CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

215904Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Date: March 18, 2022

From: Lois M. Freed, Ph.D.
Director, Division of Pharmacology/Toxicology-Neuroscience
Office of Neuroscience

Subject: NDA 215904 (ZTALMY, ganaxolone)

NDA 215904 was submitted by the sponsor (Marinus Pharmaceuticals) on July 20, 2021, to support marketing authorization for ganaxolone, a neuroactive steroid $GABA_A$ receptor positive modulator, for the treatment of seizures associated with cyclin-dependent kinase-like 5 deficiency disorder (CDD).

To support clinical development and an NDA, the sponsor conducted a battery of nonclinical studies: pharmacology (primary, secondary, safety), PK/ADME, general toxicology (up to 6 and 12 months in rat and dog), reproductive and developmental toxicology (fertility and early embryonic development in rat, embryofetal development in mouse, and combined embryofetal/pre- and postnatal development in rat), juvenile animal toxicology in rat, and a standard genetic toxicology battery (Ames assay, in vitro chromosomal aberration assay, and in vivo micronucleus assay in rat). Carcinogenicity studies in two species (26-week study in TgRasH2 mouse and 2-year study in rat) are to be conducted as post-marketing requirements (PMR), as agreed to by the Division (IND 44020 Type C Meeting Minutes, January 11, 2018)

The nonclinical studies were reviewed by Dr. Ed Fisher (Pharmacology/Toxicology NDA Review and Evaluation, NDA 215904, March 17, 2022). Dr. Fisher has concluded that the nonclinical data are adequate and recommends approval of the NDA, from a nonclinical standpoint, with several post-marketing requirements (carcinogenicity studies of ganaxolone in mouse and rat, nonclinical safety evaluation of M2, a major circulating metabolite in humans, and a brain distribution study of M47, a major circulating metabolite in humans).

In humans, two metabolites, M2 (oxy-dehydro-ganaxolone) and M47 (a ganaxolone sulfate conjugate), have been identified as major metabolites, each accounting for approximately 25% of total circulating drug-related material. At the time of NDA submission, the sponsor noted bioanalytical methods were being developed to quantitate levels of M2 in rat plasma (Pharmacology Written Summary); however, in the most recent Investigator's brochure submitted under IND, the sponsor states that M2 is a human-specific metabolite. There is currently no information on plasma levels of M47 in animal species. Although M47 is a sulfate conjugate, there are concerns that it may have pharmacological activity, depending on the extent of its distribution into the CNS (Hartneck C. Molecules 18:12012-12028, 2013). The nonclinical safety of these metabolites is to be addressed in studies conducted as PMRs.

The primary drug-related toxicity observed in the general toxicology studies of duration up to 6 and 12 months in Sprague Dawley (SD) rat and beagle dog, respectively, was sedation, which in some cases was associated with death or premature sacrifice. This is an expected effect based on the pharmacological activity of ganaxolone. Sedation and the induction of hepatic enzymes, which occurred in rat (but not dog), limited the doses of ganaxolone that could be administered. The highest doses tested in the 6- and 12-month studies were 40 mg/kg QD and 15 mg/kg QD, respectively. Plasma exposures at the final sampling time (Day 182 and Week 52, respectively) were 608.4-838.2 ng/mL ($C_{max,}$ M-F) at 40 mg/kg QD in rat (AUC data were not collected) and 2291-1756 ng/mL ($C_{max,}$ M-F) and 33888-24346 ng*hr/mL ($AUC_{(0-24h)}$, M-F) at 15 mg/kg QD in dog.

(For comparison, plasma ganaxolone exposures in humans are 262 ng/mL for C_{max} and 3000 ng*hr/mL for $AUC_{(0-24h)}$ in adults at 2000 mg, and, based on extrapolation, 300 ng/mL for C_{max} and 4000 ng*hr/mL for $AUC_{(0-24h)}$ in pediatric patients at 1800 mg.)

A full battery of reproductive and developmental toxicology studies was conducted for ganaxolone: fertility and early embryonic development (FEED) study in SD rat, embryofetal development (EFD) study in CD-1 mouse, and a combined EFD/pre- and postnatal development (PPND) study in SD rat. (An EFD study was not conducted in rabbit because sufficiently high plasma exposures could not be achieved.)

In the FEED study, there were no adverse effects on fertility, reproductive performance, or spermatogenesis at doses up to 40 mg/kg QD; however, prolonged estrus was observed at the high dose. Plasma C_{max} in males (206.4 ng/mL at the high dose) was the only TK parameter provided.

In the EFD study in mouse, an increase in fetal malformations (external, visceral) was observed at all doses (0, 50, 175, and 300 mg/kg/day) in the absence of maternal toxicity. Although the number of affected fetuses/litters was not dose-

related, TK data from separate studies in CD-1 mouse suggested saturation of exposure across the dose range tested in the EFD study. (TK data were not collected in the EFD study.)

In the combined EFD/PPND study in rat, ganaxolone (0, 10, 20, and 40 mg/kg QD) was administered to pregnant animals from gestation day (GD) 6 to GD 19 or to postnatal day 22. No adverse effects on embryofetal development parameters were observed for dams sacrificed on GD 20. However, adverse effects on postnatal growth (reduced body weight gain, delayed reflex attainment) and neurobehavioral function (decreased locomotor activity) were observed in offspring. TK data were not collected.

Although TK data are not available for these studies, data from separate studies in SD rat suggest that plasma exposures in dams were lower at all doses tested than those in humans at the maximum recommended human dose of 1800 mg/day.

To assess potential effects of direct administration of ganaxolone during postnatal development, the sponsor conducted two juvenile animal toxicology studies in SD rat, with dosing initiated on postnatal (PND) 7 in both studies and continuing to postnatal week 7 or PND 91. As in adult rat, the primary clinical signs in both studies were consistent with sedation.

In the first study (0, 12.5, 25, and 50 mg/kg QD), developmental effects consisted of reduced body weight and body weight gain and slight delays in sexual maturation in males and females at the HD. There were no adverse effects on reflex attainment or neurobehavioral function; findings on the FOB were consistent with the sedative effects of ganaxolone. No TK data were collected.

In the second study (0, 10, 22.5, 45/75/125/250 mg/kg BID; 0, 20, 45, 90/150/250/500 mg/kg/day), doses were escalated at the HD in an attempt to allow tolerance to develop to the sedative effect of ganaxolone. However, deaths due to excessive sedation occurred at the mid (MD) and high doses. Developmental effects consisted of reduced weight of reproductive organs in males at the MD and HD, delayed sexual maturation in females at all doses, and reduced brain weight in males and females at all doses. There were no adverse effects on reproductive function or neurobehavioral function. Overall, a no-effect dose for adverse effects on postnatal development was not identified. At the lowest dose tested, plasma exposures (M-F) on PND 91 were 76.9-324 ng/mL for C_{max} and 510-2020 ng*hr/mL for $AUC_{(0\text{-}24h)}$.

Because ganaxolone is a $GABA_A$ receptor positive allosteric modulator, there were concerns that administration during early postnatal development in rat would cause apoptotic neurodegeneration, as have other drugs with GABAmimetic properties (and NMDA antagonists) administered during the period of synaptogenesis (Olney JW et al. Brain Pathol 12:488-498, 2002). Therefore,

the sponsor conducted an acute neurotoxicology in neonatal rat, in which a single dose of ganaxolone (0, 10, 45, and 90 mg/kg QD) was administered on PND 7. PND 7 corresponds in humans to the last trimester of pregnancy through the first few years after birth. A single dose of MK-801 (1.0 mg/kg IP), an NMDA receptor antagonist, was administered on PND7 to a separate group of animals as a positive control.

Ganaxolone resulted in widespread neuronal death in multiple brain regions, including cortex, thalamus, and hippocampus, at all doses. At the HD, the pattern and extent of neuronal death was similar to that produced by MK-801. Neurobehavioral evaluations were not conducted. However, published studies have reported persistent adverse neurobehavioral effects of drugs (including MK-801) shown to induce neuronal death when administered to neonatal rodents (e.g., Fredriksson A, Archer T. Neurotox Res 6(6):435-456, 2004; Fredriksson A et al. Behav Brain Res 153:367-376, 2004; Jevtovic-Todorovic V et al. J Neurosci 23(3):876-882, 2003). Day 91 plasma exposures (M-F) at the lowest dose tested were 76.9-324 ng/mL for C_{max} and 540-2020 ng*hr/mL for $AUC_{(0\text{-}24h)}$.

The genotoxic potential of ganaxolone was tested in a standard battery of in vitro (Ames, mouse lymphoma) and in vivo (rat micronucleus) assays. Metabolite M2 was tested in in vitro assays; M2 was negative in an Ames assay but positive (in the absence and presence of metabolic activation) in an in vitro chromosomal aberration assay in human peripheral blood lymphocytes.

Recommendations

I agree with Dr. Fisher that the nonclinical studies of ganaxolone are adequate to support approval of the NDA from a nonclinical standpoint, considering the severity of the indication, with appropriate labeling and the following post-marketing requirements: a 6-month carcinogenicity study of ganaxolone in TgRasH2 mouse, a 2-year carcinogenicity study of ganaxolone in rat, a carcinogenicity study of metabolite M2 in rat, a juvenile animal toxicology study of metabolite M2 in rat, and a study to evaluate the distribution of metabolite M47 into brain in rat.

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/s/

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03/18/2022 11:21:12 AM

9DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	215904
Supporting document:	002
Applicant's letter date:	7/20/2021
CDER stamp date:	7/20/2021
Product:	Ganaxolone
Indication:	Treatment of seizures associated with Cyclin-dependent kinase-like 5 (CDKL5) Deficiency Disorder (CDD)
Applicant:	Marinus
Review Division:	Neurology 2
Reviewer:	Ed Fisher
Supervisor:	Lois Freed
Division Director:	Nicholas Kozauer
Project Manager:	Stephanie Parncutt

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of N215904 are owned by Marinus or are data for which Marinus has obtained a written right of reference. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application are for descriptive purposes only and are not relied upon for approval of 215904.

Note: All figures and tables in this review were excerpted from the sponsor's submission

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1 Executive Summary

1.1 Discussion of Nonclinical Findings

Ganaxolone (GNX; 3α-hydroxy-3β-methyl-5α-pregnan-20-one) is a methyl-substituted analog of the endogenous neurosteroid allopregnanolone, a derivative of progesterone. Its anticonvulsant effects are thought to result from positive allosteric modulation of the GABAA receptor.

In early primary pharmacology studies (Carter et al, 1997), the in vitro binding of GNX was consistent with activity as a positive allosteric modulator of the GABAA receptor. GNX produced concentration-dependent inhibition of specific binding of the GABAA receptor-gated chloride channel ligand [35S]TBPS to rat brain cortical membranes (IC50 of 80 nM) and enhancement of specific [3H]flunitrazepam binding to the benzodiazepine modulatory site (EC50 of 125 nM) and [3H]muscimol binding to the GABA recognition site (EC50 of 86 nM) on the GABAA receptor complex. Electrophysiological studies demonstrated that nanomolar concentrations of GNX potentiated GABA-evoked chloride currents in Xenopus oocytes expressing the human GABAA receptor subunits α1β1γ2L, α2β1γ2L or α3β1γ2L, whereas direct activation of chloride flux, in the absence of GABA, occurred only to a limited extent at micromolar concentrations.

GNX (IP or oral) was active in several traditional rodent seizure models (eg, chemically-induced convulsions, corneal kindling). GNX was not tested for activity in a Cdkl5 disease model. While Cdkl5 knockout mouse models have been generated, these do not exhibit overt seizures.

GNX (10 μM) demonstrated only minimal radioligand displacement at estrogen (5%), glucocorticoid (9%), progesterone (8%), or testosterone (14%) receptors in vitro. In an in vitro functional assay, GNX (up to 10 μM) did not exhibit significant agonist or antagonist activity at the human progesterone receptor.

In a CNS safety pharmacology study in Sprague Dawley rats (11042.RT.IV.103), GNX (9, 12, or 15 mg/kg IV) produced dose-dependent sedation as a function of time, lasting for up to 3 hrs after dosing. It was noted that, while heavily sedated, animals still responded to stimuli (toe pinch reflex) at all doses, indicating that an anesthetic level was not reached.

GNX did not inhibit the hERG channel at a concentration of 10 μM (Study 1245-007) and there were no alterations in blood pressure, heart rate, or qualitative or quantitative ECG parameters in a CV safety pharmacology study in conscious, telemetered beagle dogs (Study 1245-008) up to the highest dose tested (15 mg/kg). A dose-dependent increase in heart rate with incidences of sinus tachycardia was reported in the 12-month dog toxicity study, but there were no associated changes in QTc interval or any histopathological correlates.

GNX is extensively metabolized. In an in vitro study ([(b) (4)]08694) in which [14C]-GNX was incubated with mouse, rat, dog, and human liver microsomes, the major metabolites

were multi-hydroxy-metabolites and/or products of 20-ketone reduction. 20β-hydroxyganaxolone, 3β-hydroxymethylganaxolone, and 16α-hydroxyganaxolone were the major monohydroxylated metabolites and the major multi-hydroxy-metabolites were products of the combination of 20-keto-reduction, 16α-hydroxylation, and 3β-hydroxylation.

No metabolites unique to humans were found in the in vitro studies, but subsequent in vivo metabolism studies (8333043, 1042-GNX.AME-1001) identified a major human-specific, non-conjugated plasma metabolite, designated M2 (oxy-dehydro-ganaxolone, also designated M60b). This metabolite has been synthesized and evaluated for pharmacological activity and genotoxicity. M2 demonstrated no functional activity at GABAA receptors and was negative for mutagenicity in the Ames test; however, it produced a positive response in an in vitro chromosomal aberration assay. The sponsor has agreed to conduct appropriate follow-up nonclinical safety studies post-approval. A sulfate conjugate ([(b) (4)] designation M47) present in pooled human plasma in a similar range as that of M2 in terms of percentage of total radioactivity is also considered to represent a possible safety concern based on its abundance in plasma and literature reports indicating that sulfate conjugates of neuroactive steroids may have pharmacological activity.

[(b) (4)] several different oral formulations were evaluated in the nonclinical development program. The early pharmacology, PK, and toxicity studies, including the 6-month rat and dog and reproductive toxicity studies, used cyclodextrin-based formulations, while more recent studies (13-week rat, 12-month dog toxicity studies, repeat juvenile rat study) used a preformulated GNX suspension with the same or similar excipient composition as the clinical drug product.

Although high bioavailability was observed in single oral dose studies, in oral repeat-dose toxicology studies in mouse and rat, GNX absorption appeared to become saturated, with exposures plateauing at doses ≥ 125 mg/kg. Female rats had higher exposure than males, and exposure decreased in both sexes with repeated dosing, with greater decreases observed at higher doses. The reduction in exposure with repeated dosing was attributed to autoinduction of hepatic enzymes, which was documented in mouse and rat toxicity studies, with CYP3A and CYP2B enzyme activities being most affected. In an effort to achieve higher exposures and mitigate the dose-limiting sedative effects, which appeared to be driven by Cmax, dose escalation was used in the more recent mouse and rat toxicology studies, which allowed doses as high as 500 mg/kg to be given BID. However, plasma exposures did not increase commensurately. In dogs, there was no sex difference in exposure and no evidence of autoinduction with repeated dosing; GNX exposure increased in dogs in a dose-proportional manner up to the highest dose tested (20 mg/kg/day, limited by sedation) and accumulated with repeated dosing.

GNX-induced sedation was dose limiting in all the pivotal toxicity studies. In the 1-month study in SD rats (1245-006; oral doses of 0 (vehicle), 10, 40, and 80 mg/kg/day in males and 0, 10, 20, and 40 mg/kg/day in females, preformulated suspension), dose-dependent sedative effects were observed at all doses; clinical signs included decreased activity,

ataxia, and/or prostration that were either more severe or more frequent in females than in males. The severity and duration of sedation lessened at each dose in both sexes throughout the study, indicating accommodation and/or induction. There were no drug-related effects on mortality, body weights, hematology, clinical chemistry, urinalysis, ophthalmoscopic, organ weights, or macroscopic and microscopic examinations. CYP3A1/2 and CYP2B1/2 enzyme activities were increased in treated females. On Day 1, the highest doses tested (80 and 40 mg/kg/day) were associated with Cmax and AUC values of 228 and 439 ng/mL and 1267 and 4371 ng•h/mL in males and females, respectively. Day 15 and 29 exposures were lower than those on Day 1 in both sexes.

In the 13-week rat study (8412976; oral doses of 0, 160/250, 160/250/500, or 160/250/1000 mg/kg/day in males and 0, 60/120/250, 60/120/500, or 60/120/1000 mg/kg/day in females, preformulated suspension), dose escalation and BID dosing were used in an attempt to increase exposures. Dose-related sedation scores, with prostration and/or ataxia, were noted at all doses in both sexes. Dose-dependent increases in CYP2B and CYP3A activities were measured in GNX-treated animals. The only drug-related anatomic findings were liver and thyroid changes typically associated with hepatic enzyme induction. The NOAELs (160/250/1000 mg/kg/day in males and 60/120/1000 mg/kg/day in females) were associated with Cmax and AUC values of 103 ng/mL and 1430 ng*h/mL, respectively, in males and 459 ng/mL and 6140 ng*h/mL, respectively, in females on Day 91. Despite the higher administered doses, plasma levels did not increase appreciably between the LD and HD and decreased between Days 1 and 28.

In the 6-month rat study (SC930189; oral doses of 0 (vehicle: (b) (4) 10, 20, or 40 mg/kg/day), dose-dependent sedative effects characterized as ataxia, prostration, and/or unresponsiveness were observed in both sexes at all doses, but the degree of sedation lessened with duration of the study. The death of a HD female on Day 2 was considered possibly drug-related, likely secondary to sedation. There were no effects on body weight, clinical pathology, or gross pathology. Increased liver weights correlated with hepatocellular hypertrophy in treated females. Kidney weights were also increased in treated females but without microscopic correlate. Cmax values at the NOAEL (20 mg/kg) were 157 and 171 ng/mL in males and females, respectively. AUCs were not determined.

In the 1-month study in dogs (1245-005; oral doses of 0, 3, 10, or 15 mg/kg/day, preformulated suspension), there were no effects on mortality, but dose-dependent clinical findings of decreased activity, ataxia, prostration, salivation, and slow breathing were observed throughout the study at all doses and were considered related to the sedative effect. Sedative effects were similar throughout the study indicating little accommodation. BW and food consumption were increased in MD and HD males and females at all doses. There were no drug-related ophthalmoscopic, electrocardiographic, clinical pathology, macroscopic, or organ weight effects. A microscopic finding of testicular atrophy in 1 HD animal, associated with oligospermia/germ cell debris in the epididymides, was considered possibly drug-related. (However, no testicular toxicity was observed at the same dose in the 12-month dog study.) The highest dose (15 mg/kg/day) was associated with Cmax and AUC values of 1507 ng/mL and 15964 ng·hr/mL in males

and 1426 ng/mL and 13900 ng·hr/mL in females on Day 29, which were greater than Day 1 values.

In the 6-month dog study (SC930190; oral doses of 0 (vehicle: (b) (4) 1, 3, or 10 mg/kg), dose-dependent sedative effects (lethargy, ataxia, prostration, unresponsiveness) were observed throughout the study at all doses. There were no drug-related effects on survival or other endpoints evaluated (BW, clinical pathology, ophthalmic examinations, physical examinations, cardiovascular assessments, organ weights, and gross and microscopic pathology examinations). Day 182 Cmax values were 793 and 1200 ng/mL in HD males and females, respectively. AUCs were not determined.

In the 12-month dog study (1245-011; oral doses of 0, 3, 10, or 15 mg/kg/day, preformulated suspension), dose-dependent clinical findings related to the sedative effect (decreased activity, tremors, ataxia, prostration, and unresponsiveness) were seen at all doses. The incidence and/or duration of prostration decreased only minimally during the study. One HD male (#161) exhibited clonic and tonic convulsions at Weeks 33 and 36, respectively. Exposures were consistently approximately 2-fold higher in this dog compared to other HD animals (Cmax: 3416 ng/ml, AUC: 57966 ng•h/mL at Week 38). There was no drug-related mortality, however, and BW and food consumption were dose-dependently increased at all doses. Dose-related increases in heart rate and incidences of sinus tachycardia were observed in the ECG examination but there were no effects on QRS duration or QTc interval. There were no drug-related macroscopic or microscopic pathology changes. Drug accumulation was seen over the first 6 weeks of dosing. Week 52 Cmax values were 520, 1155, and 2291 ng/mL in males (includes #161) and 798, 1528, and 1756 ng/mL in females, and AUC(0-24h) values were 4817, 13523, and 33888 ng·h/mL in males and 5335, 15725, and 24346 ng·h/mL in females at the LD, MD, and HD, respectively.

GNX was negative for genotoxicity in both in vitro (Ames and mouse lymphoma) and in vivo (rat micronucleus) assays. The major human metabolite, M2, was negative in the Ames test but increased (SS) aberrant metaphases in 4-hour treatments in both the absence and presence of S9 mix in a mammalian chromosomal aberration assay. A 26-week carcinogenicity study of GNX in the CB6F1-Tg rasH2 transgenic mouse and a 104-week carcinogenicity study of GNX in SD rats are to be conducted postmarketing (Type C meeting minutes dated 01/11/2018).

In a 28-day carcinogenicity dose range-finding study (8412977) in which preformulated GNX (0 (vehicle: HPMC, PVA, and SLS), 125, 250, or 500 mg/kg) was administered BID by oral gavage to CByB6F1-Tg[HRAS]2Jic: Wild Type mice, findings were limited to increases (NS) in BW gain and dose-dependent increases in liver weights which correlated with increases in hepatic enzyme parameters (>2-fold increase in CYP2B and CYP3A activity, total CYP450). Since these were adaptive responses, the HD (250/1000 mg/kg/day) was a NOAEL. This dose was associated with Cmax and AUC values of 185 ng/mL and 1260 ng*h/mL, respectively, in males and 52.9 ng/mL and 968 ng*h/mL, respectively, in females on Day 26 of dosing.

In a rat fertility and early embryonic development study (1042-POl 13; oral doses of 0 (vehicle: (b) (4) 10, 20 or 40 mg/kg), alterations in estrous cyclicity were seen at the HD, but there were no effects on spermatogenesis, reproductive performance and fertility, or early embryonic development. At Week 15, the Cmax in HD males was 206 ng/mL. Cmax in females and AUCs were not determined.

In a mouse embryofetal development study (1215-007; oral doses of 0 (vehicle: (b) (4) 50, 175, or 300 mg/kg) transient clinical signs of hypoactivity and ataxia were observed at the MD and HD during the early treatment period, but maternal BW gain was unaffected. There were no effects on litter parameters at C-section, but fetal malformations were increased in all drug-treated groups compared to C: 1(1), 4(4), 5(4), and 5(3) fetuses (litters) with external/visceral malformations were observed in the C, LD, MD, and HD groups, respectively. These were not considered drug-related in the study report, based on the absence of a dose relationship, but as seen from TK data collected in another CD-1 mouse study (8412975), plasma levels plateau at doses ≥125 mg/kg, and there was a pattern of CNS defects (eg, abnormal shaped brain, exencephaly, hydrocephaly), both of which support a drug effect. Maternal plasma drug levels were not determined in this study.

In a combination embryofetal and pre- and postnatal development study in SD rats (COY 7 /961509; oral doses of 0 (vehicle: (b) (4) 10, 20 or 40 mg/kg/day), dose-dependent clinical signs (unsteadiness [moderate or severe at HD], apparent sleepiness, body tremors) were observed and maternal BW gain was decreased somewhat during part of the gestational treatment period at the HD. There were no treatment-related effects on litter parameters or fetal abnormalities at C-section on GD20. In offspring from dams allowed to litter, BW gain was decreased during lactation at the HD and during postnatal weeks (PNW) 4-19 in HD males. This was associated with delayed reflex development during the pre-weaning period and a slight delay in attainment of sexual maturation of HD male offspring. Decreased locomotor activity was seen in HD male offspring when assessed at PNW 5 and in MD and HD males when retested at PNW 11. There were no effects on learning and memory as assessed in the passive avoidance test. Offspring mating performance was unaffected by treatment. Plasma levels were not measured in the study.

Two juvenile rat studies were conducted. In the first study (COY 2/950920, oral doses of 0 (vehicle: (b) (4) 12.5, 25, or 50 mg/kg/day from PND 7 to PNW 7), clinical signs of sedation were seen at the MD and HD and decreased BW gain, delayed sexual maturation in both sexes, and altered behavior (abnormal gait, decreased grip strength in FOB) were observed at the HD. There were no changes in locomotor activity or learning and memory (passive avoidance test) and no histopathological changes, but no stage-specific testis examination was conducted. The NOAEL was 25 mg/kg/day. This study was considered inadequate based on the group sizes for neurobehavioral evaluations, inadequate assessment of learning and memory, and lack of reproductive assessments.

