling movements, twitching and tremors, and mortality in 2 M dogs, prompting lowering the dose to 80 mg/kg/day on treatment Day 23 until the end of the study. The convulsions were observed at 30-60 minutes after tapentadol administration, except for a convulsion immediately after dosing in one of the F. Food consumption was reduced in the M (HD) and F ($\geq 35\text{ mg/kg/day}$), predominantly during the first several weeks of dosing. Body weight gain was reduced at the HD. The treatment-related effects on clinical signs and body weights were reversible after the 4-week recovery period.

ECG assessments 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 the high-dose groups in Weeks 1 (120 mg/kg/day) and 13 (80 mg/kg/day). Treatment-related decreased gamma glutamyltransferase and increased serum sodium were found. Macroscopic and microscopic examinations during necropsy showed thymic atrophy and prostate gland inflammation (≥ 35 mg/kg/day) and adrenal cortical hypertrophy in the M ≥ 35 mg/kg/day). Hepatic microsomal enzyme activity analysis in liver samples recovered during the necropsy in the HD and control dogs showed a statistically significant treatment-related induction of aminopyrine N-demethylase activity in the M and F, and inhibition of glucuronyltransferase activity in the M. The NOAEL in this study (10 mg/kg/day) represented systemic exposure to the parent drug of approximately 0.04 times the clinical exposure at the MRHD of 600 mg/kg/day on an AUC basis, and on a Cmax basis, relevant to the CNS and cardiovascular effects noted during the study. Plasma O-glucuronide metabolite was not assessed.

The chronic (52-week) oral (gavage) study in M and F Beagle dogs given tapentadol once daily (10-80 mg/kg/day) confirmed the target organs toxicity seen in the shorter term oral and injection (IV and SC) studies in dog, in the CNS and cardiovascular system. One HD F was euthanized *in extremis* on dosing Day 12 due to convulsions on several days observed within 30 minutes after dosing. There were no necroscopic abnormalities in this dog. Convulsions were also seen in another HD F on multiple days throughout the dosing period up to Day 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 during the recovery period. There were also treatment-related clinical signs consistent with tapentadol mu-opioid receptor agonist effects in the dog studies, including salivation, decreased activity, recumbency, vomiting, tremor, and occasional whimpering, and fearfulness, beginning at 15-30 minutes after dosing, and lasting for up to 5 hours. Reductions in food consumption (F at ≥ 30 mg/kg/day) and body weights (HD) were observed during the first several weeks of dosing.

ECG assessments in the chronic dog study 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 decreased partial thromboplastin time (PTT) values were found in the HD dogs, which were not reversible during the 4-week recovery period. The necropsy results were negative in the standard evaluations. However, special examination of brain showed minimal to slight focal gliosis with perivascular mononuclear cell infiltration in the medulla oblongata and/or pons in 2 M and F at 30 mg/kg/day, and in 1 HD F, with no correlation to seizure incidence, and are considered to be spontaneous, in agreement with the Sponsor. In the liver enzyme activity analysis, no tapentadol effects on cytochrome P450 content were found, but 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. Also, 2-aminophenol glucuronyltransferase activity was decreased in the M and F dogs. The NOAEL in the 52-week toxicity study in dogs (10 mg/kg/day) represented systemic exposure to the parent drug that of 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, and 0.06 times on a C_{max} basis, relevant to CNS and CV observations. The systemic exposure to the O-glucuronide metabolite at the NOAEL (10 mg/kg/day) in this study represented approximately 1.7X the clinical exposure at the MRHD. The exposure to the parent drug and metabolite at the NOEL for convulsions in this study represented approximately 0.4 and 5.2 times the clinical exposures to the parent drug and metabolite, respectively, at the MRHD on an AUC basis.

In summary, the main target organs of tapentadol toxicity in the repeated dose studies in dogs, most of which were commonly observed using several routes and durations of treatment, 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 were similar in all of the studies across dose ranges given, and included salivation, restlessness, recumbency, decreased activity, rhinorrhea, panting, labored breathing, and tachypnea. In the b.i.d. study in the dogs given twice daily subcutaneous (SC) tapentadol injections for 3 months, the signs were more severe after the first than after the second daily dose, suggesting development of short term tolerance, a known phenomena with mu-opioid receptor agonist treatment. Also, the severity of the clinical signs decreased with increasing duration of treatment within several of the studies, further suggesting tolerance to opioid-induced behavioral effects. Most notable of the clinical signs were convulsions, observed in M and/or F dogs treated by SC injection for 3 months at ≥ 40 mg/kg/day (NOEL = 20 mg/kg/day SC), and by oral gavage at ≥ 50 mg/kg/day (NOEL = not determined) and ≥ 200 mg/kg/day (NOEL = 160 mg/kg) 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 up to 4 weeks duration. The convulsions were accompanied by paddling movements, tremors, and twitching. A possible relationship of the tremors observed in several studies in dogs to seizure activity was not investigated. There was no tolerance development to the treatment-related convulsant effect in the dogs. Although most of the dogs that convulsed either were sacrificed *in extremis* or received dose reductions following the seizures, a F given 80 mg/kg/day by oral gavage for 52 weeks showed convulsions on multiple days up to day 358 of dosing.

Tapentadol-related cardiovascular effects in the Beagle dog were indicated by QT prolongation in the ECG measurements across studies, at ≥ 8 mg/kg/day SC for 3 months (NOEL 4 mg/kg/day) particularly during the first week of treatment, at ≥ 35 mg/kg/day (Week 13) and 120 mg/kg/day (Week 1) in the 13-week gavage study (NOEL 10 mg/kg/day) and at 80 mg/kg/day in the 52-week oral gavage study (NOEL 30 mg/kg/day). No other ECG effects were found in the studies in dog. QT prolongation is probably associated with norepinephrine reuptake inhibition by tapentadol.

Treatment-related GI toxicity was observed in dogs given tapentadol by 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 (≥ 4 mg/kg/day). The necroscopic examinations in several oral gavage studies revealed activated lymphoid follicles in the gastric mucosa and small intestines in the 2-week oral gavage study (≥ 50 mg/kg/day), that were attributed to GI immune response by the examining pathologist. Local tissue toxicity was found in dogs given IV and SC injections, and included red foci 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 throughout the dosing periods in both 3-month SC studies (≥ 20 mg/kg/day in one and ≥ 4 mg/kg/day in the second study).

Monkey:

Two-week pilot studies were conducted in *Cynomolgus* monkeys to evaluate toxicokinetics and compare toxicity of tapentadol given by repeated IV (5 mg/kg/day) and oral (15 mg/kg/day) administration. No necroscopic examinations were performed in this study. There was occasional, slight sedation noted after the intravenous infusions, but no other clinical signs, no effects on body weights and food consumption, and no local toxicity was found upon examination of the injection sites. The results of the toxicokinetic evaluation showed extremely low oral bioavailability (<1%) suggesting extensive first-pass metabolism, and a short half life (1h by IV), with no accumulation using either route over the 14-day period.