In the repeat juvenile rat study (00398514; oral (preformulated suspension) doses of 0, 20, 45, or 90/150/250/500 mg/kg/day from PND 7 through PND 91), drug-related deaths

were seen at the MD (1 female) and HD (6 males and 4 females), primarily on PNDs 7–9. Sedation was noted prior to death in these animals (graded severe in 2), and sedative effects were seen in surviving animals at all doses, primarily during the pre-weaning period. BW gain and BWs were decreased intermittently at the MD and HD, but there were no statistically significant differences among groups in BW gain over the entire treatment period or on final BWs. Onset of puberty (vaginal opening) was dose-dependently delayed in females at all doses, reaching an approximately 4-day delay in the MD and HD groups. There were no clearly drug-related effects on neurobehavioral assessments (motor activity, auditory startle response, and learning and memory in Biel maze), during the dosing period or after the recovery period. There were also no drug-related effects on reproductive performance or spermatogenic parameters. Absolute brain weights were decreased in the reproductive subset animals at all doses in both sexes. Since relative weights were not SS different and the effect was not seen at the end of the dosing period or at the end of the 28-day recovery period in another subset of animals, this finding was not considered drug-related by the sponsor. However, the group sizes were greater in the reproductive subset (20 vs 8/sex/grp), relative weights were not calculated for females because final BW was not available due to pregnancy, and brain weight is generally spared from BW effects. Dose-related decreases in epididymis and testis weights were seen in the reproductive subset males, but there were no apparent drug-related microscopic changes and no microscopic brain abnormalities. GNX exposures (AUC0-24h) at the LOAEL for juvenile developmental toxicity were 1050/961, 524/1500, and 510/2020 ng.h/mL in males/females on PNDs 7, 36, and 91, respectively.

In an acute neurotoxicity study in neonatal rats (00398515; oral (preformulated suspension) doses of 0, 10, 22.5, or 45 mg/kg BID on PND 7), dose-related increases in the incidence and/or severity of neuronal necrosis (visible by H&E staining and confirmed by Fluoro-Jade B staining), most notably in the retrosplenial cortex, thalamic nuclei, and dorsal subiculum of the hippocampus, was seen at all doses in both sexes. The necrosis in the GNX HD group generally had the same severity and distribution pattern of neuronal necrosis as that associated with the MK-801 positive control group.

1.2 Recommendations

The application is approvable from a pharmacology/toxicology standpoint. Carcinogenicity studies of GNX in mouse and rat, a nonclinical safety evaluation of the M2 metabolite, and a brain distribution study of M47 should be conducted postmarketing.

2 Drug Information

2.1 Drug

CAS Registry Number	38398-32-2
Generic Name	Ganaxolone
Chemical Name	3α-hydroxy-3β-methyl-5α-pregnan-20-one
Molecular Formula/Molecular Weight	$C_{22}H_{36}O_2$/332.53 g/mol
Pharmacologic Class	Neuroactive steroid

2.2 Relevant IND

44020

2.3 Proposed Clinical Population and Dosing Regimen

Ganaxalone (GNX) is indicated for the treatment of seizures associated with Cyclin-dependent kinase-like 5 (CDKL5) Deficiency Disorder (CDD) in subjects 2 years of age and older. GNX is an oral suspension that is administered with food in increments of 15 mg/kg/day up to 63 mg/kg/day (up to a maximum of 1800 mg/day).

3 Studies Reviewed

Pharmacology
- Primary, secondary, safety

Pharmacokinetics
- ADME

Repeat-Dose General Toxicity
- 7-day non-GLP toxicity study in CD-1 mouse
- 1-, 3-, and 6-month pivotal toxicity studies in SD rat
- 1-, 6, and 12-month pivotal toxicity studies beagle dog

Genotoxicity
- in vitro Ames and mouse lymphoma assays of GNX
- in vivo rat bone marrow micronucleus assay of GNX
- in vitro Ames and mammalian chromosomal aberration assays of M2 metabolite

Carcinogenicity
- 1-month carcinogenicity dose range-finding study in RasH2 mouse

Reproductive and Developmental Toxicity
- Fertility and early embryonic development study in SD rat
- Embryofetal development study in CD-1 mouse
- Combined embryofetal and pre- and postnatal development study in SD rat
- 6-week juvenile development in SD rat
- 12-week juvenile development in SD rat
- Acute neurotoxicity study in neonatal SD rat

4 Pharmacology

4.1 Primary Pharmacology

Ganaxolone (GNX; 3α-hydroxy-3β-methyl-5α-pregnan-20-one) is a methyl-substituted analog of the endogenous neurosteroid allopregnanolone, a derivative of progesterone. Its's anticonvulsant effects are thought to result from positive allosteric modulation of the GABAA receptor.

In early primary pharmacology studies (Carter et al, JPET 280:1284–1295, 1997), the in vitro binding of GNX was consistent with activity as a positive allosteric modulator of the GABAA receptor. GNX produced concentration-dependent inhibition of specific binding of the GABAA receptor-gated chloride channel ligand [35S]TBPS to rat brain cortical membranes (IC50 of 80 nM) and enhancement of specific [3H]flunitrazepam binding to the benzodiazepine modulatory site (EC50 of 125 nM) and [3H]muscimol binding to the GABA recognition site (EC50 of 86 nM) on the GABAA receptor complex. Electrophysiological studies demonstrated that nanomolar concentrations of GNX potentiated GABA-evoked chloride currents in Xenopus oocytes expressing the human GABAA receptor subunits α1β1γ2L, α2β1γ2L or α3β1γ2L, whereas direct activation of chloride flux, in the absence of GABA, occurred only to a limited extent at micromolar concentrations.

In in vivo primary pharmacology studies, GNX was active in several rodent epilepsy models (Table 1).

Table 1. GNX anticonvulsant activity in rodent models

Study	Species	ROA of GNX	ED_{50} (95% CI) (mg/kg)	TD_{50} (95% CI) (mg/kg)	Protective Index[a]
Chemically-induced convulsions					
PTZ	Mouse	IP	4.3 (2.8 – 6.9)	33.4 (30.9 – 39.4)	7.8
PTZ	Rat	IP	7.8 (6.2 – 8.9)	14.2 (12.6 – 15.8)	1.8
PTZ	Rat	Oral	21.0 (18.1 – 24.3)	48.3 (38.7 – 60.3)	2.3
Bicuculline	Mouse	IP	4.6 (3.2 – 6.8)	33.4 (30.9 – 39.4)	7.3
TBPS	Mouse	IP	11.7 (8.8 – 15.7)	33.4 (30.9 – 39.4)	2.9
Aminophylline	Mouse	IP	11.5 (8.1 – 16.3)	33.4 (30.9 – 39.4)	2.9
Strychnine	Mouse	IP	> 40	33.4 (30.9 – 39.4)	< 1
4-AP	Mouse	IP	20 to 30	> 30 and < 56	NA
MES-induced convulsions					
MES	Mouse	IP	29.7 (25.3 – 34.8)	33.4 (30.9 – 39.4)	1.1
MES	Rat	Oral	58.4 (44.5 – 76.8)	48.3 (38.7 – 60.3)	0.8
Cornea-kindled convulsions					
Kindled	Rat	IP	4.5 (4.0 – 5.1)	14.2 (12.6 – 15.8)	3.2

ED_{50} = 50% maximal effective dose; GNX = ganaxolone; IP = intraperitoneal; MES = maximal electroconvulsive shock; NA = not applicable; PTZ = pentylenetetrazol; ROA = route of administration; SC = subcutaneous; TBPS = t-butylbicyclophosphorothionate; TD_{50} = behavioral toxic dose (50% population) in rotarod study.

a Protective index is calculated by dividing the ED_{50} from into the TD_{50} from the respective rotarod study.

GNX (IP administration) was effective against clonic convulsions induced by SC PTZ in mice and rats (ED50: 4.3 and 7.8 mg/kg, respectively) and against seizures induced by bicuculline (ED50: 4.6 mg/kg), IP TBPS (ED50: 11.7 mg/kg), and IP aminophylline (ED50: 11.5 mg/kg) in mice. GNX demonstrated potent anticonvulsant activity against corneal kindled stage 5 seizures in rats (ED50: 4.5 mg/kg) and increased the threshold for IV PTZ-induced clonus in mice. GNX only blocked MES-induced tonic seizures in mice at doses (ED50: 29.7 mg/kg) that produced ataxia on the rotorod (TD50: 33.4 mg/kg). GNX was also shown to be active against SC PTZ-induced convulsions after oral administration (ED50: 21 mg/kg). The profile of anticonvulsant activity was considered most similar to that of valproate. GNX was not tested for activity in a Cdkl5 disease model. While Cdkl5 knockout mouse models have been generated, these do not exhibit overt seizures.

Metabolite pharmacology

The activity of the putative major human metabolite, M2, at the GABAA receptor was compared to GNX in GABAA α1/β2/γ2 and α4/β3/γ2ion ion flux agonist and PAM (positive allosteric modulation, conducted in the presence of GABA) assays (Study US034-0011221). M2 had no functional activity at the GABAA receptor at concentrations up to 10 µM, while GNX exhibited EC50 values of 33 and 14 nM towards the GABAA α1/β2/γ2 and GABAA α4/β3/γ2 receptors, respectively, in the PAM assays.

Secondary pharmacology

In an in vitro binding assay screen (Study 100055032), GNX (10 µM) only produced significant (> 50%) inhibition against the binding of the radioligand for the GABA-gated chloride channel (95.8% inhibition).

In an in vitro binding assay conducted specifically to evaluate possible effects on cytosolic steroid receptors (Study 1042.216.046), GNX (10 µM) demonstrated only minimal radioligand displacement at estrogen (5%), glucocorticoid (9%), progesterone (8%) or testosterone (14%) receptors. In an in vitro functional assay (Indigo human PGR nuclear receptor reporter cell assay, S1500260-01) using HEK293 cells, GNX (up to 10 µM) did not activate the human progesterone receptor or inhibit progesterone activity.

Safety Pharmacology

In a CNS safety pharmacology study, single oral (gavage) doses of GNX (0, 10, 20, or 40 mg/kg) were administered to female SD rats (8/group) and neurological effects were assessed based on observations at 2, 4, 6, 8, and 24 hours postdose using a modified Irwin battery. Drug-related abnormal Irwin observations were seen at the HD (abnormal visual response, ataxia, grasping loss, abnormal righting reflex, and low carriage). A single occurrence of abnormal visual response and ataxia were seen at the MD.

The minimum sedative dose of GNX was determined in NSA mouse (Study 1042.221.014) and SD rat (Study 1042.221.013) using an open-field locomotor paradigm.

GNX (IP) administration produced dose-dependent biphasic effects on locomotor activity with increased locomotor activity at lower doses (20 and 5 mg/kg in mice and rats, respectively) and decreased locomotor activity beginning at higher doses (50 and 15 mg/kg in mice and rats, respectively) compared to C in both studies. The highest doses of GNX (50 and 20 mg/kg) produced loss-of-righting reflex. GNX was much more potent in producing sedative effects than the clinically used anticonvulsants ethosuximide and valproate.

An acute effect of GNX (0 (vehicle), 5, 10, 20, or 30 mg/kg IP) on associative learning was demonstrated in a passive-avoidance test in NSA mice (Study 1042.221.012). The HD resulted in a markedly decreased latency to enter the dark chamber compared to the vehicle control group (53.9 vs 98.5 sec, SS).

In a study comparing the sedative effects of Captisol and (b) (4) formulations of GNX (11042.RT.IV.103), both formulations produced dose-related sedation as a function of time at the doses tested (9, 12, or 15 mg/kg IV); however, the (b) (4) produced increased sedation, corresponding to higher GNX brain levels, which were 2-fold higher than those of the corresponding dose in the Captisol formulation. It was noted that though sedated, animals still responded to stimuli (toe pinch reflex) at all doses of GNX in both formulations, indicating that an anesthetic level was not reached. Sedation duration lasted from 30-120 min, depending on dose and vehicle formulation.

The effect on hERG channel currents was investigated in 2 studies using 2 different vehicles. In the first study (Study 171121.NBV) in which the vehicle was HEPES-buffered physiological saline + 0.3% DMSO + 1% BSA, GNX inhibited hERG potassium current amplitude by 0.6% at 1 µM and 1.5% at 3 µM, compared to 1.7% for the vehicle control. The second study (Study 1245-007) tested GNX in PSS containing 0.3% DMSO, which permitted evaluation of GNX at a concentration of 10 µM; no inhibition of hERG-mediated potassium currents was seen.

In a CV safety pharmacology study in telemeterized beagle dogs (Study 1245-008), single oral gavage doses (0 (HPMC, PVA, and SLS), 5, 10, and 15 mg/kg) produced no changes in body temperature, blood pressure, heart rate, or qualitative or quantitative ECG parameters.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

GNX is poorly soluble, and several oral formulations were evaluated in the nonclinical development program. The formulations used in the early single-dose PK and toxicology studies contained cyclodextrin, while the more recent toxicology studies evaluated a preformulated GNX suspension formerly referred to as the (b) (4) GNX suspension, that contains the following excipients: hypromellose, polyvinyl alcohol, sodium lauryl sulfate, methylparaben, propylparaben, sodium benzoate, (b) (4) citric acid, (b) (4) sodium citrate (b) (4), sucralose, (b) (4) simethicone emulsion, and (b) (4) water. This is the same formulation evaluated in clinical studies and resulted in the highest levels of exposure in nonclinical species compared to other oral formulations.

In a single-dose PK study in male rats (SC930057, Table 1), the plasma concentration-time profile following IV administration was biphasic and fit a two-compartment model with first order elimination. The distribution phase was very rapid with a half-life of 3 minutes. The volume of distribution at steady state was approximately 7 L/kg, greater than total body water, indicating possible tissue sequestration. The Beta half-life (HL) value was 93 minutes. Clearance was calculated to be 70.4 mL/min/kg. Following oral administration, GNX was rapidly absorbed, and Beta-HL values ranged from 5.5 to 8.6 hours. The 8-fold increase in AUCinf with the 2-fold increase in dose from 40 to 80 mg/kg suggested saturation of metabolism at the high dose. Clearance values ranged from 132 mL/min for the 40 mg/kg (b) (4)) group to 32.6 mL/min for the 80 mg/kg (b) (4)). Following oral administration, clearance values were 2- to 8-fold greater than that calculated following parenteral administration, which could be associated with reduced absorption, increased metabolism or both. Absolute bioavailability values (92-97%) indicated virtually complete absorption using the cyclodextrin formulations.

Table 1. Pharmacokinetics of GNX after single IV or PO administration to rats

	IV	Oral		
Parameter	2 mg/kg	40 mg/kg	80 mg/kg	40 mg/kg (b) (4)
C_{max} (µg/mL)	1.62	0.263	2.28	0.296
t_{max} (h)	NR	1.5	1.5	2
$AUC_{0\text{-}inf}$ (µg*min/mL)	30.9	69.7	552	107
CL (mL/min)	17.6	132	32.6	80.7
Bioavailability (%)	NA	91.6	94.5	97.3

Results are presented as mean values.

$AUC_{0\text{-}inf}$ = area under the drug-concentration vs time curve from time zero to infinity; CL = apparent clearance; C_{max} = maximum concentration; GNX = ganaxolone; (b) (4) IV = intravenous; NA = not applicable; NR = not reported; PK = pharmacokinetic; t_{max} = time of maximum concentration.

Source: Study SC930057

PK parameters determined in a single-dose PK study in male dogs (SC930058) are shown in Table 2. Following oral administration, AUC values increased greater than dose-proportionally, again suggesting saturation of metabolism, with clearance decreasing between the 40 and 60 mg/kg [redacted] (b) (4) dose groups. Absolute bioavailability values (89-91%) indicated almost complete absorption.

Table 2. Pharmacokinetics of GNX after single IV or PO administration to dogs

	IV	Oral		
Parameter	2 mg/kg	40 mg/kg	40 mg/kg	60 mg/kg [redacted] (b) (4)
C_{max} (µg/mL)	2.73	2.03	1.16	2.34
t_{max} (min)	NR	90.0	70.0	196
$AUC_{0\text{-}inf}$ (µg*min/mL)	97.5	1070	1260	3400
CL (mL/min)	294	445	622	211
Bioavailability (%)	NA	88.5	90.8	88.6

Results are presented as mean values.

$AUC_{0\text{-}inf}$ = area under the drug-concentration vs time curve from time zero to infinity; CL = apparent clearance; C_{max} = maximum concentration; GNX = ganaxolone [redacted] (b) (4) IV = intravenous; NA = not applicable; NR = not reported; PK = pharmacokinetic; t_{max} = time of maximum concentration.

Source: Study SC93058

In oral repeat-dose toxicology studies in mice, rats, and dogs, GNX was rapidly absorbed; however, in rodents GNX absorption appeared to become saturated, with exposures plateauing at about 125 mg/kg BID (see 7-day mouse and 13-week rat toxicity studies). After oral administration of GMX (10-80 mg/kg) to rabbits, plasma concentrations achieved were very low compared with rat and dog, possibly due to poor absorption and/or rapid removal from plasma by uptake or metabolism, prompting the use of mouse rather than rabbit as the second embryofetal development test species.

Distribution

In a protein binding study (Study 20200227-M005-01), values at 50 ng/mL GNX were >86.31%, > 94.63%, > 84.39% for mouse, rat, and human, respectively. Plasma protein binding values at 500 ng/mL were > 99.08%, > 99.32%, and > 99.28% for mouse, rat and human, respectively.

Tissue distribution in SD rats was evaluated in a single-dose mass balance study in which males were administered 14C-GNX formulated in [redacted] (b) (4) at a single IV dose of 2 mg/kg or single oral dose of 40 mg/kg. Based on these results, it was concluded that GNX extensively distributed to organs and tissues following oral and IV administration, with highest levels detected 1 hour after dosing, which decreased to near the limits of detection by 48 hours (Table 3). The highest concentrations of GNX were generally seen in the GI tract and liver.

Table 3. Distribution after a single oral dose (40 mg/kg) in male SD rats

	1 Hour		8 Hours		24 Hours		48 Hours	
Specimen Type	Conc. (µg/g)	% of dose	Conc. (µg/g)	% of dose	Conc. (µg/g)	% of dose	Conc. (µg/g)	% of dose
Skin	2.65	0.2	1.88	0.1	0.193	0	0.0790	0
Lymph gland	10.8	0	5.72	0	0.559	0	0.180	0
Mammary gland	7.25	0	9.47	0	1.89	0	0.136	0
Stomach	441	8.1	69.2	3.6	5.87	0.4	BDL	-
Liver	47.1	4.2	23.6	2.2	5.39	0.6	1.71	0.2
Kidney	7.93	0.1	5.65	0.1	0.909	0	0.356	0
Muscle	2.91	0	2.19	0	0.157	0	BDL	-
Heart	5.16	0	3.09	0	0.246	0	BDL	-
Lungs	6.14	0.1	4.65	0	0.585	0	BDL	-
Testes	2.19	0	1.89	0	0.172	0	BDL	-
Fat	18.0	0.1	15.4	0	2.56	0	0.198	0
Carcass	3.06	4.6	2.78	4.1	0.410	0.7	0.292	0.5
Brain	4.85	0.1	0.988	0	BDL	-	BDL	-
Spleen	3.78	0	2.24	0	0.166	0	BDL	-
Large intestine with cecum	13.0	1.3	611	61.8	36.9	4.2	4.85	0.7
Urinary bladder	3.45	0	7.15	0	1.15	0	BDL	-
Bone marrow	3.06	0	2.14	0	0.172	0	BDL	-
Adrenal gland	24.1	0	8.84	0	0.656	0	BDL	-
Pancreas	16.5	0.1	4.86	0	0.445	0	BDL	-
Eyes	1.47	0	1.22	0	BDL	-	BDL	-
Thyroid gland	5.43	0	2.86	0	BDL	-	BDL	-
Small intestine	780	67.8	107	10.7	28.1	3.3	2.28	0.3
Total	-	86.8	-	82.9	-	9.2	-	1.7

BDL = below detection level; Conc = concentration GNX = ganaxolone.
Source: Study SC930183

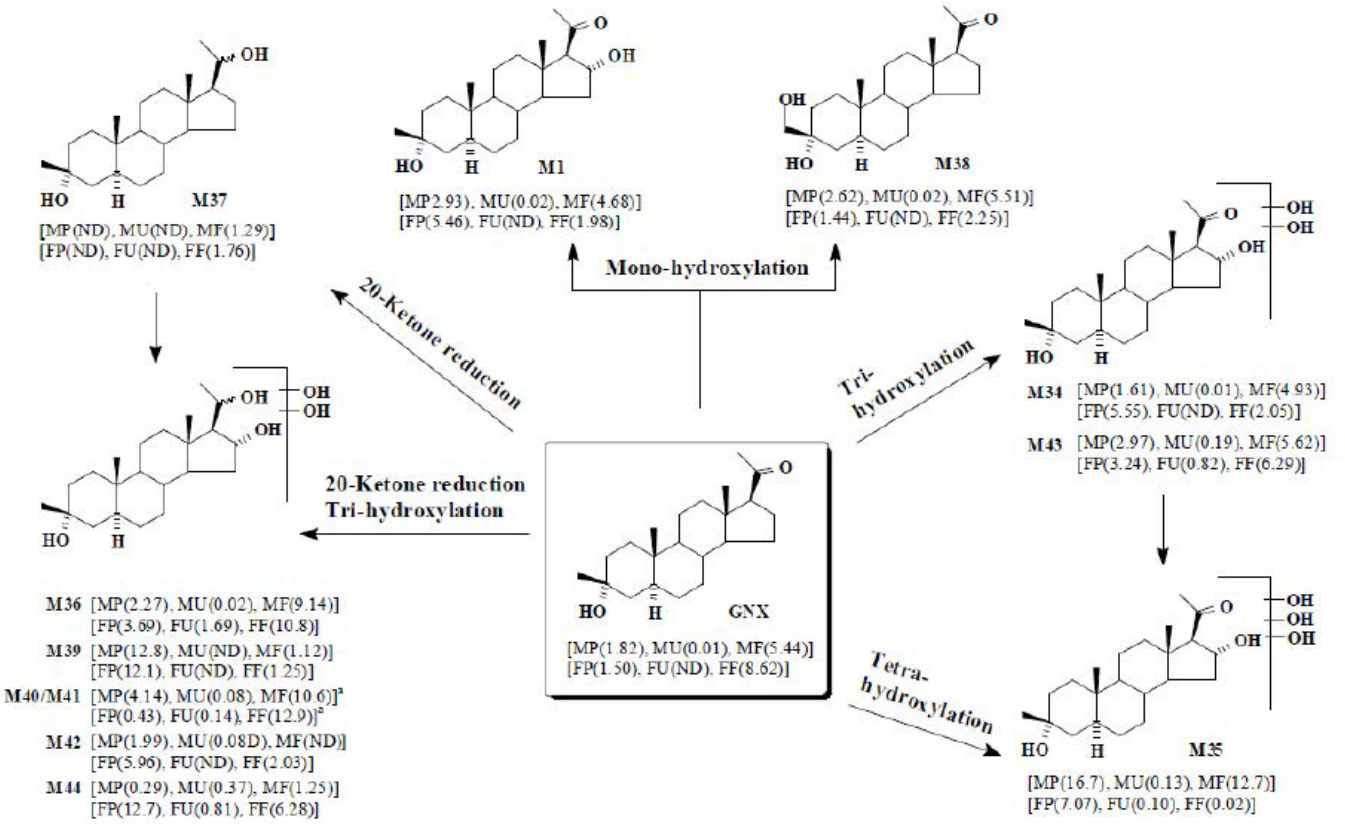
Metabolism

A metabolism study ((b) (4)08694) in mouse, rat, dog, and human liver microsomes showed that GNX was extensively metabolized, and that the major metabolites were multi-hydroxy-metabolites and/or products of 20-ketone reduction. 20β-hydroxyganaxolone (M37), 3β-hydroxymethylganaxolone (M38), and 16α-hydroxyganaxolone (M1) were the major monohydroxylated metabolites and the major multi-hydroxy-metabolites were products of the combination of 20-keto-reduction, 16α-hydroxylation, and 3β-hydroxylation. No unique human metabolites were identified in this study.

In vivo metabolism was evaluated in a study ((b) (4)07698) in which CD-1 mice received a single oral dose of 20 mg/kg [14C]-GNX (approximately 400 µCi/kg). In males, the major plasma circulating metabolite was M39 (Marinus designation), with an AUC0-t value at 22% relative to plasma total radioactivity, followed by M35 and M45, accounting for 11.7

and 8.8%, respectively (Figure 1). In females, M44 and M39 were the major circulating metabolites, with AUC0-t values greater than approximately 12% relative to plasma total radioactivity.

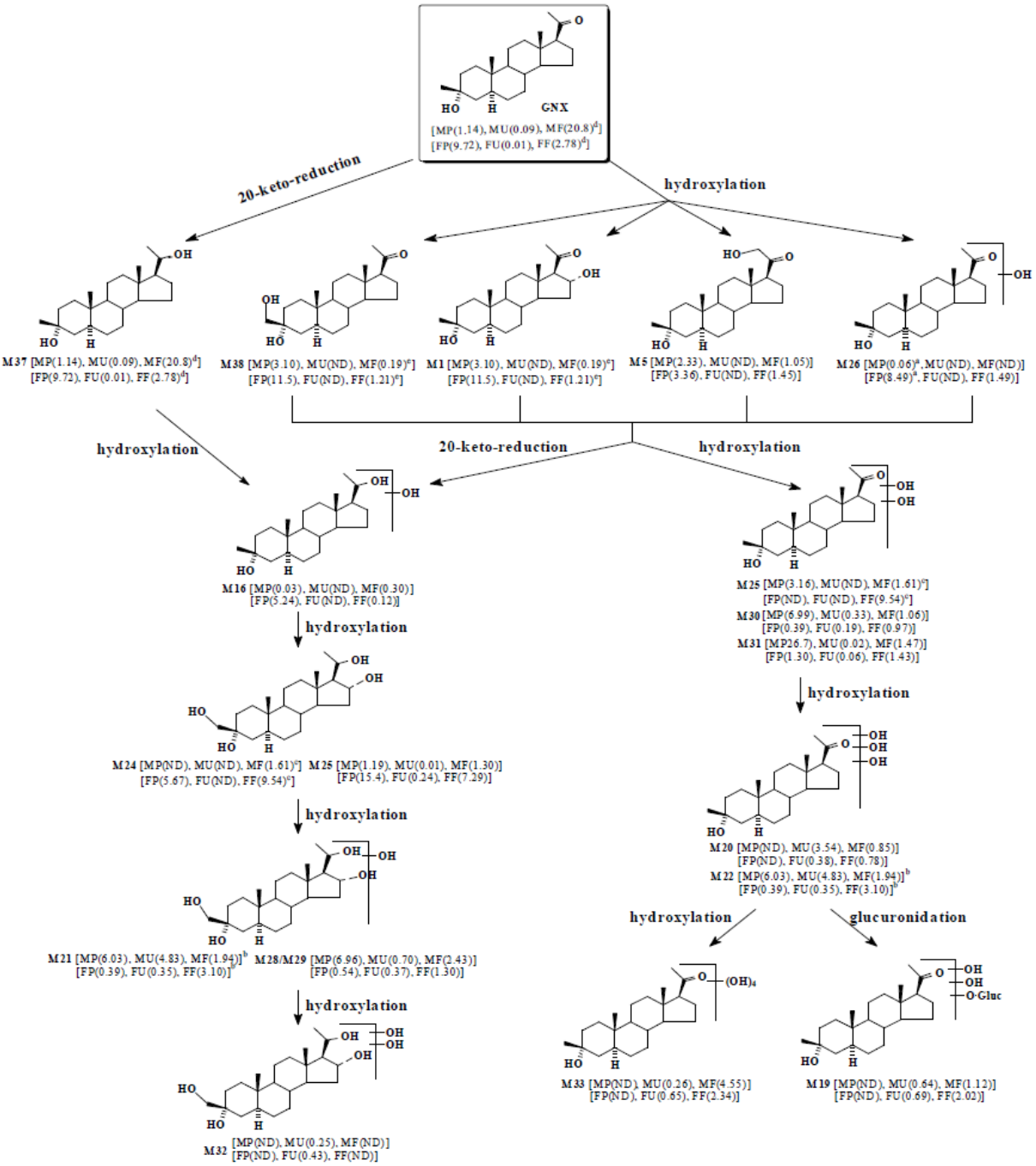
Figure 1. Proposed metabolic pathways in mice



ND = not detected; GNX = ganaxolone.
Values in parenthesis are the percent of AUC for male plasma (MP) or female plasma (FP) and percent of dose for male urine (MU) or female urine (FU), and male feces (MF) or female feces (FF).
a The value is the total value of M40 and M41.
Source: Study (b) (4) 07698

In a single-dose PK and metabolism study ((b) (4) 07699) in SD rats in which a single oral dose of 20 mg/kg [14C]-GNX (approximately 100 µCi/kg) was administered, unchanged GNX was a minor circulating component (0.97% of the AUC0-t) in males; the major metabolite was M31 (25.7% of the AUC0-t). In females, unchanged GNX was a major radioactive component in plasma (8.5% of the AUC0-t), and M1 and M23 were the major circulating metabolites (12 and 16% of the AUC0-t, respectively). (See Figure 2.)

Figure 2. Proposed metabolic pathways in rat



* Values in parentheses are the percent of AUC for male plasma (MP) or female plasma (FP) and percent of dose for male urine (MU) or female urine (FU), and male feces (MF) or female feces (FF). ND: not detected.

[a] The values are the combined values of M26 and M27.

[b] The values are the combined values of M21 and M22.

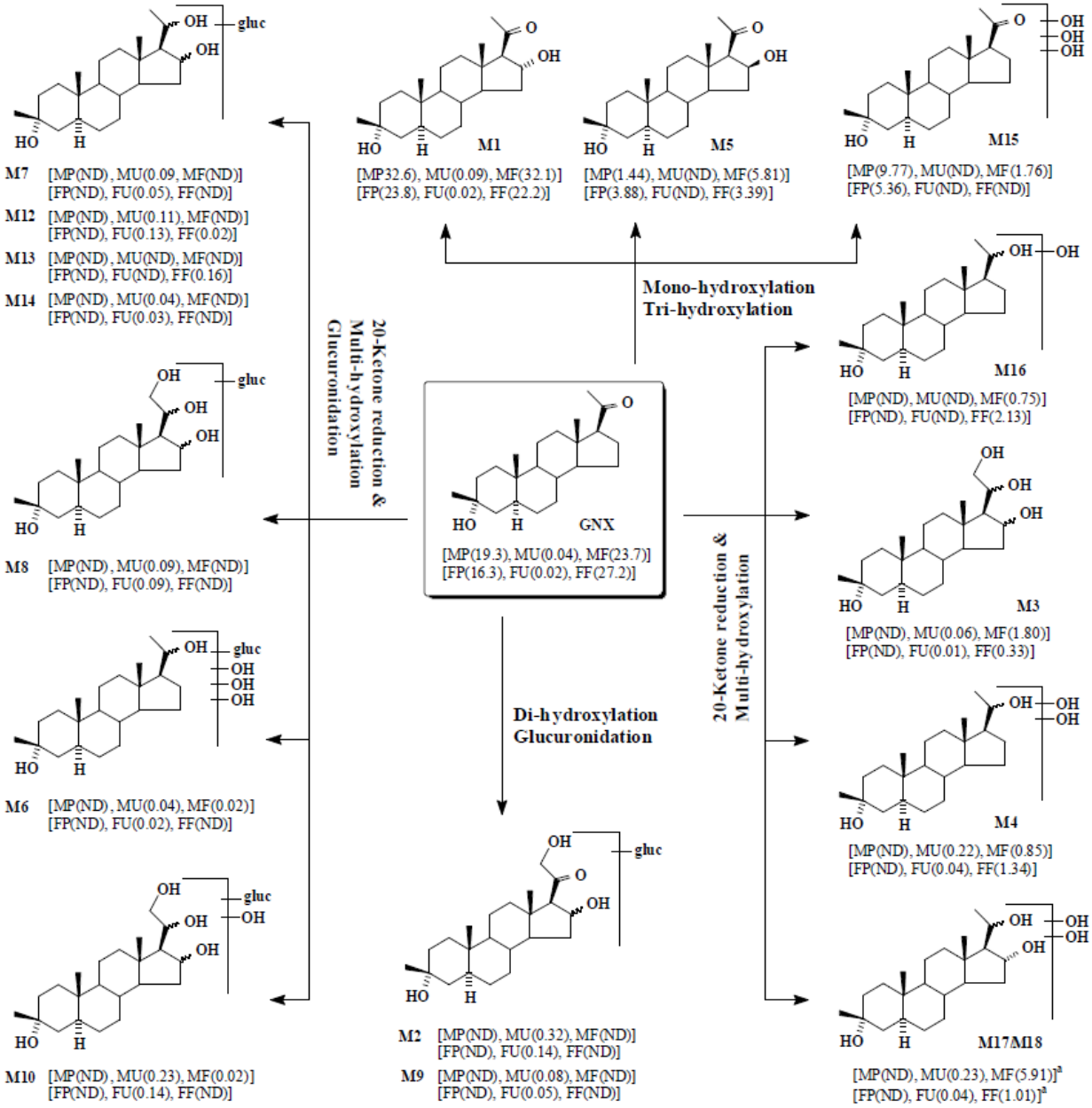
[c] The values are the combined values of M24 and M25.

[d] The values are the combined values of GNX and M37.

[e] The values are the combined values of M1 and M38.

When metabolism was evaluated in beagle dogs ([(b) (4)]07697) after a single oral dose of 10 mg/kg [14C]-GNX (approximately 15 µCi/kg), total plasma exposure to [14C]-GNX-derived radioactivity in males was largely attributed to parent and its M1 (32.6%) and M15 (9.77%) metabolites, which together accounted for 61.7% of the AUC0-t (Figure 3). In females, parent and metabolites M1 (23.8%), M5 (3.88%), and M15 (5.36%) accounted for 72% of the AUC0-t.

Figure 3. Proposed metabolic pathways in dog



*Values in parentheses are the percent of AUC for male plasma (MP) or female plasma (FP) and percent of dose for male urine (MU) or female urine (FU), and male feces (MF) or female feces (FF).

ND: not detected.

[a] The value is the total value of M17 and M18.

A more recent study (8333043) was conducted to identify and characterize the relative proportions of the major metabolites in human, dog, and rat pooled plasma samples taken following repeat oral administration of GNX, using LC-MS(/MS) determination. The reported metabolite profiling data were comparative only since values were not generated using quantitative analytical methods. The observed in vivo metabolism of the GNX was complex and showed multiple hydroxylation and subsequent conjugations. In pooled human plasma samples, approximately 30 metabolites were detected at >1% of the total chromatographic peak area. The major human metabolites were glucuronide conjugates of oxidized metabolites. However, one non-conjugated metabolite, ganaxolone+2O (m/z 365, 8.46 mins), was detected at up to 16% of total drug related exposure in human plasma but was not observed in rat or dog plasma (Tables 4 and 5). This is presumably the same metabolite, albeit with a different structure tentatively (mis)identified, as what was later determined in a human metabolism study to be oxy-dehydro-ganaxolone (M60b or M2).

Table 4. Metabolite profile in rat plasma compared to human

			Integrated Peak Area		Metabolite Coverage Ratio	Integrated Peak Area		Metabolite Coverage Ratio
Component	RT (min)	Formula	Human 800 mg bid Day 9	Male rat 20 mg/kg Day 10	Male Rat/Human	Human 800 mg bid Day 9	Female Rat 20 mg/kg Day 10	Female Rat/Human
Ganaxolone-H2O+H2	10.61	$C_{22}H_{36}O$	14414	ND	-	16246	ND	-
Ganaxolone	11.59	$C_{22}H_{36}O_2$	2953	ND	-	3148	1741	0.55
21-OH	8.95	$C_{22}H_{36}O_3$	1628	1891	1.16	1822	ND	-
16α-OH	7.78	$C_{22}H_{36}O_3$	3612	ND	-	3304	ND	-
Ganaxolone+2x(+O)	8.48	$C_{22}H_{36}O_4$	66393	1282	0.02	70397	ND	-
Ganaxolone+H2+C6H8O6	5.91	$C_{28}H_{46}O_8$	ND	ND	-	ND	ND	-
Ganaxolone-H2+C3H5NOS	8.27	$C_{25}H_{39}NO_3S$	17329	ND	-	21077	ND	-
Ganaxolone+O+C6H8O6	5.87	$C_{28}H_{44}O_9$	54644	ND	-	52651	ND	-
Ganaxolone+O+C6H8O6	6.20	$C_{28}H_{44}O_9$	20831	ND	-	20894	ND	-
Ganaxolone+H2O+C6H8O6	4.28	$C_{28}H_{46}O_9$	ND	ND	-	ND	ND	-
Ganaxolone+2x(+O)+C6H8O6	4.40	$C_{28}H_{44}O_{10}$	15149	ND	-	13738	2468	0.18
Ganaxolone+H2O+O+C6H8O6	3.45	$C_{28}H_{46}O_{10}$	ND	ND	-	ND	ND	-

ND = not detected
Components shown account for >5% of the total peak area in at least one species (rat/dog/human)
- ratio not calculated as not observed in one species

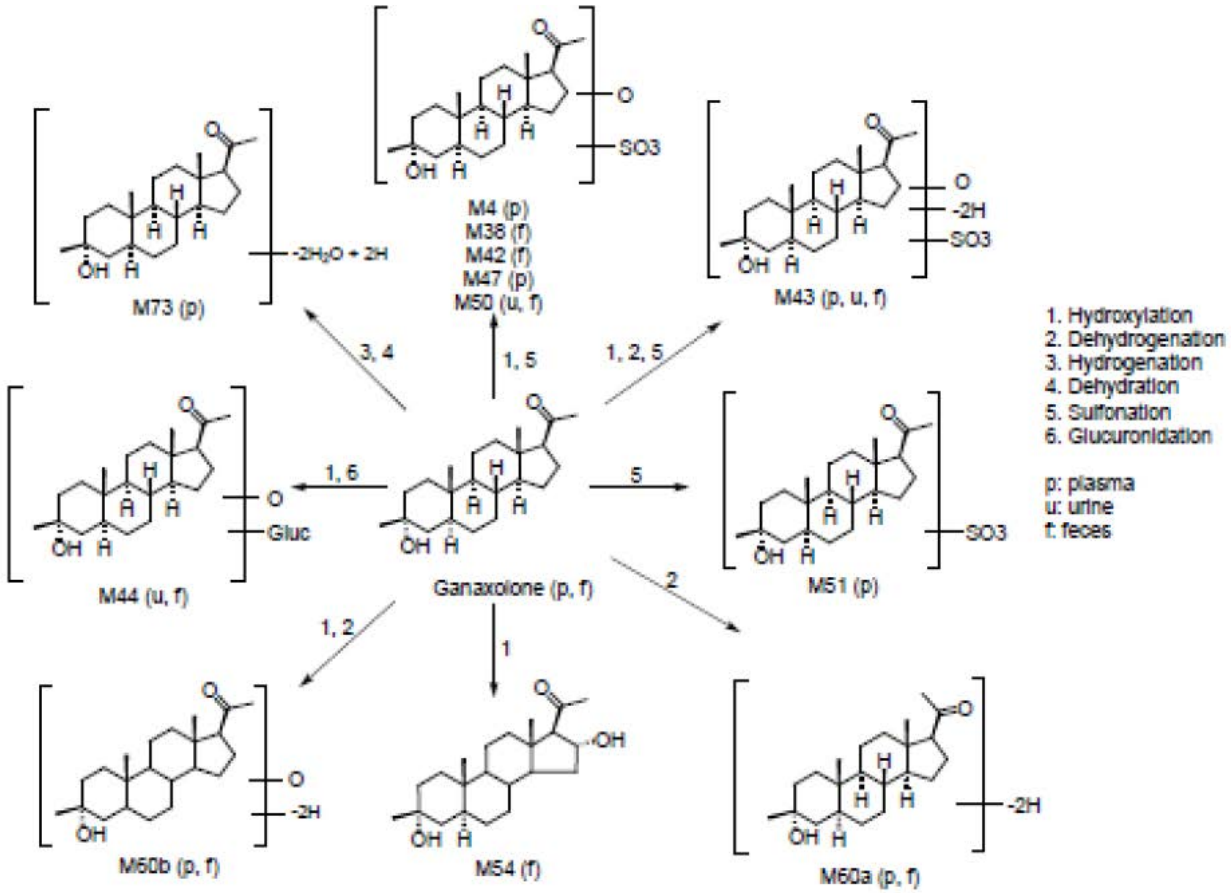
Table 5. Metabolite profile in dog plasma compared to human

			Integrated Peak Area		Metabolite Coverage Ratio
Component	RT (min)	Formula	Human 800 mg bid Day 9	Dog 10 mg/kg Day 7	Dog/Human
Ganaxolone-H2O+H2	10.61	$C_{22}H_{36}O$	25248	699	0.03
Ganaxolone	11.59	$C_{22}H_{36}O_2$	6257	3579	0.57
21-OH	8.95	$C_{22}H_{36}O_3$	924	402	0.44
16α-OH	7.78	$C_{22}H_{36}O_3$	5941	2367	0.40
Ganaxolone+2x(+O)	8.48	$C_{22}H_{36}O_4$	126663	ND	-
Ganaxolone+H2+C6H8O6	5.91	$C_{28}H_{46}O_8$	ND	10757	-
Ganaxolone-H2+C3H5NOS	8.27	$C_{25}H_{39}NO_3S$	46817	ND	-
Ganaxolone+O+C6H8O6	5.87	$C_{28}H_{44}O_9$	87992	ND	-
Ganaxolone+O+C6H8O6	6.20	$C_{28}H_{44}O_9$	36368	1527	0.04
Ganaxolone+H2O+C6H8O6	4.28	$C_{28}H_{46}O_9$	23445	ND	-
Ganaxolone+2x(+O)+C6H8O6	4.40	$C_{28}H_{44}O_{10}$	26898	ND	-
Ganaxolone+H2O+O+C6H8O6	3.45	$C_{28}H_{46}O_{10}$	22480	ND	-

ND = not detected
Components shown account for >5% of the total peak area in at least one species(rat/dog/human)
- ratio not calculated as not observed in one species

In a human pharmacokinetics and metabolism study (1042-GNX.AME-1001) in which a single 300-mg oral dose of [14C]-ganaxolone was administered, 12 metabolites were identified/characterized by liquid chromatography-high resolution mass spectrometry. The results indicated that hydroxylation was the predominant primary biotransformation pathway, while hydrogenation and sulfonation were minor primary metabolic pathways. Sulfonation, dehydrogenation, and glucuronidation were common secondary biotransformation pathways. Oxy-dehydro-ganaxolone ([redacted] (b) (4) designation M60b), a product of hydroxylation and secondary dehydrogenation, was identified as a major plasma metabolite (Figure 1, Table 6). Hydroxylation of ganaxolone with secondary sulfonation produced multiple hydroxy-ganaxolone sulfate conjugates in plasma, including M4 and M47, the latter of which appears to be a major conjugated plasma metabolite. Hydroxylation, dehydrogenation, and secondary sulfonation produced oxy-dehydro-ganaxolone sulfate (M43).

Figure 1. Proposed biotransformation pathways in human subjects



Note: Pathways are proposed based on general knowledge of metabolism and do not imply definitive pathways. Direct experimentation was not performed.

Abbreviations: f = feces; p = plasma; u = urine

Table 6. Percent of metabolite radioactivity from the 0-120-hr and 0–30-day pooled plasms samples and the exposure of each relative to GNX

Marinus Designation	(b) (4) Designation	Name	% of total radioactivity		% of GNX (0-30 day)
			1-120 hr	0-30 day	
M2	M60a/M60b	GNX+O-2H	29.43	23.39	575%
M6	M73	A ring dehydrated M4	13.42	2.3	57%
M5	M18	A ring dehydrated GNX	13.16	4.6	113%
M17	M43	M2+S	10.53	2.0	49%
M15	M47	Unknown GNX+O+S	3.95	24.35	598%
M4	M62	20-a-hydroxyGNX	3.61	2.97	73%
	Ganaxolone		3.27	4.07	100%

M2 (Marinus designation, oxy-dehydro-ganaxolone, (b) (4) designation M60b) demonstrated no functional activity in GABAA assays and was negative for mutagenicity in the Ames assay but was positive in a mammalian chromosomal aberration assay. According to the sponsor, “appropriate follow-up studies are ongoing to evaluate the potential for M2 to be genotoxic in vivo.”

Excretion

In the oral radiolabeled GNX study in rats ((b) (4) 07699, described above), more than 85% of the radioactivity was recovered within 48 hours after dosing and most of the radioactivity (>93%) was recovered within 72 hours. The majority of the [14C]GNX-derived radioactivity was excreted in the feces (64%-82%) with a smaller amount excreted in the urine (12%-29%, including cage rinse/wash).

In the oral radiolabeled GNX study in dogs ((b) (4) 07697, above), the major elimination pathway was via fecal excretion (>68%) with less recovered in the urine and cage rinse/wash (<17%). There were no apparent sex differences in excretion patterns.

Toxicokinetics

A single dose of 2000 mg administered to healthy adult volunteers was associated with a plasma exposure (AUC) of approximately 3000 ng.h/mL (1042-0404). The maximum recommended human oral (suspension) dose for the proposed indication is 63 mg/kg/day (up to a maximum of 1800 mg/day). Simulated GNX profiles were generated using a population PK model for 4 different age groups, using median weights for each age group and doses of 63 mg/kg/day up to 1800 mg administered until steady-state was achieved. At steady-state, the GNX Cmax, Cmin, and AUC0-24h were calculated (Table 6). Exposure at steady-state was similar across the different age groups of patients (approximately 4000 ng.h/mL) but higher than that measured in adults.

Table 6. Simulated GNX Steady-state Exposure in Pediatric CDD Patients (1042-CDD-POPPK-001)

Age Group	Mean Body Weight (kg)	Dose (mg)	AUC_{0-24} (ng*hr/mL)	C_{min} (ng/mL)	C_{max} (ng/mL)
2 to < 6 years	14.8	312	3903	85	247
6 to < 12 years	22.6	475	3998	84	269
12 to < 18 years	36.1	600	4106	84	293
≥ 18 years	35.1	600	4100	84	292

Notes: Dose represents the dose amount in mg administered 3 times daily.
AUC_{0-24} = 24-hour area under the GNX plasma concentration time curve; CDD = CDKL5 deficiency disorder; C_{max} = maximum GNX plasma concentration; C_{min} = minimum GNX plasma concentration.
Source: 1042-CDD-POPPK-001 Table 11.

In oral repeat-dose toxicology studies in rats, GNX absorption appeared to become saturated, with exposures plateauing at ≥ 125 mg/kg. Females had consistently higher exposures than males, and exposure decreased in both sexes with repeated dosing, with greater decreases observed at higher doses. The reduction in exposure with repeated dosing was attributed to autoinduction of liver enzymes, which was demonstrated in mouse and rat toxicity studies, with CYP3A and CYP2B enzyme activities being particularly affected. The sex difference in exposure observed in rats was attributed to the greater production of CYP3A in males. In an effort to achieve higher exposures and mitigate the dose-limiting sedative effects, which appeared to be driven by Cmax, dose escalation was used in the more recent mouse and rat toxicology studies, which allowed doses as high as 500 mg/kg to be given BID. But despite dose escalation, plasma exposures did not increase commensurately. Because of these PK factors and dose-limiting sedation, exposures at the highest doses tested in rats were similar to (F) or below (M) clinical exposures (Table 7). There was no sex difference in exposure in dogs and no evidence of autoinduction with repeated dosing; GNX exposure increased in dogs in a dose-proportional manner up to the highest dose tested (20 mg/kg/day, limited by sedation) and accumulated with repeated dosing. Exposure margins achieved were adequate in dog.

Table 7. Exposure margins at NOAELs in repeat-dose toxicity studies

Study	NOAEL (mg/kg/day)	HED[a] (mg/day)	NOAEL C_{max}[b] (ng/mL)	NOAEL AUC[b] (ng*h/mL)	Safety Margin for C_{max}[c]	Safety Margin for AUC[d]
Repeat-dose toxicology studies						
14-day rat[e] (SC930109)	60	581	M: 229 F: 479	M: 1068 F: 2900	M: 0.87 F: 1.8	M: 0.36 F: 0.97
1-month rat[f] (1245-006)	ND M: 80[g] F: 40[g]	ND 774 387	NA M: 89.3 F: 228	NA M: 374 F: 2004	NA M: 0.34 F: 0.87	NA M: 0.12 F: 0.67
13-week rat[f] (8412976)	M: 160/250/1000[h] F: 60/120/1000[h]	9677	M: 103 F: 459	M: 1430 F: 6140	M: 0.39 F: 1.8	M: 0.48 F: 2.0
6-month rat[e] (SC930189)	20	194	M: 157.4 F: 171.4	NC[i]	M: 0.60 F: 0.65	NC[i]
14-day dog[e] (SC930108)	10	333	M: 578 – 625[j] F: 241 – 433[j]	M: 3367 – 4267[j] F: 1733 – 2550[j]	M: 2.2-2.4 F: 0.9 -1.7	M: 1.1-1.4 F: 0.58-0.85
1-month dog[f] (1245-005)	ND 15[g]	ND 500	NA M: 1507 F: 1426	NA M: 15964 F: 13900	NA M: 5.8 F: 5.4	NA M: 5.3 F: 4.6
6-month dog[e] (SC930190)	10	333	M: 1200 F: 792.6	NC[i]	M: 4.6 F: 3.0	NC[i]
12-month dog[f] (1245-011)	3 15[g]	100 500	M: 520 F: 798 M: 2291 F: 1756	M: 4817 F: 5335 M: 33888 F: 24346	M: 2.0 F: 3.0 M: 8.7 F: 6.7	M: 1.6 F: 1.8 M: 11 F: 8.1
Embryofetal Toxicity Studies						
Mouse (1215 007)	300	1463	NA	249[k]	NA	0.083
Rat (COY 7/961509)	40	387	NA	2004[l]	NA	0.67

AUC = area under the concentration-time curve; C_{max} = maximum concentration; F = female; GNX = ganaxolone; HED = human equivalent dose; M = male; NA = not applicable; NC = not calculated; ND = not determined; NOAEL = no observed adverse effect level.

a HED assumes a 60 kg person, with conversion factors of 6.2 and 1.8 for rats and dogs, respectively.

b C_{max} and AUC values are from the last day of dosing.

c Safety margin for C_{max} is based on a C_{max} of 262 ng/mL from adults that received an 2000 mg/day dose of GNX in Study 1042-0404.

d Safety margin for AUC is based on a AUC of 3,000 ng*h/mL from adults that received an 2,000 mg/day dose of GNX in Study 1042-0404.

e Test article was GNX DS and the vehicle was a formulation containing cyclodextrin.

f Test article was preformulated GNX suspension.

g Adjusted NOAEL that excluded sedation effects that did not affect animal health status (eg, no body weight loss or dehydration) were reversible and were considered non-adverse in other repeat-dose toxicology studies.

h In the 13-week repeat-dose toxicology study in rats, GNX daily doses were administered as divided doses twice daily and were administered at the initial daily dose (160 and 60 mg/kg/day for males and females, respectively) from Days 1 to 4; the second daily dose (250 and 120 mg/kg/day for males and females, respectively) from Days 5 to 8; and the third daily dose (1000 mg/kg/day for both sexes) from Days 9 to 91.

i In the 6-month repeat-dose toxicology studies, blood samples for toxicokinetic analysis were only collected at 2 timepoints, timed to occur at the anticipated minimum concentration (C_{min}) and C_{max} and so AUC values could not be calculated.

j There were only 2 dogs/sex/group in the 14-day dog study.

k AUC value is from Gestation Day 6 of mice dosed with 200 mg/kg/day of the mouse dose range-finding embryofetal toxicity study (Study 1215-006), which due to a plateau in exposure is comparable to what would be achieved with the 300 mg/kg/day NOAEL in the GLP-compliant mouse embryofetal toxicity study.

l AUC value is from Day 29 in female rats dosed with 40 mg/kg/day in the 1-month repeat-dose toxicology Study 1245-006.