Overall Repeated Dose Toxicology Summary:

There were interspecies differences and similarities in target organ toxicity between the mouse, rat and dog. In the rat, the predominant treatment-related effect, after CNS clinical signs, was in the liver, evident by increased liver enzymes (e.g., ALAT, ASAT, ALP, etc) and increased liver weights, hepatocellular hypertrophy with fatty change at higher doses and longer durations, and hepatocellular necrosis. Hepatotoxicity was also a main treatment effect in mice, which showed increased liver enzymes in the clinical laboratory analyses and increased liver weights in the 2- and 13-week dietary studies and in the 13-week gavage study, and hepatocellular hypertrophy with group cell necrosis in the 13-week dietary study at high doses of 500 and 1000 mg/kg/day, in the males and females. In the dog, there were more severe CNS toxicity that included convulsions, and CV toxicity with treatment-related QT prolongation. Clinical signs were not evident in the dietary and gavage studies in mice, at up to high doses of 1000 mg/kg/day dietary for 13 weeks and 200 mg/kg/day by gavage for 13 weeks. Target organ similarities between rat and dog were in the CNS clinical signs, such as sedation, decreased body weights and food consumption in both species with tolerance development to these signs. Also, GI toxicity was found in rat and dog, but not in the mouse, and included red foci in stomach in the 4-week IV study in rat, and duodenal dilation at the highest dietary dose of 1000 mg/kg/day in rat. In the dog, gastrointestinal hemorrhages and activated lymphoid follicles in the gastric mucosa and small intestine were predominant. Local injection site toxicity was found in both rat and dog. These effects included infusion site swelling,

particularly by the SC route in rat, and red foci after IV infusion, and hemorrhage and fibrosis after SC injections in the dog. The results in both species showed greater local toxicity by SC than by IV dosing.

Most of the treatment-related toxicity, the clinical signs including convulsions, QT prolongation, and toxicity in the gastrointestinal system were reversible in the studies that used recovery period evaluations. However, injection site findings, such as hemorrhages, inflammatory infiltrates, fibrosis, phlebitis and thrombophlebitis after SC dosing in the 3-month study were only partially reversible at 4-weeks following drug withdrawal.

The results of the nonclinical toxicology studies suggest potential adverse effects in clinical treatment by mu-opioid receptor binding and norepinephrine reuptake inhibition. 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, suggest careful screening and monitoring patients with existing hepatic disease, seizure disorders and cardiovascular conditions. Most of the treatment-related toxicity observed in the nonclinical studies, such as CNS depression and hepatic changes reversible after withdrawal of treatment. These adverse effects are also monitorable to some extent, such as by periodic clinical laboratory assessments during clinical treatment. However, unexpected or severe CNS and cardiovascular toxicity may not be as easily monitored in an outpatient setting. Therefore, discussed with the patients during treatment.

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Genetic Toxicology

Tapentadol HCl was evaluated by the Sponsor in a standard battery of genetic toxicity studies. The studies included *in vitro* assays in *Salmonella typhimurium* and *Escherichia coli* (Ames Test, Reverse Mutation Assay, using both the plate incorporation test in Experiment I and pre-incubation test in Experiment II), and two independent Chromosome Aberrations Assays in Chinese Hamster V79 cells. Additionally, tapentadol was tested in the Chromosome Aberration Assay in rat bone marrow cells *in vivo*, and the Unscheduled DNA Synthesis assay in rat hepatocytes *ex vivo*.

Tapentadol was clastogenic in the first of two independent *in vitro* Chromosome Aberration studies in Chinese hamster V79 cells, resulting in a statistically significant increase in the incidence of structural chromosome aberrations at concentrations greater than 1000 mcg/ml in the presence of S9 mix. A second study, conducted to further explore the results of the positive findings in the Chromosome Aberration assay in V79 cells, revealed no increases in the frequencies of cells with aberrations at concentrations of up to the maximum concentration tested (1500 mcg/ml for 4 hours without metabolic activation with S9, and up to 1000 mcg/ml for 4 hours and 300 mcg/ml for 18 and 28 hours exposure with S9 mix).

No evidence was found of mutagenic potential by tapentadol in *Salmonella typhimurium* strains TA1537, TA 98, TA 1535, and TA100, and in *Escherichia coli* strain WP2: trp; uvrA in the Ames test using the plate incorporation and pre-incubation methods at

concentrations of up to 5000 mcg/plate. Tapentadol was also negative in the *in vivo* assay for clastogenicity in male and female Wistar rat bone marrow cells at doses of up to the maximum tolerated dose (MTD) of 40 mg/kg IV evaluated at 24 and 48 hours. Evaluation of potential mutagenicity in the hepatocytes of rats given up to 35 mg/kg IV and 350 mg/kg PO (gavage) tapentadol in the Unscheduled DNA Synthesis assay revealed no increased DNA repair synthesis induced indicative of treatment-related DNA damage and subsequent repair.

In conclusion, tapentadol was equivocal in the *in vitro* Chromosome Aberrations assay in Chinese hamster V79 cells, in the presence of metabolic activation with S9. The findings suggest potential clastogenicity by a metabolite of tapentadol HCl in the rat, from which the metabolic activating system (S9 mix) was obtained. The identity and production by human metabolism of the potentially genotoxic metabolite is not known.

Carcinogenicity

Tapentadol was negative for carcinogenicity in 104-week studies in male and female mice and rats. Male and female CD-1 mice were administered tapentadol by oral gavage at doses of 50-200 mg/kg/day for 2 years. The Sponsor reported a statistically significant treatment-related increase in hepatocellular carcinomas in the HD male mice that was found not statistically significant by Agency statistical analyses for this common tumor type in mice. Agency analyses found positive trends toward increased hepatocellular adenomas in the female mice, and a statistically significant dose response for liver adenoma + carcinoma in the male mice without statistically significant differences in the pairwise comparisons with controls. There were statistically significant trends for subcutis sarcoma in male mice, and ovarian benign granulosa cell and luteoma tumors in the female mice, also without statistically significant treatment-related increases in the pairwise comparisons. Historical control data suggested that the tumor incidences are within the background for the strain in this laboratory. The NOEL for carcinogenicity by tapentadol at the highest dose tested in mice represents approximately 1.4 times the clinical exposure to the parent drug at the maximum recommended human dose (MRHD), and approximately 8.4 times the exposure to the glucuronide metabolite at the MRHD, on an AUC basis.

Male and female Wistar rats were administered oral tapentadol by admixture in the diet, at daily doses of 10-250 mg/kg/day for 104 weeks. The results of the histopathology evaluation showed slight non-statistically significant (compared to controls) increases in the incidence of hepatocellular adenomas in the high dose females and one additional hepatocellular carcinoma in the high dose male rats. Agency statistical analyses detected positive trends in the incidence in female rats in liver hepatocellular adenoma. There was a statistically significant dose response for increased liver adenomas + carcinomas in the female rats, but no statistically significant increases over controls in any treated group. There was a positive trend for increased incidence of thymic lymphoma, but not for lymphomas at all sites, combined. Historical control data suggested that the tumor incidences in the rats are within the background for the strain in this laboratory.