6 General Toxicology

6.3 Repeat-Dose Toxicity

Study title: Ganaxolone: 7-Day Oral Gavage Toxicity and Toxicokinetic Study in Mice

Study no.:	8412975
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10 September 2019
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	MBS-J0006-060510/ 99.5%

Key Study Findings

When CD-1 mice (5/sex/grp) were administered preformulated GNX suspension for 7 days by oral gavage using an incremental dosing strategy, there were no GNX-related findings. Plasma levels (Cmax and AUC0-12) were generally similar with the increase in dose from 125 to 500 mg/kg/dose; the slight increases in GNX Cmax and AUC0-12h values were less than dose proportional.

Methods

Doses:	M: 0 (vehicle), 250, 500, or 1000 mg/kg/day
Frequency of dosing:	BID
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	Preformulated GNX suspension/ hypromellose (HPMC), polyvinyl alcohol (PVA), sodium lauryl sulfate (SLS), methylparaben, propylparaben, sodium benzoate, (b) (4) citric acid, sodium citrate (b) (4), sucralose, and simethicone
Species/Strain:	Mouse/CD-1
Number/Sex/Group:	5/sex/group
Age:	6 to 7 weeks
Weight:	M: 28.8 - 39.0 g, F: 19.9 - 29.6 g
Satellite groups:	6-18/sex/grp TK
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Table 1 Experimental design Appears this way on original

Group[a]	Subgroup	No. of Animals Males	No. of Animals Females	Dose Level[b] (mg/kg/dose)	Dose Level[b] (mg/kg/day)	Dose Concentration[c] (mg/mL)
1 (Control)	1 (Toxicity)	5	5	0	0	0
	2 (Toxicokinetic)	6	6			
2 (Low)	1 (Toxicity)	5	5	125	250	12.5
	2 (Toxicokinetic)	18	18			
3 (Mid)	1 (Toxicity)	5	5	125[d]/250[e]	250[d]/500[e]	12.5[d]/25[e]
	2 (Toxicokinetic)	18	18			
4 (High)	1 (Toxicity)	5	5	125[d]/500[f]	250[d]/1000[f]	12.5[d]/50[f]
	2 (Toxicokinetic)	18	18			

a Group 1 was administered vehicle control article only.
b Two doses were administered daily, 12 hours apart (±30 minutes). On each day of dosing, the second daily dose was based on the end dose time of the first daily dose of each sex/group/subgroup.
c Test article concentrations were based on the test article as supplied (no correction). Dose volume for all dose groups was 10 mL/kg/dose (20 mL/kg/day).
d On Days 1 through 3 of the dosing phase, animals in Groups 3 and 4 were administered two daily doses of 125 mg/kg/dose (250 mg/kg/day) at a concentration of 12.5 /mL.
e Starting on Day 4 and through the end of the dosing phase, animals in Group 3 were administered two daily doses of 250 mg/kg/dose (500 mg/kg/day) at a concentration of 25 mg/mL.
f Starting on Day 4 and through the end of the dosing phase, animals in Group 4 were administered two daily doses of 500 mg/kg/dose (1000 mg/kg/day) at a concentration of 50 mg/mL.

All animals in the GNX-treated groups were administered 125 mg/kg/dose BID (250 mg/kg/day) from Days 1 through 3, then, starting on Day 4, the BID doses were increased for the MD and HD groups so that for Days 4 through 7 doses of 125, 250, or 500 mg/kg/dose BID (250, 500, or 1000 mg/kg/day) were administered. Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, and sedation observations. Blood samples were collected for TK evaluation at 0.25, 0.5, 1, 3, 7, and 12 hours postdose on Day 7. Doses were based on the results of (b) (4) Study 8352578 (not submitted).

Observations and Results

Mortality

There were no drug-related deaths.

Clinical Signs

No drug-related clinical observations were noted.

Body Weights

There were no drug-related differences in BWs or BW change.

Toxicokinetics

TK data are shown in Table 2. Sex differences in GNX Cmax and AUC0-12 values were less than 2-fold. Exposure, based on Cmax and AUC0-12, was generally similar with the increase in dose from 125 to 500 mg/kg/dose. The slight increases in Cmax and AUC0-12h values were much less than dose proportional.

Table 2. TK parameters in CD-1 mouse

Dose Group	Dose Level (mg/kg/day)	Dose Level (mg/kg/dose)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12} (h*ng/mL)	AUC_{0-24} (h*ng/mL)	$t_{1/2}$ (h)
2	250	125	M	37.3	0.500	185	371	NC
			F	40.0	0.500	205	410	NC
			MF	38.7	0.500	195	390	NC
3	500	250	M	36.8	0.500	205	409	3.26
			F	41.9	3.00	267	534	NC
			MF	37.4	0.500	236	472	NC
4	1000	500	M	56.0	0.500	271	542	NC
			F	60.2	0.250	265	530	NC
			MF	49.6	0.500	268	536	NC

NC Not calculated due to the inability to characterize the elimination phase per SOP criteria.
Notes: Combined male and female (MF) parameters were calculated by combining concentration data for all animals (male and female) at each dose level on each interval and using these data as a separate composite profile for TK analysis. These parameters are not an average of the values calculated for males and females separately.
AUC_{0-24} results were estimated by multiplying the AUC_{0-12} results by 2.

Study title: Ganaxolone: A One Month Oral Toxicity Study in Rats

Study no.:	1245-006
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	08 Jun 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MBS-J0006-060510/ 99.5%

Key Study Findings

Oral (gavage) administration of GNX (see table) to SD rats for 4 weeks resulted in dose-dependent sedative effects (decreased activity, ataxia, and/or prostration) at all doses. Females appeared to be more sensitive than males, based on severity and frequency at the same doses. Effects decreased throughout the study indicating accommodation. There were no drug-related effects on body weights (BWs), clinical pathology, or macroscopic and microscopic examinations. CYP3A1/2 and CYP2B1/2 enzyme activities were increased in treated females. On Day 1, the highest doses tested (80 and 40 mg/kg/day) were associated with Cmax and AUC values of 228 and 439 ng/mL and 1267 and 4371 ng•h/mL in males and females, respectively. Day 15 and 29 exposures were lower than those on Day 1 in both sexes.

Methods

Doses:	M: 0 (vehicle), 10, 40, or 80 mg/kg/day F: 0 (vehicle), 10, 20, or 40 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	Preformulated GNX suspension/ Vehicle B: hypromellose, polyvinyl alcohol, sodium lauryl sulfate, methylparaben, propylparaben, and simethicone emulsion
Species/Strain:	Rat/Sprague Dawley Crl:CD(SD)
Number/Sex/Group:	10/sex/group
Age:	6-8 Weeks
Weight:	M: 225-262 g, F: 170-197 g
Satellite groups:	4-12/sex/grp TK
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Table 1

Group Assignments				
Group Number	Dose Level (mg/kg)		Number of Animals	
	Male	Female	Male	Female
Main Study				
1	0 mg/kg/day	0 mg/kg/day	10	10
2	10 mg/kg/day	10 mg/kg/day	10	10
3	40 mg/kg/day	20 mg/kg/day	10	10
4	80 mg/kg/day	40 mg/kg/day	10	10
TK				
5	0 mg/kg/day	0 mg/kg/day	4	4
6	10 mg/kg/day	10 mg/kg/day	10	10
7	40 mg/kg/day	20 mg/kg/day	10	10
8	80 mg/kg/day	40 mg/kg/day	12	10

Dose selection said to be based on the results of previous studies, but details were not provided.

Observations and Results

Mortality

There were no unscheduled deaths during the course of the study.

Clinical Signs

Dose-related sedation was observed throughout the study. Sedation scores of 4 (prostration) were observed in HD males on Days 1, 2, and 3 at 2, 4, and 8 hours postdose and in HD females on Day 1 at 2, 4, and 6 hours postdose. Nearly all HD males and females were observed with some degree of sedation during the first week with the peak effect occurring between 2 and 4 hours postdose. Decreased activity and ataxia (sedation scores of 2 and 3) were the most severe findings noted in LD and MD males and females. Females appeared to be more sensitive than males, based on scores at the doses they had in common. The severity and duration of sedation decreased throughout the study.

Body Weights

There were no drug-related differences in BWs or BW gain.

Clinical Pathology

There were no apparent drug-related changes in clinical pathology parameters.

Organ Weights

There were no group differences in organ weights.

Gross Pathology

No drug-related macroscopic observations were noted.

Histopathology

There were no drug-related microscopic findings.

Enzyme induction

A dose dependent increase in CYP3A1/2 (168, 287, and 618% compared to C) and CYP2B1/2 (124, 152, and 278% of C) activity was observed in treated females. There were no significant changes in CYP enzyme activities in males.

Toxicokinetic

TK data for GNX are shown in Table 2. Cmax and AUC values on Days 15 and 29 were consistently lower than on Day 1 in both sexes. Exposures increased approximately dose-proportionately in males and less than dose-proportionately in females.

Table 2. TK parameters in SD rat Appears this way on original

Group	Sex	Treatment	Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-t)}$ (ng·h/mL)	$AUC_{(0-inf)}$ (ng·h/mL)	$AUC_{(0-t)}$ /Dose
6	M	Ganaxolone	10	23.8	2.0	1.1	62.8	65.8	6.3
7	M	Ganaxolone	40	91.8	2.0	3.1	432	433	10.8
8	M	Ganaxolone	80	228	4.0	2.3	1267	1269	15.8
6	F	Ganaxolone	10	302	2.0	2.4	1594	1596	159.4
7	F	Ganaxolone	20	341	2.0	3.1	2186	2197	109.3
8	F	Ganaxolone	40	439	2.0	2.9	4371	4386	109.3

Ganaxolone, Day 15

Group	Sex	Treatment	Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-t)}$ (ng·h/mL)	$AUC_{(0-t)}$ /Dose	Rc[a]
6	M	Ganaxolone	10	24.4	2.0	1.3	68.0	6.8	1.1
7	M	Ganaxolone	40	77.1	1.0	2.2	258	6.5	0.6
8	M	Ganaxolone	80	136	4.0	ND	460	5.8	0.4
6	F	Ganaxolone	10	219	2.0	3.5	1307	130.7	0.8
7	F	Ganaxolone	20	272	2.0	3.9	1609	80.5	0.7
8	F	Ganaxolone	40	327	2.0	ND	2619	65.5	0.6

Ganaxolone, Day 29

Group	Sex	Treatment	Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-t)}$ (ng·h/mL)	$AUC_{(0-t)}$ /Dose	Rc[a]
6	M	Ganaxolone	10	23.3	2.0	1.3	74.2	7.4	1.2
7	M	Ganaxolone	40	71.4	2.0	4.5	350	8.8	0.8
8	M	Ganaxolone	80	89.3	4.0	ND	374	4.7	0.3
6	F	Ganaxolone	10	158	0.5	ND	1304	130.4	0.8
7	F	Ganaxolone	20	333	2.0	4.6	1790	89.5	0.8
8	F	Ganaxolone	40	228	1.0	3.9	2004	50.1	0.5

ND: Not determined. Insufficient data to determine PK parameters.

[a] Rc = Accumulation Factor = Day 15 or Day 29 $AUC_{(0-t)}$/Day 1 $AUC_{(0-t)}$

NA: Not applicable.

Study title: Ganaxolone: A 13-Week Oral Gavage Toxicity and Toxicokinetic Pre-Carcinogenicity Study in Rats

Study no.:	8412976
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	13 Nov 2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	17JM-102 and 19JM-078/99.9 and 98.3%

Key Study Findings

Oral (gavage) administration of escalating doses of GNX (see Table 1) to SD rats for 13 weeks resulted in transient clinical signs (sedation, prostrate, and/or ataxia) at all doses in both sexes and non-adverse microscopic findings in the liver (all doses in M and F) and thyroid (all doses in F), with correlative increases in organ weights. Dose-dependent increases in CYP2B and CYP3A activities were noted in GNX-treated animals. The NOAEL (1000 mg/kg/day) corresponded to Cmax and AUC values of 103 ng/mL and 1430 h*ng/mL, respectively, in males and 459 ng/mL and 6140 h*ng/mL, respectively, in females on Day 91. Despite dose escalation allowing higher doses to be administered, plasma levels decreased between Days 1 and 28 and did not increase appreciably between the LD and HD.

Methods

Doses:	M: 0 (vehicle), 160/250, 160/250/500, or 160/250/1000 mg/kg/day; F: 60/120/250, 60/120/500, or 60/120/1000 mg/kg/day
Frequency of dosing:	BID
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	Preformulated GNX suspension/ hypromellose, polyvinyl alcohol, sodium lauryl sulfate, methylparaben, propylparaben, sodium benzoate, (b) (4) citric acid, sodium citrate (b) (4), simethicone emulsion, and sucralose
Species/Strain:	Rat/Sprague Dawley Crl:CD(SD)
Number/Sex/Group:	10/sex/group
Age:	6-8 Weeks
Weight:	M: 166-265 g, F: 151-204 g
Satellite groups:	3-9/sex/grp TK
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Table 1

Group[a]	Subgroup	No. of Animals M	No. of Animals F	Males Dose Level[b] (mg/kg/dose)	Males Dose Level[b] (mg/kg/day)	Females Dose Level[b] (mg/kg/dose)	Females Dose Level[b] (mg/kg/day)	Dose Concentration[c] (mg/mL) Males	Dose Concentration[c] (mg/mL) Females
1 (Control)	1 (Toxicity)	10	10	0	0	0	0	0	0
	2 (Toxicokinetic)	3	3						
2 (Low)	1 (Toxicity)	10	10	80[d]/125[e,f]	160[d]/250[e,f]	30[d]/60[e]/125[f]	60/120[e]/250[f]	8[d]/12.5[e,f]	3[d]/6[e]/12.5[f]
	2 (Toxicokinetic)	9	9						
3 (Mid)	1 (Toxicity)	10	10	80[d]/125[e]/250[f]	160[d]/250[e]/500[f]	30[d]/60[e]/250[f]	60/120[e]/500[f]	8[d]/12.5[e]/25.0[f]	3[d]/6[e]/25[f]
	2 (Toxicokinetic)	9	9						
4 (High)	1 (Toxicity)	10	10	80[d]/125[e]/500[f]	160[d]/250[e]/1000[f]	30[d]/60[e]/500[f]	60/120[e]/1000[f]	8[d]/12.5[e]/50.0[f]	3[d]/6[e]/50[f]
	2 (Toxicokinetic)	9	9						

F = Females; M = Males.

a Group 1 was administered vehicle control article for GNX only.

b Two doses were administered daily, 12 hours apart (±30 minutes). On each day of dosing, the second daily dose was based on the end dose time of the first daily dose of each sex/group.

c Test article concentrations were based on the test article as supplied (no correction). Dose volume for all dose groups was 10 mL/kg/dose (20 mL/kg/day).

d On Days 1 through 4 of the dosing phase, males were administered two daily doses of 80 mg/kg/dose (160 mg/kg/day) at a concentration of 8 mg/mL, and females were administered two daily doses of 30 mg/kg/dose (60 mg/kg/day) at a concentration of 3 mg/mL.

e Starting on Days 5 through 8 of the dosing phase, males were administered two daily doses of 125 mg/kg/dose (250 mg/kg/day) at a concentration of 12.5 mg/mL, and females were administered two daily doses of 60 mg/kg/dose (120 mg/kg/day) at a concentration of 6 mg/mL.

f Starting on Days 9 through 91 of the dosing phase, animals in Group 2 were administered two daily doses of 125 mg/kg/dose (250 mg/kg/day) at a concentration of 12.5 mg/mL, animals in Group 3 were administered two daily doses of 250 mg/kg/dose (500 mg/kg/day) at a concentration of 25.0 mg/mL, and animals in Group 4 were administered two daily doses of 500 mg/kg/dose (1000 mg/kg/day) at a concentration of 50.0 mg/mL.

Dose selection was based on the results of a dose escalation study in SD rats (8339053) in which the intial doses were 80 mg/kg/dose BID (160 mg/kg/day) in males and 30 mg/kg/dose BID (60 mg/kg/day) in females and doses were gradually increased on Days 5 and 9 due to reach the final doses of 125, 250, or 500 mg/kg/dose BID.

Observations and Results

Mortality

There were no unscheduled deaths.

Clinical Signs

Sedation scores of 2 and 3 (decreased activity and ataxia, respectively) were noted at all doses in both sexes. A sedation score of 4 (prostrate) was noted for at least 1 male at each dose but only on Day 1 and almost exclusively 2 hours after the second dose. No animal reached a sedation score of 5 (unresponsive). All animals were normal (sedation score 1) before the first dose on the next day. Males had a higher overall incidence of sedation scores of 2 through 4 compared to females.

Body Weights

There were no drug-related differences in BWs or BW gain.

Food Consumption

There was no clear effect on food consumption.

Hematology

There were no drug-related changes in hematology parameters.

Clinical Chemistry

There were no apparent drug-related changes in clinical chemistry parameters.

Urinalysis

There were no drug-related changes in urinalysis parameters.

Organ Weights

At the terminal sacrifice, there were drug-related increases in liver weights at all doses in both sexes and in thyroid weights of females at all doses. Increased liver weights correlated with microscopic findings of periportal or diffuse hepatocyte hypertrophy and increased incidence and/or severity of periportal vacuolation in these groups.

Gross Pathology

No drug-related macroscopic observations were noted.

Histopathology

In the liver, hepatocyte hypertrophy and vacuolation were noted at all doses in both sexes and thyroid follicular cell hyperplasia was noted in females at all doses, with a dose-related incidence and/or severity.

Enzyme induction

Dose-dependent increases in CYP2B (up to 2- and 6-fold in HD males and females, respectively) and CYP3A (up to 3- and 4-fold in HD males and females, respectively) activities were observed compared to C.

Toxicokinetic

TK data for GNX are shown in Table 2. Despite higher administered doses, plasma levels decreased between Days 1 and 28 and were similar between the LD and HD.

Table 2. Plasma TK parameters for ganaxalone in SD rat

Day	Dose Group	Dose Level (mg/kg/dose)	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24} (h*ng/mL)
1	2 through 4	80	160	M	466	16.0	6880
		30	60	F	479	13.0	6740
28	2	125	250	M	134	13.0	1450
				F	345	13.0	5070
	3	250	500	M	196	12.5	1480
				F	459	13.0	4530
	4	500	1000	M	122	13.0	1410
				F	523	12.5	4810
91	2	125	250	M	99.7	13.0	1130
				F	397	12.5	5580
	3	250	500	M	77.4	1.00	1330
				F	497	13.0	5970
	4	500	1000	M	103	12.5	1430
				F	459	1.00	6140

Notes: On Day 1, all groups were administered GNX at the same dose levels/sex.
T_{max} was calculated from the first daily dose.
Doses were administered 12 hours apart (±30 minutes).
Due to the inability to properly characterize the elimination phase, $t_{1/2}$ was not calculated in most instances.

Study title: 6-Month/4-Week Repeated-dose Oral Toxicity Study of CCD-1042 in Rats

Study no.:	SC930189
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	13 Jul 1993
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	879.E.92.3/ 99%

Key Study Findings

Daily oral (gavage) administration of GNX (0 (vehicle), 10, 20, or 40 mg/kg/day; 10 mL/kg) to SD rats (10/sex/grp + 5/sex/grp TK) for 4 weeks or 6 months resulted in dose-dependent clinical signs of sedation at all doses in both sexes that may have resulted in the death of 1 HD female on Day 2. Liver weights were increased in a dose-dependent manner in females and were associated with hepatocellular hypertrophy. Based on the marked degree of sedation at the HD, 20 mg/kg was considered the NOAEL and was associated with M/F Cmax values of 157/171 ng/mL on Day 182.

Methods

Doses:	0 (vehicle), 10, 20, or 40 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4)
Species/Strain:	Rat/Sprague Dawley Crl:CD(SD)
Number/Sex/Group:	10/sex/group 4-wk interim sacrifice, 10/sex/group 6-month necropsy
Age:	8 to 9 weeks
Weight:	M: 164 to 207 g, F: 111 to 156 g
Satellite groups:	10/sex/grp TK
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Table 1. Experimental design

CCD-1042 Dose Group (mg/kg)	CCD-1042 Conc. (mg/mL)	Dose Volume (mL/kg)	4-Week Interim Necropsy (M/F)	6-Month Final Necropsy (M/F)
0	–	10	10/10	10/10
10	1	10	10/10	10/10
20	2	10	10/10	10/10
40	4	10	10/10	10/10

Dose selection was based on the results of previous dose range-finding toxicity studies (SC930057, SC930109) in which sedation was dose-limiting.

Observations and Results

Mortality

The death of 1 HD female on Day 2 was considered possibly drug-related, although the cause was undetermined. Two additional HD animals (1 M, 1 F) were determined to be asphyxiation because of food stuck in the esophagus thought to be likely associated with the exaggerated pharmacological effect (sedation) of the drug.

Clinical observations

Dose-dependent sedative effects (ataxia, prostration, and/or unresponsiveness) were observed at all doses in both sexes approximately 30 minutes after administration of the daily dose, with peak responses occurring at approximately 1 hour. The degree of sedation lessened with duration of the study in the affected males from all dose groups and in the LD and MD females.

Body Weights

No effects were observed on group BW or BW gain values.

Food Consumption

There were no effects on food consumption.

Clinical Pathology

There were no drug-related changes in hematology, serum chemistry, hormone determinations, or urinalysis.

Organ Weights

Increased (SS) liver weights were seen in females at all doses (125, 133, and 149%, respectively, compared to C). A similar trend was observed in males, but the changes were not SS. Increases in kidney weights were also observed in all drug-treated females; however, the effect was not clearly dose-related (125, 118, and 125% compared to C) and there were no changes in clinical chemistry or urinalysis parameters or microscopic correlates.

Gross Pathology

There were no drug-related gross pathology findings.

Histopathology

Dose-dependent increases in hepatocellular hypertrophy were observed in females at all doses (5/10, 7/10, and 9/9 at LD, MD, and HD, respectively).

Toxicokinetic

Plasma concentrations of GNX increased with dose and were higher in females than in males (Table 2). AUC was not calculated since there were only 2 timepoints.

Table 2. Plasma levels of GNX

Parameter	Males			Females		
	Day 1	Day 28	Day 182	Day 1	Day 28	Day 182
			10 mg/kg/day			
C_{min}[a] (ng/mL)	NA	< 16 (LOQ)	<16 (LOQ)	NA	<16 (LOQ)	26.2
C_{max}[b] (ng/mL)	<16 (LOQ)	46.2	73.6	56.3	238.6	215.8
			20 mg/kg/day			
C_{min}[a] (ng/mL)	NA	<16 (LOQ)	<16 (LOQ)	NA	<16 (LOQ)	31.3
C_{max}[b] (ng/mL)	<16 (LOQ)	110.7	157.4	171.9	367.8	171.4
			40 mg/kg/day			
C_{min}[a] (ng/mL)	NA	<16 (LOQ)	<16 (LOQ)	NA	<16 (LOQ)	<16 (LOQ)
C_{max}[b] (ng/mL)	<16 (LOQ)	257.9	608.4	595.3	1000.2	838.2

C_{max} = maximum concentration; C_{min} = minimum concentration; GLP = Good Laboratory Practice; GNX = ganaxolone; LOQ = level of quantitation; NA = not applicable, since the animals had not yet been dosed; SD = Sprague Dawley.

a C_{min} is based on plasma concentrations measured in the blood sample collected at 15 minutes prior to dosing.

b C_{max} is based on plasma concentrations measured in the blood sample collected at 90 minutes postdose.

Study title: Ganaxolone: A One Month Oral Toxicity Study in Dogs

Study no.:	1245-005
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	14 Jun 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MBS-J0006-060510/ 99.5%

Key Study Findings

Daily oral (gavage) administration of GNX (0 (vehicle), 3, 10, or 15 mg/kg/day) to dogs (3/sex/grp) for 30 days resulted in dose-related sedative effects that showed little evidence of accommodation over the treatment period. BW and food consumption were increased in MD and HD males and females at all doses. There were no drug-related ophthalmoscopic, electrocardiographic, clinical pathology, macroscopic, or organ weight effects. Testicular atrophy in 1 HD male associated with oligospermia/germ cell debris in the epididymides was considered possibly drug-related. The highest dose (15 mg/kg/day) was associated with Cmax and AUC values of 1507 ng/mL and 15964 ng·hr/mL in males and 1426 ng/mL and 13900 ng·hr/mL in females on Day 29, which were greater than Day 1 values.