Evaluation of non-neoplastic lesions in the rats showed a minimal but statistically significant treatment-related increase in centrilobular hepatocellular hypertrophy in the male and female rats. The histopathology findings in the liver are likely related to chronic, adaptive response associated with treatment-related increased metabolic enzyme activities. Increased follicular cell hypertrophy and focal follicular hyperplasia in the thyroid was observed in the HD females, probably resulting from chronic, enhanced liver enzyme activities and hypertrophy, in agreement with the Sponsor. The changes in thyroid in the females is probably not due to induction of thyroxine UDP glucuronosyltransferase activity by tapentadol, because assessments of hepatic microsomal enzyme induction and inhibition studies in liver microsomes isolated during necropsy in several studies in rats and dogs were negative for this effect. 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. The NOEL values for carcinogenicity produced by tapentadol at the highest dose tested in rats are approximately 0.7 times in the males and 2.7 times in the females the clinical exposure to the parent drug at the maximum recommended human dose (MRHD, 600 mg/day) and approximately 27 times in the male and female rats the clinical exposure to the glucuronide metabolite at the MRHD, on an AUC basis.

Reproductive and developmental toxicology

Tapentadol reproductive and developmental effects were investigated in rats and rabbits. Dose selection for the main studies was based on maximum tolerated dose (MTD) levels in the preliminary range-finding toxicity studies in pregnant and non-pregnant animals using oral gavage, and injections by the IV and SC routes. The animals were dosed by IV and SC injections in the main reproductive toxicology studies to maximize systemic exposure to the parent drug, due to the rapid and extensive first-pass metabolism by the oral route in these species.

Tapentadol was negative for adverse effects on mating and fertility at intravenous (IV) doses of up to the MTD in male and female Wistar rats. There were embryonic developmental abnormalities (pre-implantation and post-implantation losses), considered to be secondary to maternal toxicity, in agreement with the Sponsor. The NOEL values were 3 mg/kg/d for maternal toxicity, and 12 mg/kg/d for adverse effects on fertility in the male and female rats. The systemic exposure at the NOEL for adverse fertility effects represented approximately 0.41 times in the male and 0.35 times in the female rats the MRHD, on an AUC basis, based on plasma sampling in another IV study in rats.

Embryo-fetal development toxicity by tapentadol was studied in rats and rabbits. Tapentadol was negative for teratogenicity in the rats at up to maternally toxic intravenous and subcutaneous doses. However, there was a treatment-related increase the incidence and severity of fetal variations and malformations in rabbits given subcutaneous tapentadol, but not when dosed by the intravenous route.

In an IV study on developmental toxicity, pregnant Sprague Dawley rats were administered tapentadol a doses of 3-15 mg/kg/day (gestation days 6-17, inclusive). There were 2 maternal deaths each at 7 and 15 mg/kg/day, within 2-45 minutes of the first injection, preceded by convulsions, exophthalmus, flaccid position and hemorrhagic snout. Although there were reduced numbers of fetuses and implantation sites and increased late resorptions, correlating with the observed maternal toxicity (decreased maternal body weights and severe maternal clinical signs), there were no treatment-related effects on sex distribution, placenta weight, fetal weight, fetal deaths, incidence of runts, and no external, skeletal and soft tissue malformations, variations and retardations. Subsequent investigation of adverse effects on embryofetal development by SC tapentadol (10-40 mg/kg/day on Gestation days 6-17, inclusive) showed maternal toxicity at all dose levels, with dose-related increases in severity and duration of reduced body weight gain, abdominal position lasting 1-2 hours, and local toxicity (eschar formation and hemorrhagic foci) at ≥ 20 mg/kg/day. No treatment-related malformations or variations were observed in this study. However, tapentadol was embryotoxic at the HD (approximately 3 times the systemic clinical exposure at the MRHD on an AUC basis), producing developmental delay (skeletal retardation) with increased incidence of incomplete or missing ossification of the sternebra and caudal vertebral bodies when compared to control fetuses. The embryotoxic findings were correlated with maternal toxicity, particularly to treatment-related reduced food consumption and body weight gain is likely. The NOEL for embryotoxicity in rat was 20 mg/kg/day SC (approximately 1.5 times the systemic exposure at the MRHD on an AUC basis). Exposure to the glucuronide metabolite at the highest dose tested was approximately equivalent to clinical exposure at the MRHD on an AUC basis.

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

The main embryo-fetal study in rabbits was conducted using SC injection at doses of 2-12 mg/kg b.i.d. (4-24 mg/kg/day) during organogenesis. There were dose-related increases in severity and incidence of maternal miosis, abdominal position and reduced body weight gain and food consumption at all dose levels. There were statistically significant dose-related reductions in fetal body weights and fetal viability (≥ 10 mg/kg/day), with increased post-Caesarian deaths during the 24-hour incubator stay (9 fetal deaths at 10 and 7 fetal deaths at 24 mg/kg/day), when compared to the control and LD groups (3 deaths each). There were 4 runts at the HD (vs. 2 in the controls), which died during the 24 hour post-Caesarian incubator stay. Dose-related increased incidence of multiple internal malformations, with gastroschisis or thoracogastroschisis, prolapsed organs, amelia, and phocomelia were observed at 10 mg/kg/day (+0.9% incidence

compared to controls) and 24 mg/kg/day (+1.7% incidence compared to controls). Encephalocele was observed in 1 HD runt, spina bifida in 1 HD runt, kyphosis in 1 HD runt, ablepharia in 3 HD fetuses from 1 litter. Cleft palate was noted in 1 fetus at 10 mg/kg/day (0.9%), 3 fetuses from 1 HD litter and 1 additional fetus from another HD litter (3.4%). Skeletal variations (accessory 13th rib, shortened ribs, caudal vertebral bodies misaligned or fused, unossified parietal area of the skull and sternum fused or misaligned), and skeletal retardations (incomplete ossification in the frontal, parietal, interparietal, and supraoccipital skull, unossified hyoid, incomplete or unossified small sternum, and reduced, unossified or dumbbell-shaped vertebral bodies) were found with statistically significant increase at the HD compared to controls. The NOAEL for teratogenicity in this study was 4 mg/kg/d SC, representing approximately equivalent systemic clinical exposure to the parent drug and the O-glucuronide metabolite at the MRHD on an AUC basis, in the dams. The treatment-related malformations were observed in the litters from dams showing treatment-related severe clinical signs, body weight loss, and reduced body weight gain and food consumption, although not all dams showing toxicity had adversely affected fetuses. The incidences of adverse embryo-fetal findings were within the upper limit of the historical range of background incidence for the performing laboratory. However, a direct teratogenic effect by tapentadol cannot be ruled out.

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

Pup (F₁ generation) post-weaning physical development measurements showed reduced body weights and body weight gains in the HD M from PPD22 to the end of the study (pup ages 10-11 weeks), and in the 150 mg/kg/day (PPD22-37) and HD (PPD22-ages 10-11 weeks) F compared to controls. There were slight, but not significant increases in horizontal movements and rearing at (≥ 150 mg/kg/day) in the M and in all treated F groups. The results of the T-maze test on the F₁ generation showed slight, but not significant increases in test time in the learning phase in males at (≥ 150 mg/kg/day), and

in the memory phase at the HD, compared to controls. No treatment-related effects on F₁ generation sexual development, auditory function and pupil constriction were found. Specifically, there were no treatment-related effects on F mating, mean numbers of days to mate, fertility data pregnancy status, hysterectomy data (e.g., corpora lutea, implantations, concepti, etc). Toxicokinetic evaluation showed dose-related linear increases in exposure to parent drug and the glucuronide metabolite in the F₁ pregnant dams, with increased exposure to the parent drug with repeated dosing at all but the HD, and to the glucuronide at all doses, suggesting accumulation. The NOAEL for F₀ maternal toxicity was 50 mg/kg/day PO, based on reduced BWG and food consumption, representing approximately 1.5 times the clinical exposure at the proposed MRHD on an AUC basis. The NOAEL for treatment-related abnormalities in pup development was 20 mg/kg/day PO, due to pup deaths from PPD 1-4 (approximately 0.3 times the clinical exposure at the MRHD on an AUC basis). The NOAEL for the F₁ generation was 300 mg/kg/day (approximately 10 times the clinical exposure to the parent drug at the MRHD on an AUC basis); although body weights were reduced, there were no effects on mating, fertility, and neurobehavioral parameters. Maternal systemic exposure to the glucuronide metabolite at the NOAEL represented approximately 35 times the clinical exposure to the metabolite at the MRHD, on an AUC basis.

Local tolerance

Injection site adverse effects were evaluated in the intravenous (IV) and subcutaneous (SC) injection toxicity studies in rats, rabbits, and dogs. The results of 14-day studies in M and F Sprague-Dawley rats given daily injections of tapentadol at 15-120 mg/kg/day IV and 30-45 mg/kg/day SC showed infusion site swellings in the SC-treated rats. SC tapentadol in pregnant female rats produced injection site discoloration and erythemas, with weeping eroded hemorrhagic lesions, eschar formation and injection site indurations at doses of 15 mg/kg b.i.d. or higher given daily for 2 weeks. SC tapentadol injections in a dose range-finding study in pregnant rabbits at doses of 5-25 mg/kg/day for 14 days produced subcutaneous tissue lesions with dose-related increases in severity from slight to severe erythemas, of approximately 1-20 mm in size. Injection site swelling was observed in Beagle dogs given SC injections at twice daily doses of 7.5-10 mg/kg for up to 9 consecutive days. When administered for 3 months by SC injection (10-40 mg/kg b.i.d.) in dogs, tapentadol produced local tissue responses indicating severe scratching of the sites throughout the study, and necroscopic findings of ecchymoses with hemorrhages, edema and gelatinous consistency of the underlying tissues at the injection sites. A second 3-month SC toxicity study in dogs demonstrated similar local effects, with hemorrhages, inflammatory infiltrates, partially reversible fibrosis, phlebitis and thrombophlebitis, with chronic focal or multifocal perivasculitis at the highest dose of 16 mg/kg/day SC.

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

Neurotoxicity

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

The Sponsor conducted separate histopathological examinations, in addition to the standard histopathology evaluations in these studies) of rat brain from the 4-week gavage and 4-week intravenous studies, focusing specifically on the brain regions, including the retrosplenial cortex and hippocampal CA1 region, known to be sensitive to NMDA receptor antagonist-induced neuronal injury. M and F rats were administered tapentadol at 3-15 mg/kg/day IV or 300-600 mg/kg/day PO once daily for 4 weeks. Standard histopathology at the end of the dosing period included examination of brain sections (4 mcM slices, paraffin wax embedded, H&E stained) of medulla/pons, cerebellum and cerebrum. Separate microscopic examinations of the retrosplenial cortex and hippocampus CA1 region, conducted following necropsy failed to detect evidence of vacuolation and necrosis. This study is considered to be not completely adequate to detect a vacuolation response, which is best observed within a 12-hour window following an initial dose of an NMDA receptor antagonist agent. Also, it is not clear if necrosis and/or gliosis, best observed within several days of dosing, might have been detected following 4 weeks of drug exposure, under the conditions of the histopathology study, and therefore, the results of this study provide only limited evidence for an absence of NMDA receptor-induced neurotoxicity by tapentadol. However, due to low tapentadol affinity for the NMDA receptor, concern regarding this effect is minimal.

Immunotoxicity

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

Impurities

b(4)

DISCUSSION

The target organs of tapentadol toxicity observed in the nonclinical studies suggest potential adverse central nervous system (CNS), hepatic, cardiovascular, and gastrointestinal (GI) effects with clinical use. Additionally, there were equivocal signals for potential clastogenicity by a tapentadol metabolite in the evaluation of genetic toxicology in one of two *in vitro* Chromosome Aberrations assays in Chinese Hamster V79 cells, and for potential adverse effects on human pregnancy and embryo-fetal toxicity including possibly increased risk of malformations in a subcutaneous studying rabbit, but not in the rabbit assay using the intravenous route.

Dose-related CNS depression, a characteristic effect of mu-opioid receptor agonist drug action, was observed in the nonclinical studies throughout drug development, across species, doses, routes and durations of treatment. The results of the CNS depression findings predict sedative effects by tapentadol in clinical use. There may be an elevated risk of adverse CNS effects, such as somnolence, fatigue, dizziness or respiratory depression in the event of clinical overdose and/or additive effects when taken in combination with other CNS depressant drugs. The results of vital sign measurements in the clinical studies in 3515 subjects exposed in the completed Phase 1, 2, and 3 clinical studies for this submission showed a slight dose-related increase in low O₂ concentration, of 0%, 2%, and 4% in patients administered 0-30, 30-90, and 90-120 mg tapentadol, and 2 subjects were discontinued for hypoxia. The incidence of decreased oxygen saturation was slightly higher than that by oxycodone.

Tapentadol was pro-convulsant in conscious male Wistar rats pre-treated with single IV doses from 0.6-18 mg/kg, prior to intraperitoneal (IP) pentylenetetrazole injection. Convulsions were also observed in two studies in Sprague-Dawley rats administered single IV tapentadol at doses from 21.5-46.4 mg/kg. In one of those studies, tapentadol-induced convulsions were prevented by pre-treatment with diazepam and phenobarbital,

but not by naloxone. In the other study, naloxone dose-dependently reduced the incidence of convulsions by tapentadol at doses of up to 31.5 mg/kg IV.