Methods

Doses:	0 (vehicle), 3, 10, and 15 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	0.06, 0.2, or 0.3 mL/kg/day
Formulation/Vehicle:	Preformulated GNX suspension/ hypromellose, polyvinyl alcohol, sodium lauryl sulfate, methylparaben, propylparaben, simethicone
Species/Strain:	Dog/Beagle
Number/Sex/Group:	3/sex/group
Age:	7.5 to 8.5 months
Weight:	M: 7.27 to 8.74 kg, F: 5.46 to 6.79 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	None that impacted study quality or integrity

A preformulated GNX suspension was administered to beagle dogs (3/sex/grp) by oral gavage for 30 days at doses of 0 (vehicle), 3, 10, or 15 mg/kg/day. Endpoints included mortality, detailed clinical observations, daily sedation observations (5-point sedation scoring system), body weight, food consumption, ophthalmoscopic exams, clinical pathology (hematology, coagulation, and serum chemistry, urinalysis, TK analysis, and macroscopic and microscopic examinations (all dose groups).

Dose selection was based on the results of a 14-day oral gavage study (SC930108; 0 (vehicle: (b) (4)), 5, 10, or 20 mg/kg/day). Prolonged sedation (24 hours or more

after the second daily dose) led to dose reduction on Day 4/5 and eventual discontinuation of the HD group. Mild ataxia and briefer periods of lethargy or loss of righting reflex were seen throughout the study at the LD and MD.

Observations and Results

Mortality

All animals survived to their scheduled necropsy on Day 31 of the study.

Clinical Signs

Drug-related clinical signs consisted of decreased activity, ataxia, prostration, salivation, and slow breathing, all considered to be related to the sedative effect which was observed with a dose-related incidence, severity, and duration throughout the study. The most severe sedative effects were recorded in the first week of the study at 1-hour post-dose when a total of 4 occurrences of unresponsiveness (sedation score of 5, unresponsive to external stimulus) were recorded in 2 HD males. Most HD animals were still not totally recovered at the last daily observation (8 hours post-dose), but by 24 hours animals appeared normal. Effects of sedation were similar throughout the study indicating little accommodation.

Body Weights

BW gain and BWs were dose-dependently increased in the drug-treated groups. At the end of the dosing period, BW at the HD was 12/8% above C in M/F.

Food Consumption

Increased food consumption correlated with the increases in BWs.

Ophthalmic Examination

There no ophthalmological changes related to drug.

ECG

In ECGs taken pretest and pre-dose and post-dose on Day 2 and Week 4, there were no qualitative or quantitative ECG abnormalities clearly attributable to drug.

Clinical Pathology

There were no clearly drug-related changes.

Gross Pathology

There were no drug-related macroscopic or organ weight changes.

Histopathology

Drug-related changes consisted of non-adverse focal vacuolation of the zona fasciculata in the adrenal glands in MD and HD males and in females at all doses. In addition, testicular atrophy in 1 HD male was considered possibly drug-related. The adrenal changes were bilateral and characterized by small clusters of cells from the zona fasciculata that were enlarged with foamy vacuolated cytoplasm. The testicular atrophy, which was associated with mild oligospermia/germ cell debris in the epididymides, was bilateral and graded moderate based on the number of tubules affected. Atrophic tubules

were devoid of germ cells and only Sertoli cells remained. This finding was said in the study report to be unusual in a dog of that age.

Toxicokinetic

TK data for GNX are shown in Table 1. Exposures (Cmax and AUC) on Days 15 and 29 were greater than those on Day 1 and there were no obvious sex differences.

Table 1. TK parameters for GNX in dogs

Ganaxolone, Day 1

Group	Sex	Treatment	Dose (mg/kg/day)		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0\text{-}24h)}$ (ng·h/mL)	$AUC_{(0\text{-}Inf)}$ (ng·h/mL)	$AUC_{(0\text{-}24h)}$ /Dose
2	M	Ganaxolone	3	Mean	389	0.5	9.5	1939	2333	646.3
				SD	47	0.0	2.9	682	1056	227.2
3	M	Ganaxolone	10	Mean	588	0.8	9.2	4895	5730	489.5
				SD	207	0.3	0.5	473	518	47.3
4	M	Ganaxolone	15	Mean	1037	0.8	10.1	8374	10321	558.3
				SD	290	0.3	2.1	2416	3283	161.0
2	F	Ganaxolone	3	Mean	310	0.7	7.5	2241	2523	747.0
				SD	86	0.3	1.3	777	975	258.8
3	F	Ganaxolone	10	Mean	923	0.7	7.9	7060	8124	706.0
				SD	22	0.3	NC	583	NC	58.4
4	F	Ganaxolone	15	Mean	907	1.0	8.4	8243	9414	549.5
				SD	108	0.0	1.5	664	339	44.2

Ganaxolone, Day 15

Group	Sex	Treatment	Dose (mg/kg/day)		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0\text{-}24h)}$ (ng·h/mL)	$AUC_{(0\text{-}24h)}$ /Dose	Rc^a
2	M	Ganaxolone	3	Mean	409	0.7	10.4	3487	1162.6	1.7
				SD	50	0.3	4.1	1862	620.5	0.4
3	M	Ganaxolone	10	Mean	813	0.8	11.9	9429	942.9	1.9
				SD	104	0.3	NC	1916	191.6	0.4
4	M	Ganaxolone	15	Mean	1278	0.8	12.7	13363	890.9	1.6
				SD	227	0.3	2.7	3643	242.8	0.1
2	F	Ganaxolone	3	Mean	341	0.5	9.9	2703	901.0	1.2
				SD	72	0.0	4.1	1208	402.5	0.2
3	F	Ganaxolone	10	Mean	1055	0.5	10.5	8783	878.3	1.2
				SD	298	0.0	0.9	970	97.1	0.2
4	F	Ganaxolone	15	Mean	1217	0.5	12.8	12644	842.9	1.5
				SD	172	0.0	NC	2563	170.9	0.2

Ganaxolone, Day 29

Group	Sex	Treatment	Dose (mg/kg/day)		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0\text{-}24h)}$ (ng·h/mL)	$AUC_{(0\text{-}24h)}$ /Dose	Rc[a]
2	M	Ganaxolone	3	Mean	453	0.7	10.8	3999	1333.1	2.0
				SD	116	0.3	6.8	2474	824.6	0.6
3	M	Ganaxolone	10	Mean	912	0.8	12.8	10369	1036.9	2.2
				SD	97	0.3	1.7	1796	179.6	0.5
4	M	Ganaxolone	15	Mean	1507	0.7	12.4	15964	1064.3	1.9
				SD	172	0.3	2.5	5728	381.9	0.1
2	F	Ganaxolone	3	Mean	323	0.5	9.3	2972	990.6	1.3
				SD	65	0.0	3.7	1277	425.7	0.2
3	F	Ganaxolone	10	Mean	1109	0.8	11.5	11514	1151.4	1.7
				SD	270	0.3	3.9	1980	198.0	0.4
4	F	Ganaxolone	15	Mean	1426	0.8	10.7	13900	926.7	1.7
				SD	57	0.3	4.0	1759	117.3	0.4

NC: Not Calculated. SD was not calculated due to n<3.

[a] Rc = Accumulation Factor = Day 15 or Day 29 $AUC_{(0\text{-}24h)}$/Day 1$AUC_{(0\text{-}24h)}$

Study title: 6-MONTH/4-WEEK REPEATED-DOSE ORAL TOXICITY STUDY OF CCD-1042 IN THE BEAGLE DOG

Study no.:	SC930190
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	15 Jul 1993
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	879.E.92.3/ NA

Key Study Findings

Daily oral (gavage) administration of GNX (0 (vehicle), 1, 3, or 10 mg/kg/day; 2.5 mL/kg) to beagle dogs for 1 or 6 months resulted in dose-dependent sedative effects including lethargy, ataxia, and light anesthesia. There were no clearly drug-related effects on other parameters evaluated in the study. Plasma drug levels at 3 and 6 months were similar to or greater than those at 1 week. The HD (10 mg/kg/day) was associated with Day 182 Cmax values of 1200 and 793 ng/mL in males and females, respectively.

Methods

Doses:	0 (vehicle), 1, 3, or 10 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	2.5 mL/kg
Formulation/Vehicle:	Ganaxolone drug substance/ (b) (4)
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4/sex/group at 4-week and 6-month necropsy
Age:	9 to 14 months
Weight:	7 to 15 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

STUDY DESIGN

CCD-1042 Dosage (mg/kg)	CCD-1042 Conc. (mg/mL)	Volume of Delivery (mL/kg)	4-Week Interim Necropsy (M/F)	6-Month Final Necropsy (M/F)
0	--	2.5	4/4	4/4
1	0.4	2.5	4/4	4/4
3	1.2	2.5	4/4	4/4
10	4.0	2.5	4/4	4/4
		Total =	16/16	16/16

Dose selection was based on the results of the 14-day study in the beagle dog ((b) (4) # SC930108, see above) in which sedation was found to be dose-limiting.

Observations and Results

Mortality

There were no drug-related deaths during the study.

Clinical Signs

Drug-related clinical signs consisted of dose-dependent sedative effects that were observed throughout the study, with lethargy being the predominate sign at the LD and lethargy, typically progressing to ataxia, occurring in the MD group. In general, the time for total recovery was within several hours after treatment. In the HD group, extended signs of sedation were observed, characterized as ataxia usually beginning within 30 minutes after treatment, followed by prostration and unresponsiveness which generally peaked within the first 2 hours after dosing. Total recovery typically occurred within 4-6 hours of treatment.

Body Weights

BW gain and BWs were unaffected by drug.

Food Consumption

There were no effects on food consumption.

Ophthalmic Examination

No drug-related ophthalmic findings were noted.

ECG

There were no drug-related changes in ECG parameters evaluated at 1, 3, and 6 months.

Clinical Pathology

There were no drug-related changes in hematology, serum chemistry, or urinalysis parameters. Groups differences in hormone assay results were not clearly drug-related (Table 2). Estradiol levels were increased at all doses in females, but these differences were seen prior to initiation of dosing (Day -9). Progesterone values were highly variable, and changes were inconsistent, but there appeared to be a possible drug-related increase (compared to baseline and C values) in HD males. These were not considered to reflect altered endocrine function by the sponsor, based on “the absence of gross or histologic findings indicative of prolonged progesterone influence on any organ.”

Table 2. Hormone assay results

CCD-1042 Dose Group (mg/kg)		E2 (PG/mL)				FSH (NG/mL)				LH (NG/mL)				Progesterone (NG/mL)			
		Study Day															
		-9	29	85	183	-9	29	85	183	-9	29	85	183	-9	29	85	183
Males																	
0	Mean	38.85	34.33	35.94	38.61	2.48	2.53	2.17	2.27	0.73	0.49	1.83	1.43	0.05	0.02	0.06	0.08
	STD	9.48	7.15	9.28	9.35	1.69	1.35	1.64	0.83	0.70	0.27	2.01	2.02	0.12	0.04	0.12	0.12
	n	8	8	4	4	8	8	4	4	8	8	4	4	8	8	4	4
1	Mean	41.80	37.86	55.43	45.44	2.16	3.03	3.48	2.93	0.63	0.77	1.35	1.05	0.02	0.01	0.07	0.01
	STD	11.66	7.89	13.23	6.77	0.94	1.63	2.19	1.64	0.36	0.83	1.12	1.13	0.06	0.02	0.14	0.01
	n	8	8	4	4	8	8	4	4	8	8	4	4	8	8	4	4
3	Mean	33.77	30.80	32.71	25.45	2.56	3.02	2.09	2.76	0.60	0.92	1.34	1.18	0.03	0.04	0.08	0.17
	STD	12.94	12.88	12.05	15.54	1.22	1.40	0.98	0.87	0.35	0.81	1.28	1.07	0.07	0.06	0.05	0.16
	n	8	8	4	4	8	8	4	4	8	8	4	4	8	8	4	4
10	Mean	36.47	35.05	43.64	38.25	2.93	3.66	2.54	2.54	0.81	0.66	1.18	0.46	0.00	0.12	0.35	0.65
	STD	6.04	7.69	5.83	11.42	0.58	1.03	0.91	1.04	0.84	0.57	1.27	0.13	0.00	0.12	0.34	0.80
	n	8	8	4	4	8	8	4	4	8	8	4	4	8	8	4	4
Females																	
0	Mean	36.37	38.19	34.36	23.71	4.60	4.63	2.84	3.70	0.29	0.20	0.31	0.98	12.79	10.59	0.29	0.01
	STD	8.86	15.66	16.95	9.12	1.79	1.40	1.24	0.42	0.17	0.08	0.37	1.45	21.56	15.26	0.45	0.01
	n	8	8	4	4	8	8	4	4	8	8	4	4	8	8	4	4
1	Mean	59.44	44.26	43.24	65.79	3.88	4.80	4.36	3.89	0.44	0.43	0.25	1.96	15.07	6.46	4.00	1.68
	STD	45.05	18.18	12.17	39.91	1.84	2.26	2.80	1.93	0.41	0.33	0.17	3.23	19.83	10.28	7.38	1.65
	n	8	8	4	4	8	8	4	4	8	8	4	4	8	8	4	4
3	Mean	52.60	42.17	48.73	**51.29***	6.39	5.31	5.03	5.11	0.56	0.27	0.12	0.17	18.49	5.54	8.53	2.54
	STD	17.57	10.43	2.57	12.36	2.27	2.24	3.09	1.42	0.51	0.14	0.06	0.12	16.02	9.96	15.69	4.44
	n	8	8	4	4	8	8	4	4	8	8	4	4	8	8	4	4
10	Mean	**53.16***	43.58	37.08	**52.27***	3.90	5.22	3.96	4.98	0.26	0.26	0.07	0.38	12.94	2.66	0.52	26.13
	STD	14.42	10.95	9.77	2.27	1.43	1.36	0.54	1.71	0.15	0.15	0.04	0.65	15.86	3.09	0.57	17.23
	n	8	8	4	4	8	8	4	4	8	8	4	4	8	8	4	4

* Mean value of group was significantly different from control ($p \leq 0.05$).

Organ Weights

Organ weights for drug-treated males were comparable to C. There was an increase in absolute liver weight in HD females but no effects on relative weights and no microscopic correlate.

Gross Pathology

No drug-related macroscopic changes were observed.

Histopathology

There were no histopathological changes considered drug-related.

Toxicokinetics

Plasma levels of GNX are shown in Table 1. There was an unexplained decrease in Cmax values measured in HD males and females on Day 28 (approximately 61 and 68%, respectively, compared to Day 7); however, Day 91 and 182 Cmax values were substantially higher than Day 28 values and generally comparable to those on Day 7.

Table 1. TK data for GNX in 6-month dog toxicity study

Plasma Concentration (ng/mL)									
CCD-1042 Dose Group (mg/kg)		Day 7		Day 28		Day 91		Day 182	
		~C_{min}	~C_{max}	~C_{min}	~C_{max}	~C_{min}	~C_{max}	~C_{min}	~C_{max}
Males									
1	Mean	23.5	98.0	17.8	76.7	21.6	74.0	19.5	81.1
	STD	1.5	46.8	1.6	12.9	--	29.7	2.6	33.1
	n	(3)8	8	(3)8	8	(1)4	4	(3)4	4
3	Mean	62.3	210.6	45.5	202.2	22.7	177.7	46.7	255.1
	STD	23.6	77.1	16.6	87.1	4.2	81.7	19.0	145.9
	n	(7)8	8	(7)8	8	4	4	(3)4	4
10	Mean	140.1	767.1	72.8	300.3	109.0	692.6	209.5	1199.9
	STD	80.8	277.4	40.6	122.3	63.4	376.0	140.8	635.3
	n	8	8	8	8	4	4	4	4
Females									
1	Mean	25.9	64.7	17.1	52.7	23.5	50.8	20.6	57.7
	STD	7.0	14.0	--	13.4	4.6	2.6	3.2	16.9
	n	(4)8	8	(1)8	8	4	4	(2)4	(3)4
3	Mean	51.0	215.3	45.8	235.2	58.0	236.4	30.9	115.3
	STD	26.7	64.0	17.9	80.0	6.3	20.7	14.7	90.3
	n	8	8	8	8	4	4	(3)4	4
10	Mean	178.6	717.8	75.8	232.1	105.5	572.8	133.2	792.6
	STD	141.6	413.3	54.0	74.6	73.3	99.6	72.9	115.8
	n	8	8	8	8	4	4	(3)4	(2)4

a. n = 8 dogs per sex per group (4 dogs for Days 91 and 182) sampled approximately 24 hours after the last dose (~C_{min}) and again approximately 2 hours after drug administration (~C_{max}). The number of samples above the limit of quantitation is in parenthesis with the number of samples assayed. The mean and standard deviation for the number of dogs having values at or above the limit of quantitation (16 ng/mL) are presented.

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Study title: Ganaxolone: A 1-Year Oral Toxicity Study in Dogs

Study no.:	1245-011
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	27 Jul 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MBS-J0012-060710, MBS-J0021-060912, MBS-J0025-070504/ 98.9%

Key Study Findings

Oral (gavage) administration of GNX (0 (vehicle), 3, 10, and 15 mg/kg/day) to beagle dogs for 52 weeks resulted in dose-dependent clinical findings related to the sedative effect (decreased activity, tremors, ataxia, prostration, and unresponsiveness) at all doses. The incidence and/or duration of prostration decreased only minimally during the course of the study. One HD male (#161) exhibited clonic and tonic convulsions at Weeks 33 and 36, respectively. Exposures were consistently approximately 2-fold higher in this dog compared to other HD animals (Cmax: 3416 ng/ml, AUC: 57966 ng•h/mL at Week 38). There was no drug-related mortality. BW and food consumption were dose-dependently increased at all doses. Dose-related increases in heart rate and incidences of sinus tachycardia were observed in the ECG examination but there were no effects on QRS duration or QTc interval. There were no drug-related macroscopic or microscopic pathology changes. Drug accumulation was seen over the first 6 weeks of dosing. Week 52 Cmax values were 520, 1155, and 2291 ng/mL in males (includes #161) and 798, 1528, and 1756 ng/mL in females, and AUC(0-24h) values were 4817, 13523, and 33888 ng·h/mL in males and 5335, 15725, and 24346 ng·h/mL in females at the LD, MD, and HD, respectively.

Methods

Doses:	0 (vehicle), 3, 10, and 15 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	0.06, 0.2, or 0.3 mL/kg/day
Formulation/Vehicle:	Preformulated GNX suspension/ hydroxypropylmethylcellulose, sodium lauryl sulfate, polyvinylalcohol, simethicone and methyl and propyl paraben in water
Species/Strain:	Dog/Beagle
Number/Sex/Group:	3/sex/group
Age:	6 to 7.5 months
Weight:	M: 7.27 to 8.74 kg, F: 5.46 to 6.79 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	None that impacted study quality or integrity

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male[a]	Female[a]
1	0	9	9
2	3	9	9
3	10	9	9
4	15	9	9

[a]The first four animals/sex/group were euthanized and submitted to necropsy after 39 weeks on study (interim). The remaining five animals/sex/group were euthanized and submitted to necropsy after 52 weeks on study (terminal).

Preformulated GNX suspension was administered to beagle dogs (9/sex/grp) by oral gavage QD for 53 weeks (interim necropsy at 39 weeks) at doses of 0 (vehicle), 3, 10, or 15 mg/kg/day. Endpoints included mortality, detailed clinical observations, daily sedation observations (5-point sedation scoring system), body weight, food consumption, ophthalmoscopic exams, ECG, clinical pathology (hematology, coagulation, and serum chemistry, urinalysis, TK analysis, and macroscopic and microscopic examinations (all dose groups).

Dose selection was based on the results of the 1-month ((b) (4) #1245-005) and the 6-month ((b) (4) # SC930190) dog studies described above.

Observations and Results

Mortality

All animals survived to their scheduled necropsy at 9 or 12 months.

Clinical Signs

Drug-related clinical signs consisted of decreased activity, tremors, ataxia, prostration, and unresponsiveness, which were all considered to be related to the dose-related sedative effect. HD animals were unresponsive for up to 4 hours postdose, primarily during the first month of study, but by 24 hours most animals were said to appear normal. After 3 months, the duration of prostration observed in males decreased slightly. The incidence and duration of prostration were generally less in females during the course of the study.

One HD male (#161) was observed with a clonic convulsion (muscle contractions intermittent, muscles alternatively contract and relax) in Week 33 and a tonic convulsion (prolonged muscle contraction, tetany with opisthotonus) in Week 36. This dog was said to be the most severely affected animal in the HD group and was given several drug holidays during the study (Days 10, 11, 12, 16 and 314-320). Plasma drug levels in this dog were found to be the highest in the group throughout the study (Cmax of 3400 ng/ml and AUC (0-24h) of 57900 ng•h/mL on Day 260).

Body Weights

BWs and BW gains were increased compared to C at all doses in both sexes throughout the study (Table 1).

Table 1.

Mean Body Weight, kg								
	Males				Females			
Dose Level (mg/kg/day)	Pretest	Week 52	Change in Body Weight %	Change in Body Weight kg	Pretest	Week 52	Change in Body Weight %	Change in Body Weight kg
0	9.10	10.86	19.3	+1.8	6.79	8.14	19.9	+1.4
3	9.08	12.34	35.9	+3.3	6.88	9.12	32.6	+2.2
10	9.16	13.44	46.7	+4.3	6.62	9.86	48.9	+3.2
15	9.13	13.37	46.4	+4.2	6.67	10.19	52.8	+3.5

Food Consumption

Increased food consumption correlated with the increases in BWs.

Ophthalmic Examination

There no ophthalmological changes related to drug.

ECG

In ECGs taken pretest and after 13, 26, 38, and 52 weeks (predose and 1 hour postdose), a dose-related increase in heart rate and incidence of sinus tachycardia (4 MD and 4 HD animals with at least 1 incidence of sinus tachycardia, which was reported on 4 separate occasions in HD animal #172) were observed. There was a physiologically normal shortening of the PR and QT interval in response to the higher heart rate but no effect on QRS duration or QTc interval.

Clinical Pathology

There were no clearly drug-related changes in hematology or coagulation parameters.

Cholesterol values were increased (up to 50%) compared to C and pretest values in MD and HD males and females at all time points (Weeks 13, 26, 38, and 52). Other slight differences (increase in AST, ALT, potassium, and phosphorus values in HD males) were considered incidental or toxicologically insignificant.

Urinary volume was increased and specific gravity decreased in HD males at 6, 9, and 12 months, indicating a possible mild diuretic effect.

Gross Pathology

There were no drug-related macroscopic or organ weight changes.

Histopathology

There were no drug-related microscopic findings.

Toxicokinetic

TK data for GNX are shown in Table 2. It appeared that steady state was achieved by Week 6. Accumulation factors (Rc) for Week 6 were 1.77, 2.48, and 2.56 in males, and 1.55, 2.31, and 2.44 in females, at the LD, MD, and HD, respectively. Accumulation factors did not increase appreciably from Week 6 to Week 52.

Table 2.