Convulsions were observed in dog, in the repeated dose toxicology studies by the subcutaneous route in a 3-month study, and by oral dosing during dose-escalation in a maximum tolerated dose selection (MTD) study, and in the 13- and 52 week evaluations. No convulsions were observed in the 3-month SC study in dogs given doses from 4-16 mg/kg/day. Higher SC doses, in the other 3-month study produced convulsions in 1/4 dogs at 40 mg/kg/day and in 2/4 dogs at the highest dose given of 80 mg/kg/day. In the oral MTD study, convulsions were observed upon dose escalation from 280 to 350 mg/kg PO in 1/2 dogs, and after de-escalation of the dose from 350 to 320 mg/kg (1/4 dogs), from 320 to 280 mg/kg (3/4 dogs), and from 280 to 200 mg/kg (4/4 dogs). In the 13-week oral toxicology study, there were convulsions in 1/8 dogs at 80 mg/kg/day and 2/8 dogs at the dose of 120 mg/kg/day. The results of the 12-month chronic oral toxicology study showed convulsions in 2/8 dogs at the highest dose of 80 mg/kg/day. The no effect levels (NOEL) for convulsions in the oral studies (35 mg/kg/day PO the 13-week study, and 30 mg/kg/day PO in the 52-week study) provide no safety margin for human exposure, approximately 0.2, based on comparative systemic exposure at the MRHD of 600 mg/kg/day in a 70 kg patient on an AUC basis, suggestive of human risk. Exposure to the tapentadol O-glucuronide metabolite in oral toxicology studies of the dog represented approximately 5 times the systemic exposure compared to that produced by the MRHD in human, on an AUC basis, and additionally, this is of less concern because the glucuronide metabolite was found throughout drug development to lack receptor-binding affinity and pharmacodynamic activity. On a peak plasma level (C_{max}) basis, the NOEL for convulsions in the dogs in the 13-week and 52-week oral studies provided a low safety factor of approximately 0.34 for the parent drug, and therefore no safety margin for the proposed MRHD. It is notable that although tolerance to most of the tapentadol-induced CNS effects was observed as treatment duration increased, there was no evidence of tolerance development to the convulsive effects of tapentadol. Seizures were observed in a female dog throughout the 1-year dosing period up to Day 358 of the study. This might be expected if the convulsions observed in the nonclinical toxicology studies were related to NE reuptake inhibition, to which there is considerably less tolerance development than to effects of opioid agonist agents. Support for a non-opioid mechanism of action for a pro-convulsant effect by tapentadol in the animal studies was shown by reversibility of the convulsions with diazepam and phenobarbital, but not by naloxone in 2 single dose IV studies in rat.

The results of a CNS Safety Pharmacology study conducted in rat to explore the timecourse of tapentadol-induced respiratory depression, convulsions and deaths in relation to plasma and CSF parent drug, O-glucuronide and sulfate metabolite concentrations showed treatment-related respiratory depression, cyanosis and convulsions starting 15-20 minutes after infusion, and also late peaks in the incidences of convulsions with cyanosis and deaths at ≥ 12 hours after dosing, and after parent drug and glucuronide metabolite were nearly completely cleared from plasma and CSF (below level of detection). Therefore, the late convulsions, cyanosis and deaths could not be attributed to plasma and CSF parent or glucuronide and sulfate metabolite concentrations. The

Sponsor speculated that potential products of parent drug or metabolite enrichment in deeper CNS compartments, not detected in plasma and CSF, may have produced these effects, but this issue was not further addressed. The findings in this study are noted but not of great concern at this time because there were no clear or repeatable findings in the toxicology studies in rat and dog of delayed onset neurobehavioral signs or findings of neurobehavioral toxicity after drug and metabolite clearance from plasma in the toxicology studies, whole body autoradiography in a tissue distribution assay in rat showed decreases in CNS radioactivity (representing parent drug and all metabolic products) from peak levels at 0.25 h to nearly complete clearance to below detection levels at 8 hours, the results of the microscopic examinations in brain in the toxicology studies including the special histopathology evaluation of potential NMDA receptor target site neurotoxicity found no clearly treatment-related histopathologic evidence of neurotoxicity, and there were no findings in the clinical studies of convulsions or results indicating a potential for delayed or extended adverse CNS and respiratory effects by tapentadol after clearance of the drug in humans.

QT prolongation has been associated with norepinephrine reuptake inhibition by other drugs, and is associated with drug potency at the rapidly-activating delayed-rectifier potassium current at the I_{Kr} receptor. Tapentadol was shown to inhibit norepinephrine reuptake in the pharmacology studies, and is one of the proposed pharmacodynamic mechanisms action for analgesia in this submission. The results of several nonclinical studies suggest a potential for treatment-induced cardiac findings including QT interval prolongation and arrhythmias as multiple tapentadol signals were found *in vitro* and *in vivo* in the toxicology studies in dog. The results of a study in Chinese Hamster Ovary cells transfected with the hERG channel showed no effect on the repolarizing cardiac potassium current at concentrations at and below 221 mcg/L (approximately equivalent to the peak clinical plasma concentration (C_{max}) at steady state at the MRHD), but reversibly reduced the rapid component of the delayed rectifying potassium current at 10 and 100-fold higher concentrations of 2210 and 22100 mcg/l (-22% and -73%, respectively, $IC_{50} = 7978$ mcg/L). *Ex vivo* studies in isolated cardiac tissues showed prolongation of the action potential duration in isolated rabbit papillary muscles at 6630 mcg/l, induced bradycardia with slowing of atrio-ventricular conduction and ventricular depolarization in the spontaneously beating guinea pig Langendorff preparation at ≥ 663 mcg/L, induced negative chronotropic and inotropic effects in isolated atrial and papillary muscle preparations, and reduced isometric aortic contractions in potassium-depolarized rat aortic strips with lidocaine-blocked sodium channels ($IC_{50} = 153$ mM, 33813 ng/ml). The Safety Pharmacology studies conducted in the conscious and anesthetized dog revealed no prolongation effects on QT and corrected (QTc) interval after oral administration of tapentadol, but QT prolongation was observed in several toxicology studies in the conscious dog, including when tapentadol was given by subcutaneous injection at doses of ≥ 8 mg/kg/day in the 3-month study (NOEL = 4 mg/kg/day), and orally at doses of ≥ 35 mg/kg/day for 13 weeks, 120 mg/kg/day for 1 week (NOEL 10 mg/kg/day) and 80 mg/kg/day throughout dosing for 52 weeks (NOEL 30 mg/kg/day). There was some tolerance development to the effects on QT interval, in the 3-month study. The potential for QT prolongation was addressed clinically during drug development, and found to be negative in the definitive clinical thorough QT

prolongation studies (Studies HP5503/25 and R1331333-PAI-1018). However, the no effect levels (NOEL) for QT prolongation in the subcutaneous and oral toxicology studies in dog provide no safety margin for human exposure, approximately 0.04 to 0.2, based on comparative systemic exposure at the MRHD, suggestive of human risk. Therefore, the

_____ taken into consideration during treatment.

Hepatotoxic effects were found in most of the toxicology studies on tapentadol in rodents treated by the oral route, but in only one of several studies by the intravenous route. Liver enzymes (e.g., ALAT, ASAT, ALP) were increased in the 4-week IV study (NOEL 7 mg/kg/d), and in the oral studies of 4 weeks at ≥ 425 mg/kg/day (NOEL 300 mg/kg/day), 13 weeks at ≥ 500 mg/kg/day dietary and ≥ 200 mg/kg/day by gavage (NOEL 250 and 100 mg/kg/day, respectively), and 26 weeks at ≥ 150 mg/kg/day for ALP and \geq mg/kg/day for ASAT and ALAT (NOEL 75 mg/kg/day). Clearly, increased liver enzyme effects were noted at lower doses with corresponding NOEL values, with increasing duration of treatment, suggesting increased potential for adverse effects in liver with prolonged administration. Additionally, liver weights were increased in the 13-week dietary (≥ 250 mg/kg/day, NOEL not determined) and gavage (≥ 60 mg/kg/day, NOEL not determined), and in the 26-week gavage (≥ 150 mg/kg/day, NOEL 75 mg/kg/day). The histopathology indices of hepatotoxicity in these studies included hepatocellular hypertrophy in the 13-week dietary (≥ 500 mg/kg/day, NOEL 250 mg/kg/day) and gavage (≥ 60 mg/kg/day, NOEL not determined), and 26-week gavage (≥ 150 mg/kg/day, NOEL 75 mg/kg/day) studies. Fatty change in the liver was observed in the 13-week and 26-week studies. Liver necrosis was found only in the 4-week intravenous study at doses of ≥ 15 mg/kg/day (NOEL 7 mg/kg/day). In no study were Kupffer cell activation or liver fibrosis observed.

The hepatotoxic effects were reversible in the recovery periods in all studies, and the results of the 2-year carcinogenicity studies showed no progression to neoplasms. The observation of treatment-related hepatotoxicity in the rats but not in the dogs might be predicted if these effects are due to adaptations from increased hepatic metabolic activity, as proposed. Tapentadol undergoes extensive first-pass hepatic metabolism, and is metabolized to the O-glucuronide to a greater extent in the rats than in the dogs. Also, tapentadol is metabolized to a greater extent in all animal species tested than in humans, and therefore may be less likely to show treatment-related hepatotoxicity that is related to metabolic activity in humans. The results of safety measurements in total of 3515 subjects exposed in the 31 completed Phase 1, 2, and 3 clinical studies for this submission showed no subjects with ALT or AST levels of 3 times the upper limit of normal with below normal alkaline phosphatase and elevated bilirubin, and no statistically significant differences between placebo and tapentadol-treated groups in percent of subjects with ALT or AST greater than 3 times or 5 times the upper limit of normal, respectively. In a clinical evaluation in patients with hepatic impairment, the results showed a slight increase of 1.71 times in AUC in patients with mild impairment and an increase of 4.22 times in patients with moderate impairment. Therefore, although the potential for tapentadol-induced hepatotoxicity in humans at the proposed doses and

durations of treatment are not of great concern, the possibility cannot be ruled out that there may be some patients, such as those with liver disease, or taking tapentadol for prolonged periods or at doses higher than indicated, who may be at increased risk of hepatotoxicity.

GI findings in the Safety Pharmacology studies of reduced gut motility in rodent and vomiting or retching in the toxicology studies in dog suggest the potential for tapentadol-induced constipation and nausea, well-known mu-opioid receptor secondary effects.

Tapentadol showed no evidence of genotoxic potential in the Ames test and *in vivo* in the Mouse Micronucleus test. However, the results were equivocal in the Chromosome Aberration assay in Chinese Hamster Ovary (CHO) V79 cells. Tapentadol was clastogenic in the first of two independent assays at concentrations greater than 1000 mcg/ml in the presence of metabolic activation with S9. These results suggest genotoxic potential by an unidentified metabolite under the conditions of the study. A second assay in CHO cells, conducted to further explore the results of the positive findings in the Chromosome Aberration assay, revealed no increases in the frequencies of cells with aberrations at concentrations of up to the maximum tested of 1500 mcg/ml for 4 hours without S9 and up to 1000 mcg/ml for 4 hours and 300 mcg/ml for 18 hours exposure with S9 mix. However, the potential for clastogenicity by tapentadol in humans cannot be rejected. The results of the genetic toxicity studies are presented in the label. Although not required for the acute indication, 2-year carcinogenicity studies were conducted in mice and rats. The results were negative for carcinogenicity in 2-year studies in mice and rats. The carcinogenicity studies received ExecCAC concurrence on the protocols used and on the final study results.

The study of fertility and early embryonic development to implantation in the rat showed no evidence of tapentadol effects on mating and fertility. Adverse effects on early embryonic development were reported at IV doses of 6 mg/kg/day and above, and included decreased numbers of corpora lutea and implantations, decreased numbers of live conceptuses, and increased percent pre- and post-implantation loss. These effects are considered to be associated with findings of severe maternal toxicity noted in the same dose range. The results of the fertility study in rat provided a safety margin for human risk of adverse effects on fertility at the no effect level of 12 mg/kg/day of approximately 0.4 in males and 0.35 in females, at the MRHD on an AUC basis (based on extrapolation from toxicokinetic analyses in a separate 4-week intravenous study in rats).

The results of the embryo-fetal development study in pregnant rats given tapentadol by subcutaneous injection during the period of organogenesis showed no evidence of treatment-related malformations at doses of up to 40 mg/kg/day. At this dose, the safety margins for human risk for teratogenicity is approximately 3 for the parent drug and approximately 1 for the glucuronide metabolite. However, embryotoxicity was found, with increased incidences of skeletal retardations (incomplete or missing ossification of sternebra and caudal vertebral bodies) indicating developmental delay at doses of 40 mg/kg/day and above, which also resulted in severe maternal toxicity. The exposures at this dose represented approximately 1.5 times for the tapentadol, and equivalent exposure

to the glucuronide metabolite the systemic exposures in humans at the MRHD on an AUC basis.

The results of the embryo-fetal development study in Himalayan rabbits administered tapentadol by subcutaneous injection during organogenesis showed dose-related increases in the incidence of runts and multiple malformations that included thoracogastroschisis, prolapsed organs, amelia, phocomelia, encephalocele, spina bifida, cleft palate, ablepharia, and skeletal malformations. Although the malformations were observed predominantly in fetuses of dams that showed severe treatment-induced maternal toxicity, and the incidence of malformations were within the upper limits of historical control range for the laboratory, except for ablepharia which slightly exceeded the upper historical range, a potential for tapentadol-induced teratogenicity cannot be entirely ruled out. It is noted, however, that no external and skeletal malformations were observed in rabbits given tapentadol by intravenous injections at up to maximum tolerated doses during organogenesis, in another study. In the clinical studies conducted for this submission, there were 6 reports of pregnancy in patients on the study drug. No abnormal pregnancies were reported and 3 normal infants were delivered.