Mean (SD)[a] Toxicokinetic Parameters

Day 1

Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$[b] (h)	$AUC_{(0\text{-}24h)}$ (h·ng/mL)	$AUC_{(0\text{-}24h)}$/Dose (h·ng/mL/mg/kg)
3	M	421 (82.0)	0.6 (0.2)	9.4 (1.7)	2064 (649)	688 (216)
10	M	769 (180)	0.9 (0.5)	8.6 (1.4)	5880 (2635)	588 (264)
15	M	1082 (281)	0.7 (0.3)	10.0 (2.2)	9896 (4641)	660 (309)
3	F	441 (119)	0.6 (0.2)	8.6 (1.6)	2244 (814)	748 (271)
10	F	773 (123)	0.6 (0.2)	9.0 (2.2)	5117 (1533)	512 (153)
15	F	1104 (394)	0.9 (0.5)	7.7 (1.2)	7791 (2433)	519 (162)

Week 6

Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$[b] (h)	$AUC_{(0\text{-}24h)}$ (h·ng/mL)	$AUC_{(0\text{-}24h)}$/Dose (h·ng/mL/mg/kg)	Rc
3	M	383 (119)	0.6 (0.2)	12.0 (2.9)	3533 (883)	1178 (294)	1.77 (0.39)
10	M	1036 (205)	0.7 (0.3)	13.4 (3.2)	12794 (3743)	1279 (374)	2.48 (1.05)
15	M	1706 (614)	2.1 (2.4)	17.0 (9.0)	23291 (8961)	1553 (597)	2.56 (0.93)
3	F	464 (184)	0.6 (0.2)	12.3 (3.4)	3517 (1524)	1172 (508)	1.55 (0.26)
10	F	1075 (165)	0.7 (0.3)	13.3 (5.1)	11422 (2491)	1142 (249)	2.31 (0.48)
15	F	1308 (292)	1.7 (2.4)	14.0 (3.0)	17922 (6151)	1195 (410)	2.44 (0.88)

Week 13

Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$[b] (h)	$AUC_{(0\text{-}24h)}$ (h·ng/mL)	$AUC_{(0\text{-}24h)}$/Dose (h·ng/mL/mg/kg)	Rc
3	M	442 (121)	0.5 (0.0)	13.6 (4.3)	4193 (1995)	1398 (665)	2.06 (0.82)
10	M	1263 (379)	0.9 (0.5)	14.6 (3.9)	15049 (5095)	1505 (510)	2.93 (1.44)
15	M	1863 (514)	1.3 (1.0)	19.0 (8.3)	26265 (9978)	1751 (665)	2.96 (1.30)
3	F	497 (123)	0.6 (0.2)	12.4 (3.1)	4050 (2009)	1350 (670)	1.76 (0.40)
10	F	1131 (240)	0.7 (0.3)	14.7 (4.4)	12320 (4166)	1232 (417)	2.47 (0.80)
15	F	1722 (372)	1.3 (1.1)	14.8 (4.8)	20930 (7115)	1395 (474)	2.93 (1.31)

Week 26

Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$[b] (h)	$AUC_{(0\text{-}24h)}$ (h·ng/mL)	$AUC_{(0\text{-}24h)}$/Dose (h·ng/mL/mg/kg)	Rc
3	M	503 (174)	0.6 (0.2)	16.0 (4.8)	4404 (1825)	1468 (608)	2.11 (0.64)
10	M	1297 (489)	0.8 (0.3)	17.7 (7.6)	15443 (6581)	1544 (658)	2.92 (1.33)
15	M	1922 (656)	1.2 (0.5)	20.7 (5.1)	27789 (9246)	1853 (616)	3.11 (1.18)
3	F	574 (168)	0.6 (0.2)	14.0 (6.7)	4267 (1813)	1423 (604)	1.90 (0.47)
10	F	1168 (276)	0.9 (0.5)	16.2 (6.3)	14659 (5491)	1466 (549)	2.95 (1.01)
15	F	1751 (411)	1.1 (0.4)	14.6 (3.1)	22313 (7712)	1488 (514)	3.13 (1.48)

Week 38							
Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$[b] (h)	$AUC_{(0\text{-}24h)}$ (h·ng/mL)	$AUC_{(0\text{-}24h)}$/Dose (h·ng/mL/mg/kg)	Rc
3	M	553 (174)	0.5 (0.0)	13.7 (3.5)	4626 (1734)	1542 (578)	2.23 (0.47)
10	M	1392 (408)	0.8 (0.3)	15.8 (4.1)	15737 (6370)	1574 (637)	3.02 (1.45)
15	M	2086 (697)	1.8 (2.3)	20.1 (7.5)	31195 (11358)	2080 (757)	3.56 (1.58)
3	F	656 (161)	0.6 (0.2)	13.1 (2.4)	4954 (2754)	1651 (918)	2.15 (0.57)
10	F	1164 (235)	0.8 (0.5)	13.7 (3.5)	12583 (4080)	1258 (408)	2.56 (0.84)
15	F	1767 (301)	1.3 (0.5)	16.7 (5.3)	23626 (7305)	1575 (487)	3.45 (1.92)
Week 52							
Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$[b] (h)	$AUC_{(0\text{-}24h)}$ (h·ng/mL)	$AUC_{(0\text{-}24h)}$/Dose (h·ng/mL/mg/kg)	Rc
3	M	520 (185)	0.7 (0.3)	15.5 (4.6)	4817 (2088)	1606 (696)	2.38 (0.50)
10	M	1155 (431)	1.2 (0.8)	18.4 (7.2)	13523 (4109)	1352 (411)	3.15 (1.88)
15	M	2291 (796)	1.1 (0.5)	25.0 (9.6)	33888 (11314)	2259 (754)	4.04 (2.31)
3	F	798 (157)	0.5 (0.0)	19.0 (2.6)	5335 (2762)	1778 (921)	2.30 (0.71)
10	F	1528 (401)	0.7 (0.3)	18.4 (9.9)	15725 (5935)	1572 (593)	3.24 (1.12)
15	F	1756 (676)	1.7 (0.7)	17.6 (7.9)	24346 (11623)	1623 (775)	3.61 (1.97)

[a]All values within () indicate the standard deviation.
[b]Half-life may have been underestimated since the sample collection time interval was 24 hours.
Rc: Accumulation Factor = Day X $AUC_{(0\text{-}24h)}$ / Day 1 $AUC_{(0\text{-}24h)}$

Cmax values in HD males, excluding animal number 161, ranged from approximately 1000 to 2400 ng/mL; female values ranged from 1200 to 2200 ng/mL. In comparison, HD male #161, which exhibited convulsions twice in the study, showed a plasma Cmax value of approximately 3400 ng/mL on Days 260 and 364 and had predose values from Day 36 to 364 ranging from 1287 to 1605 ng/mL compared to 261 to 876 ng/mL for the other HD males.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Mutagenicity Test on CDD-1042 in the Salmonella-Escherichia coli/Mammalian-microsome Reverse Mutation Assay with a Confirmatory Assay

Study no.:	16601-0-409R
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	13 Dec 1994 (B1) and 20 Dec 1994 (C1)
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	NA

Methods and Results

Concentrations for the mutagenicity assay (0, 50, 100, 250, 500, 1000, and 5000 µg per plate) were selected based on the results of a range-finding study. The assay was conducted using 3 plates per dose in the presence and absence of rat liver S9 mix. In the initial mutagenicity assay (Table 1) and the confirmatory assay all data were acceptable and no positive increases in the number of revertants per plate were observed with any of the tester strains (Salmonella typhimurium TA98, TA100, TA1535, TA1537, and E coli WP2uvrA) either in the presence or absence of S9. All criteria for a valid study were met.

Table 1.

		MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACKGROUND LAWN*
	DOSE/PLATE	TA98		TA100		TA1535		TA1537		WP2uvrA		
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: Rat Liver												
VEHICLE CONTROL		22	7	87	28	10	1	4	1	15	2	1
TEST ARTICLE	50.0 µg	22	6	91	14	9	2	8	2	20	8	1
	100 µg	21	7	86	22	10	3	4	1	23	5	1
	250 µg	22	3	87	10	12	1	6	1	19	4	1
	500 µg	20	8	83	3	9	3	5	1	17	6	1sp
	1000 µg	20	3	74	11	12	3	8	3	22	2	1mp
	5000 µg	18	5	78	5	10	1	7	2	21	7	6hp
POSITIVE CONTROL **		868	13	902	80	123	16	120	22	841	81	1
MICROSOMES: None												
VEHICLE CONTROL		13	7	78	9	7	2	6	1	14	3	1
TEST ARTICLE	50.0 µg	13	4	81	11	6	3	5	1	14	5	1
	100 µg	14	1	69	7	10	2	6	2	17	2	1
	250 µg	14	2	67	4	7	2	5	3	16	3	1
	500 µg	10	3	72	6	8	3	5	1	13	4	1sp
	1000 µg	15	3	71	9	8	5	5	3	12	2	1mp
	5000 µg	16	4	65	5	8	5	6	0	10	3	6hp
POSITIVE CONTROL ***		113	9	534	34	417	4	251	26	899	108	1

Study title: Ganaxolone Metabolite (M2): A Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli

Study no.:	9603168
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	09 Apr 2021
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	M2/NA

Methods and Results

Testers strains (Salmonella typhimurium TA98, TA100, TA1535, TA1537, and E coli WP2uvrA) were treated with M2 at a range of concentrations up to 5000 µg/plate in the presence and absence of rat liver S9 mix, using the plate incorporation version of the bacterial reverse mutation test. Substantial reductions in revertant colony counts were obtained following exposure of strain TA1535 to M2, in the absence of S9 mix, indicating that the metabolite was toxic to this strain at the highest concentrations tested (1581 and 5000 µg/plate). Precipitation was observed at concentrations ≥1581 µg/plate in both the presence and absence of S9 mix. No substantial increases in revertant colony numbers were obtained with any of the tester strains, following exposure to M2 at any concentration, in either the presence or absence of S9 mix (Table 1). Two instances of mean colony counts above the 98% tolerance limit of the negative historical control data were noted (both instances were observed in the absence of S9 mix, one with strain TA1537 at a concentration of 158 µg/plate and the other with strain TA100 at a concentration of 50 µg/plate), however, since both of these increases were not concentration-related and were not above the 2-fold threshold for a positive response, they were considered to be due to normal variation and not genotoxicity.

Table 1.

Metabolic Activation	Treatment	Concentration or Dose Level (µg/plate)	Mean Revertant Colony Counts (SD)				
			TA1535	TA1537	TA98	TA100	WP2 uvrA
Without activation	DMSO		22 (7)	14 (5)	24 (10)	129 (7)	34 (15)
	M2	1.58	17 (1)	NT	NT	NT	NT
		5.0	15 (6)	NT	NT	NT	NT
		15.8	10 (3)	17 (7)	34 (9)	136 (25)	41 (3)
		50	18 (5)	17 (3)	25 (1)	173[a] (16)	43 (4)
		158	15 (9)	22[a] (3)	32 (6)	123 (27)	46 (3)
		500	17 (4)	15 (3)	29 (5)	144 (17)	42 (6)
		1581	11 (3)	13 (1)	22 (3)	106 (6)	31 (3)
		5000	8 (2)	11 (3)	23 (4)	122 (11)	30(6)
	sodium azide	0.5	359 (15)	-	-	394 (14)	-
	9-aminoacridine hemihydrate	50	-	417 (81)	-	-	-
	2-nitrofluorene	1	-	-	151 (20)	-	-
	4-nitroquinoline-N-oxide	0.5	-	-	-	-	331 (167)
With activation	DMSO		15 (4)	12 (4)	37 (2)	139 (15)	42 (3)
	M2	15.8	18 (5)	17 (7)	40 (7)	172 (7)	45 (3)
		50	17 (7)	15 (2)	39 (3)	149 (6)	54 (7)
		158	17 (3)	22 (4)	39 (9)	164 (11)	43 (2)
		500	17 (3)	10 (6)	40 (11)	164 (17)	48 (7)
		1581	14 (7)	12 (1)	33 (5)	128 (3)	43 (4)
		5000	16 (10)	13 (5)	25 (5)	164 (4)	35 (6)
	2-aminoanthracene	5	305 (8)	-	-	-	-
	Benzo[a]pyrene	5	-	120 (7)	248 (36)	921 (25)	-
	2-aminoanthracene	20	-	-	-	-	200 (2)

CTD = Common Technical Document; DMSO = Dimethyl sulfoxide; GLP = Good Laboratory Practice.

a Increase in revertant colony counts above the 98% tolerance limit of the negative historical control data

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Evaluation of CoCensys Compound 1042 Using the L5178Y/tk+/- Mouse Lymphoma Cell Mutagenesis Assay (MLA) with Colony Sizing, With and Without Metabolic Activation

Study no.:	93020
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	08 Apr 1993
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	879.E.92.3/ NA

Methods and Results

In the range-finding assay at concentrations ranging from 0.1 to 1000 µg/mL, concentration-related reductions in cell survival were induced by the vehicle (b) (4)) in the absence and presence of metabolic activation. As a result, the vehicle was changed to (b) (4) alone.

In the definitive assay, after a 2-day expression period, L5178Y mouse lymphoma cells were exposed to GNX in (b) (4) or (b) (4) alone in the presence (4 GNX concentrations ranging from 20 to 60 µg/mL and concentrations of (b) (4) ranging from 0.67% to 2.00%) and absence (6 GNX concentrations ranging from 4 to 48 µg/mL; concentrations of (b) (4) ranging from 0.13% to 2%) of metabolic activation. Aliquots of 1000 cells were obtained from each culture by serial dilution and cloned to determine cloning efficiency. After adding 1 µg/mL of TFT, the remaining cells were cloned to determine mutant frequency.

In the confirmatory assay, GNX concentrations ranging from 5 to 80 µg/mL in the absence of activation and from 4 to 44 µg/mL in the presence of activation were tested along with the corresponding concentrations of (b) (4) Concentration-dependent reductions in cell survival that appeared to be induced by the vehicle were observed in the presence and absence of activation in the definitive and confirmatory assays.

No significant increases in induced mutant frequencies were observed in the cultures exposed to GNX in either the definitive (Table 1) or confirmatory assay.

Table 1.

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Results from Definitive L5178Y/tk$^{+/-}$ Mouse Lymphoma Mammalian Cell Mutagenesis Assay of CoCensys Compound 1042 and the (b) (4) in the Absence and Presence of Metabolic Activation.

Chemical	+/- S9	Conc./ml	RSG(%)	RTG (%)	MF x 10^{-6}	IMF x 10^{-6}	Notes
(b) (4)	-	100.0 µl	95.11	92.40	87		
	-	100.0 µl	104.89	107.88	84		
CCD 1042	-	20.0 µg	67.65	70.49	77	0	
CCD 1042	-	60.0 µg	9.46	6.87	102	17	
(b) (4)	-	0.67 %	76.48	82.45	78	-	
	-	2.00 %	6.77	5.55	84	-	
	-	5.56 µg	19.42	13.48	521	434	♦♦
	-	11.11 µg	2.30	0.60	1,016	976	♦♦
	+	100.0 µl	108.02	107.02	214		
	+	100.0 µl	91.98	92.84	223		
CCD 1042	+	4.0 µg	92.21	80.07	187	-	
CCD 1042	+	20.0 µg	29.45	22.55	234	16	
CCD 1042	+	40.0 µg	8.12	6.67	226	8	
CCD 1042	+	48.0 µg	3.76	4.03	245	27	
(b) (4)	+	0.13 %	75.39	77.16	228	9	
	+	0.67 %	50.90	53.54	239	20	
	+	1.33 %	13.88	15.49	223	4	
	+	1.60 %	13.21	10.94	284	65	
	+	1.87 %	11.57	16.77	178	-	
	+	2.00 %	9.27	8.93	276	58	
	+	11.11 µg	2.48	0.04	521	416	♦♦

RTG(%) = Percent relative total growth; MF = mutant frequency; IMF = induced mutant frequency; ♦♦ = IMF ≥ 100 x 10^{-6}.

(b) (4)

Study title: Ganaxolone metabolite (M2): In Vitro Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes

Study no.:	9603173
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	09 Apr 2021
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	M2/ NA

Methods and Results

Human peripheral blood lymphocytes were treated in the absence and presence of rat liver S9 for 4 hours and continuously without S9 for 21 hours. For each treatment condition in the main test, the highest concentration selected for detailed analysis produced approximately a 50% decrease in the RMI (mitotic index relative to the concurrent negative control). In addition, the next 2 or 3 lower concentrations were also examined. The highest concentrations selected for evaluation of chromosome aberrations, in each treatment condition, were 333 µg/mL for the 4-hour treatment in the absence of S9 mix (51 % RMI), 256 µg/mL for the 4-hour treatment in the presence of S9 mix (49% RMI), and 128 µg/mL for the 21-hour treatment in the absence of S9 mix (57% RMI). 300 metaphases were scored at each point chosen for assessment.

Precipitation was observed at concentrations ≥ 256 µg/mL in the 21-hour treatment only. Statistically significant increases in the incidence of aberrant metaphases, which were also above the 95% upper control limit of the laboratory negative historical control range and exhibited a dose-relationship when evaluated with a trend test, were observed in both 4-hour treatments, in the absence and presence of S9, at a concentration of 256 µg/mL (Table 1). In the 4-hour treatment in the absence of S9, the next higher concentration tested, 333 µg/mL, the incidence of aberrant metaphases was above the 95% upper control limit of the laboratory negative historical control range but was not statistically significant. It was concluded that M2 was positive for clastogenicity in the absence and presence of S9 mix.

Table 1.

Treatment	Conc. (μg/mL)	MI	RMI (%)	Number of Cells Examined	% Aberrant Cells	Number of Aberrations					Incidental Observations †				
						b	e	B	E	Other	(g	G	R	P	C)
4-hour treatment in the absence of S9 (0S9)															
DMSO	-	7.5	100	300	0.3	1	0	0	0	0	2	0	0	0	1
M2	64.0	7.2	96	300	1.0	2	0	1	0	0	0	0	0	0	0
	128	5.1	68	300	1.7	4	0	1	0	0	0	0	0	0	0
	256	5.4	71	300	3.0*	10	1	5	0	0	4	0	0	0	0
	333	3.9	51	300	2.0[a]	7	0	1	0	0	1	0	0	0	0
4-hour treatment in the presence of S9 (+S9)															
DMSO	-	10.8	100	300	0.7	2	0	0	0	0	0	0	0	0	0
M2	64.0	9.1	85	300	0.3	0	0	1	0	0	0	0	0	0	0
	128	7.4	69	300	1.7	8	0	4	0	0	2	1	0	0	0
	256	5.3	49	300	4.3*	20	3	5	0	0	3	0	0	1	0
CP	4.0	6.9	64	300	12.7*	31	4	14	0	0	0	0	0	0	0
21-hour treatment in the absence of S9 (0S9)															
DMSO	-	5.7	100	300	0.7	1	0	1	0	0	5	0	0	0	0
M2	32.0	4.5	79	300	1.7	2	0	3	0	0	2	0	0	0	0
	64.0	3.2	56	300	1.7	1	0	4	0	0	3	0	0	0	0
	128	3.2	57	300	1.7	2	0	3	0	0	5	0	0	0	1
MMC	0.05	6.6	116	300	7.7*	7	4	14	1	0	2	0	0	0	0

DMSO= Dimethyl Sulfoxide; M2 = Ganaxolone-M2 metabolite; MI = Mitotic index; RMI = Relative Mitotic Index (DMSO = 100%); b = Chromatid break; e = Chromatid exchange; g = Chromatid gap; B = Chromosome break; E = Chromosome exchange; G = Chromosome gap; other = Includes pulverized chromosomes and cells with > 8 aberrations; R = Endoreduplication; P = Polyploidy; C = Premature centromere division; † = g, G, P, R and C are excluded from the calculation of % aberrant cells; [a] = Increase in incidence of aberrant cells above the 95% control limits; CP = Cyclophosphamide monohydrate; MMC= Mitomycin C

Results of statistical analysis using one-tailed Fisher's exact test: * when $p \leq 0.05$ (significant), Otherwise, $p > 0.05$ (not significant)

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mutagenicity Test on CDD 1042 in an In Vivo Rat Micronucleus Assay

Study no:	16601-0-454 (b) (4)
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	19 Dec 1994
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	90002 CSO#C-017/ NA

Methods and Results

In the dose range-finding study, GNX (50, 100, 150, or 200 mg/kg) was administered to SD rats (3/sex/dose) by oral gavage and the MTD was estimated to be 140 mg/ml based on mortality at the 2 highest doses (1/6 and 6/6, respectively). In the micronucleus assay, SD rats (5/sex/dose) were administered GNX (0 (vehicle: (b) (4) 35, 70, or 140 mg/kg; 20 mL/kg) or cyclophosphamide (60 mg/kg; 10 mL/kg) by oral gavage and euthanatized 24, 48, or 72 hours after dosing for extraction of the bone marrow. GNX did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes (Table 1).

Table 1.

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E. MALES	FEMALES	TOTAL	RATIO PCE:NCE MEAN ± S.E. MALES	FEMALES
VEHICLE CONTROL (b) (4)		24	0.28 ± 0.06	0.10 ± 0.00	0.19 ± 0.04	0.89 ± 0.09	1.04 ± 0.06
	20 ml/kg	48	0.12 ± 0.07	0.10 ± 0.04	0.11 ± 0.04	1.15 ± 0.12	0.98 ± 0.08
		72	0.06 ± 0.04	0.12 ± 0.06	0.09 ± 0.03	0.88 ± 0.10	0.78 ± 0.15
POSITIVE CONTROL Cyclophosphamide	60 mg/kg	24	1.60 ± 0.30*	1.28 ± 0.36*	1.44 ± 0.23*	0.87 ± 0.10	0.70 ± 0.07
TEST ARTICLE		24	0.16 ± 0.07	0.06 ± 0.04	0.11 ± 0.04	1.03 ± 0.10	1.04 ± 0.10
	35 mg/kg	48	0.08 ± 0.04	0.10 ± 0.05	0.09 ± 0.03	1.02 ± 0.07	0.95 ± 0.07
		72	0.08 ± 0.05	0.16 ± 0.07	0.12 ± 0.04	0.91 ± 0.11	0.92 ± 0.11
		24	0.12 ± 0.04	0.12 ± 0.02	0.12 ± 0.02	1.12 ± 0.25	0.89 ± 0.14
	70 mg/kg	48	0.04 ± 0.02	0.20 ± 0.03	0.12 ± 0.03	1.18 ± 0.29	0.84 ± 0.08
		72	0.18 ± 0.07	0.14 ± 0.07	0.16 ± 0.05	1.03 ± 0.11	1.09 ± 0.08
		24	0.12 ± 0.05	0.20 ± 0.00	0.15 ± 0.03	0.91 ± 0.12	0.57 ± 0.16
	140 mg/kg	48	0.18 ± 0.04	**	0.18 ± 0.04	1.19 ± 0.19	**
		72	0.15 ± 0.05	**	0.15 ± 0.05	0.78 ± 0.09	**

* Significantly greater than the corresponding vehicle control, $p<0.05$.
**No females survived from the 48 and 72 hour harvest groups.

8. Carcinogenicity

A 26-week carcinogenicity study in the CB6F1-Tg rasH2 transgenic mouse and a 104-week carcinogenicity study in SD rats are to be conducted postmarketing.

8.1 Dose Selection

Study title: Ganaxolone: Pre-carcinogenicity 4-Week Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study in Mice (CByB6F1-Tg[HRAS]2Jic: Wild Type)

Study no.:	8412977
Study report location:	4.2.3.4.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07 Nov 2019
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	19JM-078/ 98.3%

Key Study Findings

When the preformulated GNX suspension was administered BID by oral gavage to CByB6F1-Tg[HRAS]2Jic: Wild Type mice, findings were limited to increases (NS) in BW gain and dose-dependent increases (SS at all doses in males and in MD and HD females) in liver weights, which correlated with increases in hepatic enzyme parameters: >2-fold increases (compared to C) in CYP2B (180% to 341%) and CYP3A activity (291% to 645% of control) at all doses, in CYP450 content in HD males, and in CYP4A activity in HD males and females. Since these were adaptive responses, the HD (250/1000 mg/kg/day) was the NOAEL. This dose was associated with Cmax and AUC values of 185 ng/mL and 1260 ng.hr/mL in males and 52.9 ng/mL and 968 ng.hr/mL in females on Day 26 of dosing.

Methods

Doses:	0 (vehicle), 250, 250/500, and 250/1000 mg/kg/day
Frequency of dosing:	BID
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	Preformulated GNX suspension/ HPMC, PVA, SLS, methylparaben, propylparaben, sodium benzoate, (b) (4) citric acid, sodium citrate (b) (4), sucralose, and simethicone
Species/Strain:	Mouse/ CByB6F1-Tg[HRAS]2Jic: Wild Type
Number/Sex/Group:	10/sex/group
Age:	8 to 9 weeks
Weight:	M: 24.1 to 32.9 g, F: 18.2 to 27.4 g
Satellite groups:	3-18/sex/grp TK
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Preformulated GNX suspension (0 (vehicle), 250, 250/500, and 250/1000 mg/kg/day) was administered BID by oral gavage (10 mL/kg) to CByB6F1-Tg[HRAS]2Jic: Wild Type mice (10/sex/grp). Due to enzyme induction and dose-limiting sedation, the MD and HD groups were administered the same dose as the LD group (125 mg/kg BID) for the first 3 days of dosing and then 250 and 500 mg/kg BID, respectively, for the remaining 25 days of dosing, with the HD considered the maximum feasible dose. Endpoints included mortality, clinical observations, sedation observations, body weights, food consumption, and clinical and anatomic pathology. Blood samples were collected from up to 3 TK animals/sex/group/time point in the LD, MD, and HD groups on Days 8 and 26 at approximately 0.5, 3, 12 (prior to the second daily dose), 12.5, 15, and 24 hours postdose.

Table 1. Study design

		No. of Animals		Dose Level[b]		Dose Concentration[c]
Group[a]	Subgroup	Males	Females	(mg/kg/dose)	(mg/kg/day)	(mg/mL)
1 (Control)	1 (Toxicity)	10	10	0	0	0
	2 (Toxicokinetic)	3	3			
2 (Low)	1 (Toxicity)	10	10	125	250	12.5
	2 (Toxicokinetic)	18	18			
3 (Mid)	1 (Toxicity)	10	10	125[d]/250[e]	250[d]/500[e]	12.5[d]/25[e]
	2 (Toxicokinetic)	18	18			
4 (High)	1 (Toxicity)	10	10	125[d]/500[f]	250[d]/1000[f]	12.5[d]/50[f]
	2 (Toxicokinetic)	18	18			

a Group 1 was administered vehicle control article only.
b Two doses were administered daily, 12 hours apart (±30 minutes). On each day of dosing, the second daily dose was based on the end dose time of the first daily dose of each sex/group/subgroup.
c Test article concentrations were based on the test article as supplied (no correction). Dose volume for all dose groups was 10 mL/kg/dose (20 mL/kg/day).
d On Days 1 through 3 of the dosing phase, animals in Groups 3 and 4 were administered two daily doses of 125 mg/kg/dose (250 mg/kg/day) at a concentration of 12.5 mg/mL.
e Starting on Day 4 and through the end of the dosing phase, animals in Group 3 were administered two daily doses of 250 mg/kg/dose (500 mg/kg/day) at a concentration of 25 mg/mL.
f Starting on Day 4 and through the end of the dosing phase, animals in Group 4 were administered two daily doses of 500 mg/kg/dose (1000 mg/kg/day) at a concentration of 50 mg/mL.

Observations and Results

Mortality

There were no drug-related deaths.

Clinical Signs

No GNX-related clinical observations were noted.

Body Weights

Increases in BW gain were seen in treated animals, but the differences were not clearly dose-related and there were no SS differences in BW gain over the treatment period or in BW at the end of dosing.

Food Consumption

BW gain generally correlated with increased food consumption in males but not in females.

Hematology

There were no drug-related changes in hematology parameters.

Clinical Chemistry

There were no apparent drug-related changes in clinical chemistry parameters.

Organ Weights

At the terminal sacrifice, there were drug-related increases liver weights (reported as liver/gall bladder) at all doses in both sexes (Table 2).

Table 2.

	GNX							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	250	250/ 500	250/ 1000	0	250	250/ 500	250/ 1000
Liver/Gall Bladder								
Absolute Weight (g)	1.2382	120*	122*	130*	0.9292	109	125*	128*
Body Weight Ratio (%)	4.6554	113*	118*	121*	4.3708	108	123*	122*
Brain Weight Ratio (%)	269.6137	116*	121*	126*	196.6442	108	123*	126*

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as percentage control mean value.

Gross Pathology

No drug-related macroscopic observations were noted.

Histopathology

No drug-related microscopic changes were reported.

Enzyme induction

Drug-related increases in testosterone 16ß-hydroxylase activity, a marker for CYP2B activity (180% to 341% of control), and testosterone 6ß-hydroxylase activity, a marker for CYP3A activity (291% to 645% of control), were seen at all doses in both sexes (Table 3). CYP3A induction was greater in males and CYP2B induction greater in females. An increase (2-fold) in total cytochrome P450 content was seen in HD males and increased (2-fold) CYP4A activity was seen in HD males and females.

No notable changes in microsomal protein yield (92.7% to 144% of control), CYP2E activity (104% to 123% of control), or UDPGT activity (118% to 126% of control) were observed for any GNX-treated group.

Table 3. Microsomal enzyme analysis

		Percent of Control					
	Group	2		3[a]		4[b]	
	Dose Level (mg/kg/day)	250		250/500		250/1000	
Parameter	Sex	M	F	M	F	M	F
Protein yield		92.7	144	125	114	94.1	134
Total cytochrome P450 content (CYP450)		144	125	164	118	**203**	133
Ethoxyresorufin O-deethylase (CYP1A)		115	65.5	127	61.1	86.0	**210**
Testosterone 16β-hydroxylase (CYP2B)		180	**243**	**221**	**269**	**253**	**341**
Testosterone 6β-hydroxylase (CYP3A)		**423**	**291**	**611**	**321**	**645**	**355**
Lauric acid 11-hydroxylase (CYP2E)		108	123	111	112	120	104
Lauric acid 12-hydroxylase (CYP4A)		156	188	137	196	**201**	**219**
Uridine diphosphoglucuronosyltransferase (UDPGT-4-MU)		121	126	126	118	124	123

F Female.
M Male.
Note: Values in bold represent a notable change, which is ≥2-fold increase compared to the control values.
a Animals were administered 250 mg/kg/day from Day 1 through Day 3 and beginning on Day 4 until the end of dosing phase were administered 500 mg/kg/day.
b Animals were administered 250 mg/kg/day from Day 1 through Day 3 and beginning on Day 4 until the end of dosing phase were administered 1000 mg/kg/day.

Toxicokinetic

TK parameters for GNX on Days 8 and 26 are shown in Table 4. Cmax and AUC0-24 values generally increased with dose (but less than dose proportionally) and were generally similar between Days 8 and 26 in the LD and MD groups and only slightly higher at the HD, indicating no consistent accumulation of GNX beyond Day 6 of dosing.

Table 4. GNX TK parameters in mouse

Day	Dose Group	Dose Level (mg/kg/dose)	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24} (h*ng/mL)	$t_{1/2}$ (h)
8	2	125	250	M	32.8	12.5	385	NR
				F	46.1	12.5	469	NR
				MF	40.8	12.5	429	NR
	3	250	500	M	39.7	15.0	629	NR
				F	90.9	12.5	808	NR
				MF	61.8	12.5	719	NR
	4	500	1000	M	38.9	12.5	551	NR
				F	60.3	12.5	896	NR
				MF	49.6	12.5	724	NR
26	2	125	250	M	22.0	12.5	259	NR
				F	37.0	15.0	573	NR
				MF	27.7	12.5	416	NR
	3	250	500	M	33.8	12.5	385	NR
				F	69.8	15.0	770	NR
				MF	51.2	15.0	603	NR
	4	500	1000	M	185	12.0	1260	4.67
				F	52.9	12.5	968	NR
				MF	108	12.0	1100	NR

NR = Not reported due to the inability to characterize the elimination phase per SOP criteria.

Notes: Combined male and female (MF) parameters were calculated by combining concentration data for all animals (male and female) at each dose level on each interval and using these data as a separate composite profile for TK analysis. These parameters are not an average of the values calculated for males and females separately.

Doses were administered twice daily, 12 hours apart (±30 minutes).

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Fertility and Early Embryonic Development in the Rat by Oral Gavage Administration

Study no.:	COY 17/973803
Study report location:	4.2.3.5.1
Conducting laboratory and location:	(b) (4) (b) (4) (b) (4)
Date of study initiation:	23 May 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot # 0105 M/0090M, 99.5%

Key Study Findings

When GNX (0, 10, 20, or 40 mg/kg/day) was administered to male and female rats prior to and throughout mating and continuing in females during early gestation, clinical signs of sedation were seen in HD males and at all doses in females; BW gain was decreased slightly (NS) pre-pairing in HD males and females. Alterations in estrous cyclicity were seen at the HD, but there were no clearly drug-related effects on spermatogenesis, reproductive performance and fertility, or early embryonic development. A Cmax value of 206 ng/mL was measured in HD males at Week 15. Cmax in females and AUCs were not determined.

Methods

Doses:	0 (vehicle), 10, 20 or 40 mg/kg
Frequency of dosing:	QD
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	Preformulated β-cyclodextrin complex suspension/ (b) (4)
Species/Strain:	Sprague Dawley Crl:CD(SD) rats
Number/Group:	24/group
Age:	Males: 6 weeks; Females: 11 to 12 weeks
Weight:	M: 91 - 138 g, F: 179 - 209 g
Satellite groups:	none
Deviation from study protocol:	None that impacted study quality or integrity

GNX (0 (vehicle), 10, 20 or 40 mg/kg) was administered once daily by gavage to males (24 animals/group) for 10 weeks prior to pairing and up to termination (Week 15) and to females (24 animals/group) for 2 weeks prior to pairing and through to implantation (GD 7). Vaginal smears for assessing mating performance were taken daily from females during the mating period through terminal sacrifice on Day 14 of pregnancy. At necropsy maternal organs were examined for the number of corpora lutea, implantation sites, live fetuses, and embryofetal deaths. Males were sacrificed and examined macroscopically,

organ weights were recorded, sperm parameters evaluated, and selected reproductive tissues (testes and epididymides in C and HD and males that failed to produce pregnancy) were examined microscopically. Females were sacrificed on GD14, litter values were determined, and macroscopic examinations, organ weight determinations, and microscopic examinations of reproductive organs (ovaries from females that failed to mate or was found non-pregnant) were performed. Blood samples were taken from males in the last week of dosing for TK analysis.

Group/ colour code	Treatment Ganaxolone (mg/kg/day)#	Concentration Ganaxolone (mg/mL)	Vehicle	Dose volume (mL/100 g)	No of rats		Animal numbers	
					M	F	M	F
1: White	Control	0	*	1	24	24	1 - 24	97 - 120
2: Yellow	10	1	*	1	24	24	25 - 48	121 - 144
3: Green	20	2	*	1	24	24	49 - 72	145 - 168
4: Red	40	4	*	1	24	24	73 - 96	169 - 192
Health check							193 - 202	203 - 212

\# Material as supplied (25 mg/mL suspension in β-cyclodextrin complex)
* Vehicle consisted of [redacted](b) (4) and water for irrigation. Control animals also received [redacted](b) (4) at the same concentration as present in the high dose formulations [redacted](b) (4)

Dose selection was said to be based on the results of the oral juvenile (COY 2/95920) and PPND (COY 7/961509) studies in the rat, both performed in the same laboratory as the current study.

Observations and Results

Mortality:

There was no drug-related mortality.

Clinical signs:

Clinical signs consisted of unsteady gait (HD males, all doses in females), uncoordinated movements (MD and HD females), collapsed posture (HD females), and eyelids partially closed (HD females).

Body weight and food consumption:

BW gain was decreased slightly (NS) in HD males during the 10 weeks prior to mating and in HD females over the 2-week pre-pairing period (Table 1). There were no drug-related effects on BW gain of pregnant females up to GD 14.

Table 1. BW gain prior to and during after mating
Males

Mean gain (g/rat) between Week				
0 - 10	304	283	283	277
sd	46.2	34.7	54.7	62.3
% of control	-	93	93	91
0 - 14	349	327	335	329
sd	56.7	42.5	62.3	69.1
% of control	-	94	96	94

Females

Mean gain (g/rat) between Week				
0 - 2	23	25	27	19
sd	9.3	11.0	5.7	8.5
% of control	-	109	117	83

Toxicokinetics

Male TK values for GNX are shown in Table 2. AUCs were not calculated.

Table 2. Plasma GNX concentrations in male rats in Week 15

Time h	Mean plasma concentration (ng/mL) in Group/dosage (mg/kg/day)		
	2♂ 10	3♂ 20	4♂ 40
0.5	25.6	53.8	206.4
0.75	10.5	27.1	63.0
1.0	12.7	64.1	112.1
1.5	16.1	52.7	152.8
2.0	7.3	29.7	61.6
4.0	4.1	0.0	17.5
8.0	0.0	0.0	3.9
24.0	0.0	0.0	0.0
Cmax	25.6	64.1	206.4
Tmax	0.5h	1.0h	0.5h

Necropsy

Organ weights

There were no drug-related effects on specified organ weights.

Sperm assessments

There were no group differences in sperm count or motility.

Macroscopic pathology

No drug-related effects were noted.

Microscopic pathology

One male in each of the C and HD groups (nos. 20 and 90, respectively) showed minimal unilateral seminiferous tubular germ cell degeneration/depletion (Table 3). In addition, an additional male in each of these groups (nos. 9 and 93, respectively) had marked unilateral seminiferous tubular germ cell degeneration/depletion, with a marked reduction in the numbers of spermatozoa in the right epididymis (left examined for animal 93). These were not considered drug-related, based on group incidences.

In animals that failed to successfully mate, marked seminiferous tubular degeneration/depletion was seen bilaterally in the testes of a MD male (55), with a marked reduction of epididymal sperm. No microscopic findings were detected in the testes and epididymis of 2 HD rats (77 and 80) that failed to successfully mate. No microscopic findings were detected in the ovaries of a MD female (151) or 2 HD females (173 and 176) that failed to mate.

Table 3. Microscopic pathology incidence summary

	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	---------- Males ----------				---------- Females ----------			
Animals on study	24	24	24	24	24	24	24	24
Animals completed	24	0	1	24	0	0	1	2
Ovaries								
Examined	0	0	0	0	0	0	1	2
No abnormalities detected	0	0	0	0	0	0	1	2
Testes								
Examined	24	0	1	24	0	0	0	0
No abnormalities detected	22	0	0	22	0	0	0	0
Seminiferous tubular germ cell degeneration/depletion - unilateral (Total)	2	0	0	2	0	0	0	0
Minimal	1	0	0	1	0	0	0	0
Marked	1	0	0	1	0	0	0	0
Seminiferous tubular germ cell degeneration/depletion - bilateral (Total)	0	0	1	0	0	0	0	0
Marked	0	0	1	0	0	0	0	0
Right Epididymis								
Examined	24	0	1	24†	0	0	0	0
No abnormalities detected	23	0	0	23	0	0	0	0
Reduced numbers of spermatozoa (Total)	1	0	1	1	0	0	0	0
Marked	1	0	1	1	0	0	0	0

† Left epididymis examined for animal 93

Mating Performance and Litter Data

Assessment of vaginal smears taken daily for females prior to mating revealed an increased incidence (5/24 females) of extended estrous in HD females compared to C and historical control data (Table 4). Three pairings, 1 MD (male 55: female 151) and 2 HD (male 77: female 173, male 80:176), did not result in a pregnancy, despite positive evidence of mating. In the unsuccessful pairings at the HD, both females showed extended estrous prior to mating. In the unsuccessful MD pairing, the female showed an irregular cycle, and the male was found to be probably infertile at histological examination. No histopathology changes were noted in any of the other apparently infertile animals. The effect on estrous cyclicity appeared to be reflected in slight reductions in mating performance indices at the HD (Table 4).

A total of 24, 24, 23, and 22 females in C, LD, MD, and HD groups, respectively, had viable young at sacrifice on GD 14. There were no clearly drug-related group differences in the mean numbers of corpora lutea, implantations, live young, and the associated pre- and post-implantation losses (Table 5).

Table 4. Mating performance

	Number of animals in category group/dosage (mg/kg/day)				
	1 Control	2 10	3 20	4 40	Historical means[a]
Males					
Paired	24	24	24	24	
Mating	24	24	24	24	
Achieving pregnancy	24	24	23	22	
Females					
Paired	24	24	24	24	
Mating	24	24	24	24	
Achieving pregnancy	24	24	23	22	
Performance indices					
Mating rate (%)	100	100	100	100	100%
Conception rate (%)	100	100	96	92	96%
Fertility index (%)	100	100	96	92	96%
Pre-coital interval (days)					
1 - 4 (%)	24(100)	22(92)	21(88)	24(100)	94%
5 - 8 (%)	0	2(8)	3(13)	0	6%
9 - 12	0	0	0	0	0%
13 - 16	0	0	0	0	0%
17 - 20	0	0	0	0	0%
Oestrous cycles					
Regular 4 or 5 day cycle (%)	21(88)	18(75)	18(75)	19(79)	80%
Irregular cycle† (%)	1(4)	5(21)	5(21)	0	18%
Extended oestrousø (%)	1(4)	0	1(4)	5(21)	2%
Acyclic§ (%)	1(4)	1(4)	0	0	0%
Mean number of cornified smears*	5.0	4.7	5.2	5.3	3.3

† At least one cycle of 2, 3 or 6 to 10 days
ø At least 4 consecutive days of predominantly cornified cells
§ At least 10 days without cornified cells
* Smears showing predominantly cornified cells before mating
a From 3 comparative studies

Table 5. Litter data

Group dosage (mg/kg/day)		1 Control	2 10	3 20	4 40	Historical data† Mean	 Range
Dams with live young							
	No. of litters	24	24	23	22		
Group mean values							
	No. of corpora lutea	16.8	16.3	16.8	15.9	14.2	12.5 - 16.8
	No. of implantations	16.2	15.2	16.1	15.0	13.4	11.7 - 15.9
	No. of *in utero* deaths:						
	early	0.4	0.4	0.3	0.7	0.5	0.2 - 0.9
	late	-	0.2	-	0.2	0.1	0 - 0.2
	early and late	0.4	0.6	0.3	0.9	0.6	0.3 - 1.0
	No. of live young	15.8	14.5	15.8	14.2	12.8	11.2 - 14.9
	Pre implantation loss (%)	3.4	8.4	4.0	5.3		
	Post implantation loss (%)	2.4	4.5	1.9	5.8		
Litter incidence#	*n*						
in utero deaths:	0	16	16	17	12		
early	1	7	6	5	8		
	2	1	2	1	1		
	5	-	-	-	1		
in utero deaths:	0	24	21	23	20		
late	1	-	1	-	1		
	2	-	2	-	-		
	3	-	-	-	1		

\# No. of litters with *n* losses/*in utero* deaths

$p > 0.05$

† Of 15 studies performed in these laboratories using the same strain of rats

9.2 Embryofetal Development

Study title: Oral (Gavage) Embryo-Fetal Developmental Toxicity Study in the Mouse

Study no.:	1215-007
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	16 Jul 1998
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	G 8074, purity not provided

Key Study Findings

Oral (gavage) administration of GNX (0 (vehicle), 50, 175, or 300 mg/kg) to pregnant CD-1 mice throughout organogenesis (GD 6-15) resulted in transient clinical signs of hypoactivity and ataxia at the MD and HD during the early treatment period but no effects on maternal BW gain. There were no effects on litter parameters at C-section. Fetal malformations were increased in all drug-treated groups compared to C: 1(1), 4(4), 5(4), and 5(3) fetuses (litters) with external/visceral malformations were observed in the C, LD, MD, and HD groups, respectively. These were not considered drug-related in the study report, based on the absence of a dose relationship, but in TK data collected in another CD-1 mouse study (8412975) plasma levels plateaued at doses ≥125 mg/kg, and there seemed to be a pattern of CNS defects, both of which support a potential drug effect. Maternal plasma drug levels were not determined.

Methods

Doses:	0, 50, 175, or 300 mg/kg/day
Frequency of dosing:	QD
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Preformulated β-cyclodextrin complex/ (b) (4)
Species/Strain:	Mouse/ Crl:CD-1 (ICR) BR
Number/Group:	24/group
Age:	5 to 8 weeks
Weight:	20-30 g
Satellite groups:	no
Study design:	Dosing GD 6-15, C-section GD 18
Deviation from study protocol:	None that impacted study quality or integrity

GNX was administered to time-mated female mice once daily by oral gavage on GDs 6 to 15, inclusive. The following parameters and end points were evaluated: mortality,

clinical signs, body weights, food consumption, maternal reproductive (ovarian and uterine) parameters (GD 18), fetal weights, and fetal examinations (external, visceral, and skeletal).

Doses were based on the results of a mouse embryofetal development dose range-finding study (#1215-006) in which GNX (0 (vehicle), 50, 125 and 200 mg/kg) was administered once daily by gavage to pregnant CD-1 mice (8/grp) from GDs 6 to 15, maternal BW gain was decreased slightly (10%, NS) at the HD but there was no clear evidence of developmental toxicity. The no-effect dose for embryofetal toxicity (200 mg/kg/day) was associated with maternal Cmax values of 657 and 63 ng/mL at 1h on GDs 6 and 15, respectively.

In a more recent 7-Day oral DRF study (8412975) in CD-1 mice using the clinical formulation ((b) (4) GNX suspension) and BID dosing, TK parameters determined on Day 7 showed that increases in Cmax and AUC values were much less than dose proportional (Table 1). At these doses, all animals survived to their scheduled sacrifice, and there were no drug-related clinical observations or sedation and no effects of body weight or food consumption.

Table 1.

Dose Group	Dose Level (mg/kg/day)	Dose Level (mg/kg/dose)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12} (h*ng/mL)	AUC_{0-24} (h*ng/mL)	$t_{1/2}$ (h)
2	250	125	M	37.3	0.500	185	371	NC
			F	40.0	0.500	205	410	NC
			MF	38.7	0.500	195	390	NC
3	500	250	M	36.8	0.500	205	409	3.26
			F	41.9	3.00	267	534	NC
			MF	37.4	0.500	236	472	NC
4	1000	500	M	56.0	0.500	271	542	NC
			F	60.2	0.250	265	530	NC
			MF	49.6	0.500	268	536	NC

Observations and Results

Mortality

There were no drug-related deaths.

Clinical Signs

Transient clinical signs (hypoactivity and ataxia) were observed at the MD and HD with a dose-related incidence and severity (ataxia graded severe at HD). All animals recovered within a few hours or by the next day. However, these observations were detected primarily on the first day of treatment.

Body Weight

There were no effects on maternal BW gain.

Cesarean Section Data

There were no effects on litter parameters.

Fetal evaluations

Fetal malformations were increased in all drug-treated groups compared to C (Table 2). There was no clear dose relationship; fetal and litter incidences were similar across doses, although the average percent malformed fetus values increased dose-dependently. At the HD, 5 fetuses from 3 litters showed external and/or visceral malformations (edema of the head, brain abnormal shape, skull flattened, pelvic kidney, cleft palate); at the MD, 5 fetuses/4 litters were malformed (exencephaly, open eye(s), retinal dysplasia, abnormal shaped brain, diaphragmatic hernia); at the LD, 4 fetuses/4 litters showed malformations (exencephaly, hydrocephaly, diaphragmatic hernia); and in the C group, 1 fetus was malformed (hydrocephaly) (Table 3).

Table 2. Fetal abnormalities

	Group 1 - 0 mg/kg/day	Group 2 - 50 mg/kg/day	Group 3 - 175 mg/kg/day	Group 4 - 300 mg/kg/day
Total number of litters with live fetuses	22	21	23	22
Total number of live fetuses	225	251	265	243#
Number of fetuses examined externally	225 (1)	251 (1)	265 (1)	243
Number of fetuses examined viscerally	106	120	126	118
Number of fetuses examined skeletally	119	130	139	125
Number of fetuses with external/visceral malformations	1	4	5	5
Number of litters affected	1	4	4	3
Number of fetuses with skeletal malformations	0	0	0	0
Number of litters affected	0	0	0	0
Total number of malformed fetuses	1	4	5	5
Average per cent malformed fetuses	0.4	1.6	1.9	2.1
Total number of litters affected	1	4	4	3
Percentage of litters examined	4.5	19.0	17.4	13.6
Number of fetuses with external/visceral variations	14	6	6	8
Number of litters affected	10	6	6	6
Number of fetuses with skeletal variations	118	129	136	125
Number of litters affected	22	21	23	22

Remark: Number(s) in parenthesis refer to dead fetuses examined additionally

including the fetuses of animal no.74

Table 3. External/visceral malformations

Key	Type of defect		Group 1 - 0 mg/kg/day	Group 2 - 50 mg/kg/day	Group 3 - 175 mg/kg/day	Group 4 - 300 mg/kg/day
	External/visceral malformations					
5	Exencephaly	F	0	1	2	0
		L	0	1	2	0
		%	0.0	4.8	8.7	0.0
17	External hydrocephaly	F	1	1	0	0
		L	1	1	0	0
		%	4.5	4.8	0.0	0.0
19	Brain abnormal shape	F	0	0	1	2
		L	0	0	1	1
		%	0.0	0.0	4.3	4.5
34	Skull flattened	F	0	0	0	1
		L	0	0	0	1
		%	0.0	0.0	0.0	4.5
36	Edema: head	F	0	0	0	1
		L	0	0	0	1
		%	0.0	0.0	0.0	4.5
54	Open eye(s)	F	0	0	1	0
		L	0	0	1	0
		%	0.0	0.0	4.3	0.0
60	Retinal dysplasia	F	0	0	1	0
		L	0	0	1	0
		%	0.0	0.0	4.3	0.0
84	Cleft palate	F	0	0	0	1
		L	0	0	0	1
		%	0.0	0.0	0.0	4.5
305	Diaphragmatic hernia	F	0	2	1	0
		L	0	2	1	0
		%	0.0	9.5	4.3	0.0
397	Pelvic kidney	F	0	0	0	1
		L	0	0	0	1
		%	0.0	0.0	0.0	4.5

F = number of fetuses affected L = number of litters affected % = percentage of litters affected

9.3 Pre- and Postnatal Development

Study title: The Effect on Pre- and Post-natal Development Including Embryofoetal Development and Maternal Function in the Rat (Gavage administration)

Study no.:	COY 7/961509
Study report location:	4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	25 Sep 1995
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	# 0777L OPOS, 99%

Key Study Findings

In a combination rat embryofetal and pre- and postnatal development study, GNX (0 (vehicle: (b) (4) 10, 20 or 40 mg/kg/day) was administered once daily by gavage to pregnant SD rats (48/grp) from GD 6 through GD 19 (EFD, 20–23/grp) or continuing through delivery until weaning (PPND, 25-28/group). Dose-dependent clinical signs (unsteadiness [moderate or severe at HD], apparent sleepiness, body tremors) were observed and BW gain was decreased (10% compared to C, SS) during GDs 6-16 at the HD. There were no treatment-related effects on litter parameters or incidences of fetal abnormalities at C-section on GD20. In offspring from dams allowed to litter, BW gain was decreased (NS) during lactation at the HD, and postweaning BW gain was decreased (SS) from postnatal weeks (PNW) 4 to 19 in HD males. This was associated with a slight (NS) delay in attainment of specific developmental landmarks (righting reflexes) during the pre-weaning period and a slight (NS) delay in attainment of sexual maturation of HD male offspring. Decreased (SS) locomotor activity was seen in HD male offspring when assessed at PNW 5 and in MD and HD males when retested at PNW 11. There were no effects on learning and memory as assessed in the passive avoidance test. Offspring mating performance was unaffected by treatment.

Methods

Doses:	0 (vehicle), 10, 20, 40
Frequency of dosing:	QD
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	β-CD complex suspension/ (b) (4)
Species/Strain:	Crl: CD BR VAF/PLUS strain
Number/Group:	48/group
Satellite groups:	none
Study design:	EFD/PPND, dosing GD6-GD19 or GD6-PND22
Deviation from study protocol:	None

GNX (0 (vehicle), 10, 20 or 40 mg/kg/day) was administered once daily by gavage (10 mL/kg) to pregnant SD rats (48/grp) from GD 6 through GD 19 for those (20-23/group) that were sacrificed on GD 20 for assessment of effects on EFD or until weaning PND 22) for the remainder (25-28/group) for evaluation of effects on PPND.