Tapentadol administration in a pre- and post-natal development study in rat resulted in no effects on post-natal neurobehavioral parameters, learning and memory, mating, and fertility at up to the maternally oral toxic dose of 300 mg/kg/day given during pregnancy through lactation (post-partum day 21) (approximately 10 times the clinical exposure at the proposed MRHD on an AUC basis). However, there were adverse effects on the fetuses and pups, including developmental delay (incomplete ossification), decreased pup body weights and body weight gains, and increased incidence of pup mortality in the 4 days after birth, at doses representing systemic exposure of approximately 0.3 times for the parent drug and 35 times for the glucuronide metabolite the human exposure at the MRHD on an AUC basis. Adequate exposures to the fetuses and pups were confirmed using plasma sampling demonstrated transfer of tapentadol across the placenta and in milk.

In conclusion, the reproductive toxicology studies on tapentadol conducted in rat and rabbit revealed a potential risk of harm to the developing human fetus at doses that were also associated with maternal toxicity, although there were no indications in these studies of adverse effects on fertility and post-natal learning and behavior. The degree to which maternal toxicity was responsible for the observations across studies of developmental delay indicated by skeletal retardations and reduced fetal and pup weights, and increased fetal and pup mortality, and to malformations that were seen in rabbits treated during organogenesis, vs. direct reproductive toxicity by tapentadol cannot be determined unequivocally. Therefore, these results should be clearly and completely described in the label, and tapentadol should not be used during pregnancy unless the benefit of tapentadol treatment clearly outweighs the potential risks to the developing fetus.

The results of a 4-week oral study in rats, with examination of leukocyte population morphology, distribution, and function (T- and B-lymphocytes, monocytes and granulocytes) provided no evidence of potential immunotoxic response by oral tapentadol

in clinical use. The Sponsor evaluated the potential for inducing morphological lesions in areas known to be sensitive to NMDA-receptor binding activity (e.g., retrosplenial cortex, CA1 region), early in drug product development in response to Agency recommendations. The results of special histopathology examination of rat brain in 4-week oral and intravenous studies showed no evidence of neuronal injury, vacuolation or necrosis that may present potential risk in human use. These examinations are considered to be inadequately conducted, but due to low tapentadol affinity for the NMDA receptor, this is not presently of great concern.

The target organ findings were at doses providing low (or no) safety margins in most of the nonclinical studies, to support the proposed maximum recommended human dose (MRHD). The safety factors for the parent drug tapentadol and the major O-glucuronide metabolite from the main nonclinical toxicology studies conducted to support the safety of clinical tapentadol administration are presented in the following table (by the reviewer):

Species	Study	Doses Studied (mg/kg/d) (all studies included vehicle controls)	Target Organ Findings ²	Dose at NOAEL (mg/kg/d) ²	AUC _{0-inf} (ng.h/ml) [AUC glucuronide]	Multiple of MRHD (AUC, or mg/m ² basis) ² [mult for glucuronide]
Rat	Acute LD50	-	-	>1000	-	>113X (mg/m ²) ^a
Rat	13-wk dietary	250,500,1000	Hepatocel. hypertrophy, fatty change	250	~2100(M+F)	4X
Rat	13-wk gavage	60,200,400	↑ASAT,ALAT,liver wts, hepatocel. hypertrophy	60	~4800 [~650,000]	10X [39X]
Rat	26-wk gavage	75,150,300,(450)	↑ASAT,ALAT,LDH, liver wts, fatty change, hypertrophy	75	~625 [150671]	1.25X [9X]
Dog	13-wk gavage	10, 35, (120)/80	Convulsions(80/120)	10	17.1-18.9	0.04X
Dog	52-wk gavage	10, 30, 80	Convulsions(80)	30	203(M+F) [86308]	0.4X [5.2X]
Rat	Segment I I.V.	3, 6, 12	Negative	12	Not collected (estim. based on another IV study)	1.1X(F),0.17XM (mg/m ²) 0.35X(F),0.41X(M) AUC (est)
Rat	Segment II SC	10, 20, 40	Negative (teratogen) EmbrFet tox: devel delay at matern. tox. doses	40	1563 [18514]	3X [1X]
Rabbit	Segment II SC	4, 10, 24	Mat. Tox: sedation, IBW&food cons. EmbrFet tox: malformations in fetuses from dams with severe maternal tox at ≥10	24	5743	10X
Rat	Segment III gavage	50,150,300 (F1: 25, 75, 150/100)	Negative	300	Fo: 1273 [138947] F1: 263-313 [F1: 67534]	Fo: 2.5X [8.4X] F1: 0.5X-1X [juvenile?]
Mouse	CA Gavage	50, 100, 200	Negative	200	763(M)-633(F) [139018(M+F)]	1.4X [~8.4X]
Rat	CA Dietary	10, 50, 125, 250	Negative	250	458(M)-1705(F) [449039(M+F)]	0.7X(M)-2.7X(F) [~27X]

^aAUC: based on clinical tapentadol AUC of 500 ng.h/ml and tapentadol o-glucuronide AUC of 16,500 ng.h/ml at 600 mg/day, presented for last measurement taken (e.g. Week 52 for the 52-week study)

^b mg/m²: based on 600 mg/day, 70 kg patient

^c data not collected where missing from table

² Excluding clinical signs of sedation, body weight and food consumption changes

^b Based on single human dose of 100 mg, 70 kg patient

After oral administration, tapentadol is rapidly and extensively metabolized to the O-glucuronide metabolite by UDP-glucuronosyltransferases (predominantly UGT1A9, UGT2B7 and UGT1A6 in human), providing up to 14 times the exposure compared to circulating parent drug, and therefore the metabolite was comprehensively evaluated in the nonclinical studies. The glucuronide metabolite was found to have no binding affinity for opioid and 30 other receptor, ion channel and transporter targets. Also, tapentadol-O-glucuronide had no effects in the primary antinociceptive activity and secondary pharmacodynamics studies, and in toxicologic evaluation. Exposure to the main metabolite was confirmed by toxicokinetic measurements in the animal studies, and was found in quantitative comparison assays to be higher than in humans, thus providing adequate safety margins for human exposure. Due to the high capacity of the UGT metabolic enzyme system, potential drug interactions by saturation in the presence of other drugs that are cleared by glucuronidation are unlikely. Evaluation of interactions with drugs that inhibit the UGT enzymes, found only very slight inhibition of tapentadol glucuronidation, except for probenecid which showed approximately 45%-60% reduction in tapentadol glucuronidation.

b(4)

CONCLUSIONS:

In conclusion, the target organ effects in the nonclinical studies were predominantly characteristic class effects of mu-opioid receptor agonist and norepinephrine reuptake inhibitor drugs. Partial to complete tolerance developed to many of the tapentadol-induced CNS clinical signs, both acutely (between twice daily doses) and chronically over the course of the study treatment periods. Also, most of the target organ effects were reversible during the post-treatment recovery periods. It is noted that the major target organ findings, such as treatment-related convulsions, QT prolongation, and indices of hepatotoxicity were addressed in human studies during clinical development of the proposed drug product. Potential adverse hepatic effects could be monitored to some extent during clinical use, by periodic clinical laboratory assessments. The potential for treatment-induced convulsions, _____, clastogenicity and adverse effects on pregnancy and embryo-fetal development may not be feasibly monitored, particularly in the outpatient population and therefore should be clearly described in the appropriate precautionary sections of the product label.

b(4)

UNRESOLVED TOXICOLOGY ISSUES

There are no unresolved toxicology issues and no further animal studies are needed at this time.

RECOMMENDATIONS

Tapentadol HCl can be approved for the proposed indication under NDA 22-304, from a pharmacology and toxicology perspective.

The appropriate nonclinical studies were conducted in support of the safety and labeling of oral Immediate Release Tapentadol HCl for the clinical indication as proposed under NDA 22-304. No further nonclinical studies are needed at this time for marketing approval.

SUGGESTED LABELING: Revisions to the sections of the label on Pregnancy, Carcinogenesis, Mutagenesis, Impairment of Fertility, and Animal Toxicology and/or Pharmacology will be needed, to include additional information from the nonclinical toxicology findings, and to refine the language. Detailed recommendations will be provided in a separate Labeling Review.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

3.7. APPENDIX/ATTACHMENTS

Minutes of the ExecCac Meeting of August 26, 2008

Appendix 1

Executive CAC

Date of Meeting: August 26, 2008

Committee: David Jacobson-Kram, Ph.D., ONDIO/PharmTox, Chair

Abby Jacobs, Ph.D., ONDIO/PharmTox, Member

Bayo Lanionu, Ph.D., DMIHP, Alternate Member

Paul Brown, Ph.D., ONDIO/PharmTox, Member

Adam Wasserman, Ph.D., DAARP, Team Leader

Kathleen Young, Ph.D., DAARP, Presenting Reviewer

Author of Draft: Kathleen Young, Ph.D.

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

NDA # 22-304

Drug Name: Tapentadol

Sponsor: Ortho-McNeil Pharmaceutical, Inc.

Background: Tapentadol is a new molecular entity that is being developed for the oral treatment of moderate to severe, acute and chronic pain at doses of 100 mg up to 6 times/day (600 mg/day, and up to 700 mg on the first day of treatment). Tapentadol is active primarily through agonist activity at the mu- and sigma2 receptors, and also inhibits norepinephrine uptake.

The results of non-clinical pharmacology studies showed the analgesic potency of tapentadol to be 2X-3X that of morphine, although the affinity for the mu-opioid receptor was 1/50 the affinity of morphine.

Tapentadol was evaluated in a standard battery of genetic toxicity studies and found to be equivocal for clastogenicity. A positive response was found in one of two *in vitro* Chromosome Aberration studies in Chinese hamster V79 cells, showing increased incidence of structural chromosome aberrations at concentrations greater than 1000 mcg/ml in the presence of metabolic activation with S9. No evidence of genetic toxicity by tapentadol was found in the Ames test, the *in vivo* assay for clastogenicity in rat bone marrow cells, and in rat hepatocytes in the Unscheduled DNA Synthesis assay.

Mouse Carcinogenicity Study

A 2-year oral gavage study was conducted in CD-1 mice given tapentadol doses of 50 (LD), 100, (MD1) and 200 (MD2) mg/kg/day. The high dose group (HD) received the following treatments, with dose adjustments during the study: 200 mg/kg/day (Weeks 1-14), escalation to 300 mg/kg/day (Weeks 15-27) upon Agency recommendation, and subsequent reduction to 200 mg/kg/day (Weeks 29-91) following observations of increased mortality after the dose escalation. An additional, dose-escalation high dose group (n=9/sex/group) was used to test tolerability of the high dose per Agency recommendations. The additional group was given 200 mg/kg/day during Weeks 1-13 and 300 mg/kg/day during Weeks 14-28, followed by dose reduction to 200 mg/kg/day during Weeks 29-91. Dosing was terminated in the MD2 (200 mg/kg/day) male mice during Week 100 and in the MD female mice during Week 99, due to excessive mortality (20 surviving animals). The high dose groups were terminated in Week 92, also due to low survival. The surviving mice were kept to the end of the 104-week period without

treatment for histopathologic examination. All animals, including the mice found dead and sacrificed in extremis were examined microscopically. The doses were originally selected based on the results of a 13-week oral dose selection study and received prior Agency concurrence (see ExecCAC meeting of December 9, 2003).

The duration of treatment and survival in the 2-year study was adequate in all groups for valid statistical evaluation of the parameters examined. According to the Sponsor, there was a treatment-related increase in hepatocellular carcinomas in the high dose male mice (incidence 4/51 compared to 0 – 1 per group in the controls, low dose and mid dose mice), that was found not statistically significant by Agency statistical analyses for this common tumor type in mice. There were statistically significant trends for subcutis sarcoma in male mice. However, there were no statistically significant treatment-related increases in any dosed group compared to concurrent controls. Historical control data suggested that the tumor incidences are within the background for the strain in this laboratory.

Rat Carcinogenicity Study

A 2-year study was conducted in Wistar rats, at tapentadol oral doses of 10, 50, 125, and 250 mg/kg/day administered by admixture in the diet (n=50/sex/group). Two additional groups received negative control (pelleted standard rat maintenance diet). The doses were based on the results of a 13-week preliminary oral (dietary) toxicity study, and the protocol received ExecCAC concurrence (see minutes of ExecCAC meeting of January 22, 2002, IND 61,345).

Survival in the 2-year study was adequate in all groups at the end of the dosing period for valid statistical evaluation of the parameters examined. Agency statistical analyses detected positive trends in the incidence of hepatocellular adenoma in the female rats ($p < 0.025$) with incidence of 2% and 4% at 125 and 250 mg/kg/day, respectively. There was a statistically significant dose response for increased liver adenomas + carcinomas in the female rats, but no statistically significant increases over controls in any treated group. Historical control data suggested that the tumor incidences are within the background for the strain in this laboratory.

Executive CAC Recommendations and Conclusions:

2-Year Mouse:

The Committee concurred that the study was adequate and was negative for carcinogenicity.

2-Year Rat:

The Committee concurred that the study was adequate and was negative for carcinogenicity.

David Jacobson-Kram, Ph.D.

Chair, Executive CAC

cc:\

/Division File, DAARP

/Adam Wasserman, Ph.D., Team leader, DAARP

/Kathleen Young, Ph.D., Reviewer, DAARP

/Matthew Sullivan, RPM, DAARP

Reviewer: Kathleen Young, Ph.D.

NDA No. 22-304

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/s/

David Jacobson-Kram

9/2/2008 12:20:58 PM

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

/s/

Kathleen Young
9/24/2008 12:37:33 PM
PHARMACOLOGIST

Adam Wasserman
9/24/2008 12:53:42 PM
PHARMACOLOGIST

I concur with the recommendation of Dr. Young that
Tapentadol HCl may be approved from the nonclinical
perspective.