According to the study report, doses were selected by the sponsor based upon results of a study in the neonatal rat performed in the same laboratory (COY 2/950920) and other information known to the sponsor (including a 6-month toxicity study in the rat).

Observations and Results

F0 Dams

Mortality

Three dams (1 from each dose group) were sacrificed prior to the scheduled termination, but none of these deaths was considered clearly drug-related.

Clinical signs

Clinical signs of sedation (unsteadiness, apparent sleepiness, body tremors) were noted at all doses (ranging in severity from moderate to severe at the HD to very slight at the LD) from half an hour after dosing. These gradually diminished in severity and were not apparent at initial animal check the following morning (approximately 22 hours after dosing). Although the signs at the HD were considered to be fairly severe, according to the report, “they did not compromise the ability of the dams to retain their litter in utero or during lactation.”

Body weight

BW gain was decreased (10%, SS) over the dosing period GD 6-16 at the HD compared to C. There were no group differences thereafter during gestation or lactation.

Cesarean Section Data

There were no apparent drug-related effects on litter parameters on PND 20 (Table 1). Differences in litter size reflected differences in numbers of implantations, which occurs prior to initiation of dosing.

Table 1. Litter data at Day 20 of pregnancy

Group Dosage (mg/kg/day)		1 Control	2 10	3 20	4 40
Dams with live young					
No. of litters		20	20	20	20
Group mean values					
No. of corpora lutea		14.6	14.7	14.7	14.4
No. of implantations		14.0	13.6	13.3	13.0
No. of *in utero* deaths:					
- early		0.8	0.9	0.6	0.4
- late		0.1	0.0	0.0	0.2
- early and late		0.8	0.9	0.6	0.6
No. of live young		13.2	12.8	12.8	12.4
Sex ratio (% males/litter)		49.4	50.9	54.9	45.8
Litter weight (g)		51.29	48.02	49.68	48.51
Foetal weight (g)		3.91	3.78	3.91	3.95
Litter incidence#	'n'				
Pre-implantation losses	0	12	11	9	7
	1	5	5	4	5
	2	2	1	2	5
	3	1	1	2	2
	4		1	1	
	5			2	
	6				1
	7		1		
in utero deaths: early	0	10	10	10	14
	1	5	5	9	5
	2	5	3	1	
	3		2		1
in utero deaths: late	0	19	20	20	16
	1	1			4
in utero deaths: early and late	0	10	10	10	10
	1	5	5	9	9
	2	4	3	1	
	3	1	2		1

No. of litters with 'n' losses/*in utero* deaths

Fetal evaluations

Malformation incidences (0, 5, 2 and 1 fetuses in C, LD, MD, and HD litters) did not appear to be affected by treatment, and there were no clearly drug-related effects on incidences of visceral or skeletal variations (Table 2). "Squat fetus" was seen in 4 fetuses from 1 litter

at the LD; this was considered a spontaneous genetic defect based on historical experience in the lab. The other malformations were microphthalmia in 1 LD fetus, orbital socket reduced in size in 1 MD fetus, diaphragmatic hernia in 1 MD fetus, and forelimb flexure in 1 HD fetus.

Table 2. Fetal abnormalities

Category	No. of affected foetuses/ litter (n)	Group/dosage (mg/kg/day)			
		1 Control	2 10	3 20	4 40
		No. of litters with 'n' foetuses affected			
Number of litters examined		20	20	20	20
Malformation	0	20	18	18	19
	1		1	2	1
	2				
	3				
	4		1		
Visceral anomaly	0	11	14	12	14
	1	6	5	5	5
	2	2	1	3	1
	3	1			
Skeletal anomaly	0	10	10	13	11
	1	6	6	5	6
	2	1	2		3
	3		1	2	
	4	3			
	7		1		

Delivery and pre-weaning litter data

Gestation length was dose-dependently increased (SS at the HD), and dystocia in 1 HD dam (No. 176) that was sacrificed was considered possibly drug-related (Table 3). There were no clearly drug-related effects on in utero losses, litter size at birth and through to weaning, or pup losses at birth or from birth to weaning. Slight decreases in pup BW gain to weaning (5% at HD, NS) and BW at weaning (4% at HD, NS) were seen at the MD and HD, and these were associated with slight delays (SS at HD) in reflex development.

Table 3. Litter data from birth to weaning

Group/ dosage (mg/kg/day)	No. of litters	Impl. sites	Implant loss %	At birth					At Day 4				At Day 8			
				Litter size		Pup loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)
				Total	Live											
1 Control	23	13.6	4.0	13.1	13.1	0.0	82.6	6.3	12.9	1.4	125.9	9.8	12.9	1.7	217.5	17.0
2 10	26	13.6	6.4	12.9	12.7	1.3	82.2	6.6	12.3	4.3	120.4	10.0	12.2	5.4	206.2	17.2
3 20	26	13.6	5.6	12.9	12.6	2.4	80.2	6.4	12.0	7.3	115.0	9.6	11.8	8.3	187.9	15.8
4 40	23	13.8	4.2	13.2	13.1	1.2	85.2	6.5*	13.0	1.8	121.4	9.4	12.9	2.5	198.5	15.5

Group/ dosage (mg/kg/day)	At Day 12				At Day 16				At Day 21			
	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cum. loss %	Litter wt (g)	Mean pup wt (g)
1 Control	12.8	2.0	320.8	25.2	12.8	2.0	427.3	33.6	12.8	2.0	594.5	46.8
2 10	12.2	5.7	305.0	25.6	12.1	6.0	412.6	34.8	12.1	6.0	572.1	48.2
3 20	11.7	8.9	286.4	24.1	11.7	8.9	385.3	32.6	11.7	8.9	537.3	45.4
4 40	12.7	3.7	298.4	23.6	12.7	4.3	398.9	31.8	12.6	4.6	562.0	45.0

* $p \leq 0.05$

Group/ dosage (mg/kg/day)	No. of litters	Duration of pregnancy	Mean age (days post coitum) for attaining:			Pupil reflex (Day 20)
			Surface righting	Startle response	Air righting	% successful
1♀ Control	23	21.4	24.1	34.4	37.4	100
2♀ 10	26	21.6	24.2	34.7	37.3	100
3♀ 20	26	21.7	24.3	34.8	37.7	100
4♀ 40	23	21.8*	24.5*	34.5	38.0*	100

* $p \leq 0.05$

Postweaning offspring data

Body weight

BW gain from Week 4 and final BWs were decreased in HD males (Table 4).

Table 4. Bodyweights - group mean values (g) – F1 generation

Week	Group/dosage (mg/kg/day) #							
	1♂ Control	2♂ 10	3♂ 20	4♂ 40	1♀ Control	2♀ 10	3♀ 20	4♀ 40
4N	90	92	92	86	83	86	84	81
5	149	151	150	142	129	133	129	123
6	220	219	218	207	170	172	169	163
7	286	285	286	269	200	202	197	193
8	349	347	347	327	228	231	222	220
9	396	394	391	369	248	253	245	241
10	434	434	430	405	267	273	264	259
11	465	463	462	435	282	284	274	273
12P	496	496	494	464	295	296	286	284
13	521	519	518	488	333	334	322	319
14	549	553	551	519	370	372	365	360
15	566	563	566	533	445	441	436	432
16	571	568	573	540	364	375	370	358
17	590	588	590	555	396	402	394	391
18	608	607	609	572	392	397	390	382
19	625	623	625	583	385	377	374	368
20						413	453	409
21								404
Group mean weight gain (g/rat)								
4 - 12	406	404	402	379 *	212	210	202	204
% Control	-	100	99	93	-	99	95	96
4 - 19	534	531	533	498 *				
% Control	-	99	100	93				

\# Treatment refers to F0 adult females - F1 generation untreated
* $p \leq 0.05$
N Nominal age
P Animals paired for 20 days

Sexual maturation

Sexual maturation (preputial separation) was slightly delayed (NS) in HD males (Table 5).

Table 5. Sexual maturation - group mean values - Fl generation

Balanopreputial skinfold cleavage

Group/ dosage (mg/kg/day)#	Number of males showing balanopreputial skinfold cleavage on Day *post partum*													Total	Mean age (days)	Bodyweight (g) on day of occurrence
	40	41	42	43	44	45	46	47	48	49	50	52	53			
1 Control	1	-	2	2	3	4	4	1	1	2	-	-	-	20	45.0 (44.8)	241.1
2 10	1	3	-	3	2	5	2	2	-	1	-	1	-	20	44.6 (44.6)	238.2
3 20	-	-	3	3	5	5	2	-	2	-	-	-	-	20	44.4 (44.6)	235.7
4 40	-	1	1	3	3	4	2	-	1	2	1	1	1	20	45.9 (45.9)	238.4

Vaginal opening

Group/ dosage (mg/kg/day)#	Number of females showing vaginal opening on Day *post partum*													Total	Mean age (days)	Bodyweight (g) on day of occurrence
	31	32	33	34	35	36	37	38	39	40	42	43	46			
1 Control	-	2	4	8	1	3	-	-	-	-	1	-	1	20	35.0 (34.8)	123.5
2 10	2	3	3	4	5	1	1	1	-	-	-	-	-	20	34.0 (34.1)	121.5
3 20	2	1	6	3	2	2	-	1	1	1	-	1	-	20	34.8 (34.6)	124.2
4 40	-	1	5	4	5	2	-	2	1	-	-	-	-	20	34.8 (35.0)	120.7

Treatment refers to F0 females, F1 generation untreated
No statistical significance ($p > 0.05$)
Values in parentheses are adjusted for bodyweight as covariate

Neurobehavioral assessment

Locomotor activity

When tested in an Actimat device at PNWs 5 and 11, locomotor activity was decreased at all doses compared to C (SS at MD and HD; Table 6). There were no apparent drug-related differences in females (only tested at PNW 5).

Table 6. Locomotor activity - males

Group dosage (mg/kg/day)#	Week 5 Mean amount of time (in secs) spent in categories of activity (5 minute period)			
	No. tested	Total	Low level	High level
1 Control	20	219	138	118
2 10	20	205	131	106
3 20	20	214	135	113
4 40	20	* 202	131	** 101

Week 11						
Group/ dosage (mg/kg/day)#	No. tested	Mean amount of time (in secs) spent in categories of activity (10 minute period)			Mean amount of time (in secs) spent in high level activity during:	
		Total	Low level	High level	First 5 minute block	Last 5 minute block
1 Control	20	376	262	163	87	76
2 10	20	340	238	147	76	70
3 20	20	* 335	236	140	77	63
4 40	20	* 347	* 241	147	75	71

Treatment refers to F0 parent females - F1 generation untreated
* $p < 0.05$, ** $p < 0.01$

Learning and memory

When animals were tested at 7 - 8 weeks of age, there were no drug-related effects on performance in the passive avoidance test.

Reproduction

When offspring were mated starting on PND 84, there were no apparent effects on mating performance (precoital interval, pregnancy rate (100% in all groups)) or litter parameters (implantation rate, implantation losses, litter size at birth, pup weights).

9.4 Juvenile Animal Toxicity Studies

Study Title: CCD 1042 EFFECTS ON THE NEONATAL RAT BY DIRECT TREATMENT FROM DAY 7 POST PARTUM TO 7 WEEKS OF AGE (GAVAGE ADMINISTRATION)

Study no.:	COY 2/950920
Study report location:	4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 4, 1994
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	94297-·02, 94297-03, 94297-04/99%

Key Study Findings

Daily oral (gavage) administration of GNX (0 (vehicle: βCD), 12.5, 25, or 50 mg/kg/day; 10 mL/kg) to neonatal/juvenile SD rats (2 'batches" of 10/sex/grp) from PND 7 to PND 49 resulted in clinical signs of sedation (MD, HD), decreased BW gain (HD), primarily during the first week of treatment, delays in the age of attainment of sexual maturation in both sexes (HD), and altered behavior (abnormal gait, decreased grip strength, decreased activity) in the FOB performed in PNW 5 (HD). There were no changes in activity at PNW 5 or learning and memory (passive avoidance only) at PNW 7 and no histopathological changes at the end of dosing (but no stage-specific testis examination or sperm analysis). No TK data were collected. The NOAEL was 25 mg/kg/day.

Methods

Doses:	0 (Control), 12.5, 25.0 and 50.0 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	βCD (b) (4) purified water
Species/Strain:	Rat/Crl: CD® BR VAF/Plus strain
Number/Sex/Group:	10/sex/group (Batch 1) + 10/sex/group (Batch 2)
Age:	PND 7 – PNW 7
Weight:	6-7g at start of dosing
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

GNX (CCD 1042; 0, 12.5, 25.0 and 50.0 mg/kg/day) was administered by oral gavage to two batches (10/sex/group each) of SD rats from PND 7 to PNW 7 (Table 1). Assessments of preweaning development and sexual maturation were carried out on all animals. Neurobehavioral evaluations were performed on animals from 1 batch only and included, for Batch 1, a functional observation battery and, for Batch 2, accelerating

rotarod, actimat, and passive avoidance testing. Ophthalmoscopy and clinical pathology were performed on Batch 1 animals prior to terminal sacrifice. Microscopic examination was carried out on a full range of tissues from C and HD animals in Batch 1.The second batch was retained untreated for 2 weeks to assess reversibility of any findings at the end of treatment but only organ weights and macroscopic examinations were conducted.

Table 1. Study design

Group/ colour code	Treatment CCD 1042 (mg/kg/day)#	Concentration CCD 1042 (mg/ml)	Vehicle	Dose volume (ml/100 g)	No. of pups			
					Batch 1		Batch 2	
					♂	♀	♂	♀
1: White	Control	0	βCD	1	10 (5)	10 (5)	10 (7)	10 (5)
2: Yellow	12.5	1.25	βCD	1	10 (5)	10 (5)	10 (6)	10 (6)
3: Green	25.0	2.5	βCD	1	10 (5)	10 (5)	10 (7)	10 (7)
4: Red	50.0	5.0	βCD	1	10 (5)	10 (5)	10 (6)	10 (7)

( ) Number of contingency animals at weaning
Material as supplied
βCD β cyclodextrine mixture - see dose preparation

Dose selection was based on the results of a dose range-finding study performed in the same laboratory (COY1/943149) in which a dose of 60 mg/kg/day showed signs of toxicity with immediate loss of righting reflex, reduced bodytone, reduction in body temperature, tremors, and loss of consciousness in some animals lasting for several hours. At 40 mg/kg/day similar effects were observed but without loss of consciousness.

Observations and Results

Mortality

There were no drug-related deaths during the study.

Clinical Signs

Drug-related clinical signs primarily consisted of sedative effects. At the HD, all animals showed signs of lethargy, reduced body tone, unsteady gait and, in most animals, apparent sleepiness (although animals said to be fully alert on handling) on occasion throughout the study. Females were affected more than males. In addition, 2 animals showed intermittent body tremors on 1 or 2 occasions during PNW 5, and one of these animals also appeared to be unconscious during this time (1 occasion). At the MD, signs of lethargy and/or reduced body tone were noted occasionally, although not all animals were affected. No treatment-related signs were observed at the LD.

Body Weights

BW gain and BWs were decreased at the HD, mostly during the first week of dosing (Table 2).

Table 2. Bodyweights (g) – Batch 2

Week	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 12.5	3♂ 25.0	4♂ 50.0	1♀ Control	2♀ 12.5	3♀ 25.0	4♀ 50.0
0	7	7	7	7	6	7	6	7
0.4	11	11	12	12	10	11	12	11
0.5	13	13	14	13	12	13	13	13
0.6	15	15	16	15	14	15	15	15
1	17	17	18	18	16	17	17	17
1.1	19	19	20	18	18	19	19	17
1.2	21	22	22	20	20	22	22	20
1.3	24	24	25	22	23	24	24	21
1.4	26	27	28	24	25	27	27	24
1.5	30	31	31	27	28	30	30	26
1.6	32	33	33	28	31	33	32	28
2	34	36	35	31	33	35	34	30
2.1	37	38	38	32	35	37	37	32
2.2	40	40	40	35	37	39	39	34
2.3	42	42	42	36	40	42	41	36
2.4	44	45	45	38	42	44	44	38
2.5	47	48	48	41	45	47	47	41
2.6	50	51	52	45	47	50	51	44
3	55	57	57	49	52	55	56	48
4	89	91	93	82	85	85	88	79
5	144	146	149	134	130	128	135	122
6	213	216	220	199	175	168	175	163
7	281	282	290	262	207	202	208	197
8	355	352	360	325	238	231	237	231
9	418	389	408	364	255	247	250	259
Bodyweight gain (g/rat)								
Weeks				**				**
1 - 2	17.9	18.7	17.3	13.0	17.5	18.2	17.0	12.7
sd	1.99	1.97	1.23	1.94	1.82	1.87	2.18	1.34
%control	-	104	97	73	-	104	97	73
Weeks								
2 - 6	177.9	179.5	184.3	167.6	140.6	132.2	140.3	132.9
sd	10.58	8.84	6.82	14.32	14.77	12.15	12.53	12.40
%control	-	101	104	94	-	94	100	95

** $p \leq 0.01$

Treatment commenced Week 1, withdrawn from commencement of passive avoidance test in Week 7

Pre-weaning development

There were no effects on the age of attainment of surface righting, startle response, air righting, or pupil reflex.

Sexual maturation

There was a slight delay in the age of attainment of sexual maturation in both males and females at the HD (Table 3). This appeared attributable to the BW effect.

Table 3. Sexual maturation in male and female rats

Batch 2

Group/ dosage (mg/kg/day)	Number of males with balano preputial skinfold cleavage on Day post partum										Total	Mean age of attainment (days)	Bodyweight (g) on day of attainment
	41	42	43	44	45	46	47	48	49	50			
1 Control			3	4			2		1		10	44.8 (44.6)	250
2 12.5			1	6			2		1		10	45.0 (44.9)	248
3 25.0	2		1	4		1	1	1			10	44.2 (44.1)	249
4 50.0		1		4		1	2	1	1		10	45.5 (45.8)	243

Values in brackets are adjusted for bodyweight as covariate
No statistical significance ($p > 0.05$), Kruskal-Wallis test on mean ages, or covariate analysis

Batch 1

Group/ dosage (mg/kg/day)	Number of females with vaginal opening on Day post partum									Total	Mean age of attainment (days)	Bodyweight (g) on day of attainment
	30	31	32	33	34	35	36	37	45			
1 Control		1		2	4	3				10	33.8 (33.8)	128
2 12.5				2	4	4				10	34.2 (34.0)	130
3 25.0		1		1	5	3				10	33.9 (34.1)	123
4 50.0			1	1	2	3	1	1	1+	10	35.6 (34.6)	130

\+ Excluded from covariate statistical analysis
Values in brackets are adjusted for bodyweight as covariate
No statistical significance ($p > 0.05$), Kruskal-Wallis test on mean ages, or covariate analysis

Functional observational battery

Swaying/lurching gait was observed in HD males (2/10) and females (2/10) when an FOB was conducted in Batch 1 animals prior to dosing at PNW 5 (Table 4). Decreased (SS) hindlimb grip strength and decreased activity were noted in HD females.

Table 4. FOB

Summary of observations - group values Week 5

Group	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
Dosage (mg/kg/day)	0	12.5	25.0	50.0	0	12.5	25.0	50.0
No. of animals	10	10	10	10	10	10	10	10
OBSERVATIONS:								
HOME CAGE								
Posture, sitting	10	9	7	9	9	8	6	9
REMOVAL FROM CAGE								
Removing, easy	10	10	10	10	10	10	10	10
Handling, easy	9	8	9	8	10	8	10	9
Salivation	0	1	0	1	0	2	0	0
Vocalising	2	0	2	0	1	1	3	0
IN THE ARENA								
Grooming	3	3	5	2	3	2	5	5
Arousal, alert	10	10	10	10	10	9	10	10
Defecation	3	6	1	4	0	0	0	1
Urination	2	3	0	2	1	0	1	5
GAIT								
Walking on toes	7	7	8	2	7	6	4	5
Swaying	0	0	0	2	0	0	0	2
Hunched	0	1	0	0	0	0	0	0
MANIPULATIONS								
Approach, a reaction	10	10	10	9	10	10	10	10
touch, a reaction	10	8	9	10	10	10	8	10
startle (present)	10	10	10	10	10	10	10	10
Righting, immediate	10	9	8	10	9	10	10	10
Tail pinch, a reaction	10	10	9	10	10	10	10	9
Pupil reflex (present)	10	8	10	8	9	9	10	10
GROUP MEANS								
Activity counts	16	18	14	18	20	18	21	15**
Rearing counts	13	15	12	13	13	13	13	12
Temperature (°C)	37.6	37.6	37.6	37.7	38.0	38.2	38.5	38.3
Bodyweight (g)	161	160	162	158	150	147	142	136*
Grip strength (kg)								
Forelimb	0.39	0.37	0.41	0.42	0.41	0.42	0.43	0.44
Hindlimb	0.44	0.43	0.47	0.48	0.54	0.52	0.52	0.47*
Foot splay (cm)	9.3	9.0	9.5	9.6	8.8	8.2	8.5	8.7

Numbers reflect the number of animals showing the response or with the indicated score
* $p \leq 0.05$, ** $p \leq 0.01$

Post-weaning neurobehavioral evaluations

There were no drug-related effects in Batch 2 animals on rotarod (PNW 4), locomotor activity (PNW 5), or passive avoidance (PNW 7).

Ophthalmoscopy

No changes considered drug-related were observed in C and HD animals from Batch 1 examined at the end of dosing.

Clinical Chemistry

There were no clearly drug-related changes in clinical chemistry parameters; however, inorganic phosphorus was slightly increased (up to 15%, SS) at the MD and HD.

Urinalysis

There were no drug-related changes in urinalysis parameters.

Organ Weights

Liver weights were increased slightly (9%, SS at HD) in Batch 1 males at the end of the treatment period, but no differences were seen in Batch 2 animals after a 2-week recovery period.

Gross Pathology

There were no drug-related macroscopic changes.

Histopathology

There were no drug-related microscopic changes.

Toxicokinetics

TK data were not collected.

Study title: A Twice Daily Oral (Gavage) Juvenile Toxicity Study with Ganaxolone in Sprague Dawley Rats

Study no.:	00398514
Study report location:	4.2.3.5.4
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	19 Nov 2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	18JM-102 and 19JM-038/ 99.9 and 99.4%

Key Study Findings

Juvenile SD rats were administered GNX (0 (vehicle), 20, 45, 90/150/250/500 mg/kg/day) BID by oral gavage from PND 7 through PND 91, followed by a 28-day recovery period. Drug-related deaths were seen at the MD (1 female) and HD (6 males and 4 females) and generally occurred between PNDs 7–9, except for 1 HD male found dead on PND 14. For these animals, sedation (grade 3 in 2 animals) was generally noted prior to death. Sedative effects were also seen in surviving animals at all doses, primarily during the pre-weaning period. BW gain and BWs were decreased (SS) intermittently at the MD and HD, but there were no SS differences among groups in BW gain over the entire treatment period or final BWs. Age of vaginal opening was dose-dependently increased (SS) at all doses, reaching an approximately 4-day delay in the MD and HD groups. There were no clearly drug-related effects on neurobehavioral assessments (motor activity, auditory startle response, and learning and memory in Biel maze), during the dosing period or after the recovery period. There were also no drug-related effects on reproductive performance or spermatogenic parameters. Following the reproductive assessment in Subset B rats, absolute brain weights were decreased at all doses in both sexes. Since relative weights were not SS different and the effect was not seen at the end of the dosing period in Subset A animals or at the end of the 28-day recovery period in Subset A animals this finding was not considered drug-related by the sponsor. However, the group sizes were greater at the Subset B necropsy (20 vs 8/sex/grp), relative weights were not calculated for females because final BW was not available due to pregnancy, and brain weight is generally spared from BW effects. Dose-related decreases (SS at MD and/or HD) in epididymis and testis weights were seen in Subset B males, but there were no apparent drug-related microscopic changes (stage aware testicular microscopic examination performed in C and HD) and no microscopic brain abnormalities (expanded histopathology examination according to Bolon et al, 2013). GNX exposures (AUC0-24h) at the LOAEL for juvenile developmental toxicity were 1050/961, 524/1500, and 510/2020 ng.h/mL in males/females on PNDs 7, 36, and 91, respectively.

Methods

Doses:	0 (vehicle), 20, 45, 90/150/250/500 mg/kg/day
Frequency of dosing:	BID
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	hypromellose, polyvinyl alcohol, sodium lauryl sulfate, methylparaben, propylparaben, sodium benzoate, (b) (4) citric acid, sodium citrate (b) (4), sucralose, (b) (4) simethicone emulsion, purified water, cherry artificial flavor (b) (4)
Species/Strain:	Rat/ Sprague Dawley (Crl:CD(SD))
Number/Sex/Group:	48-51/sex/grp + 33-34/sex/grp TK (see Table 1 below)
Age:	PND 7 to PND 91

Juvenile SD rats were administered GNX twice daily (0 (vehicle), 20, 45, 90/150/250/500 mg/kg/day) by oral gavage for 12 weeks, from PND 7 through PND 91, followed by a 28-day recovery period (Table 1). TK assessments were conducted on PND 7, 36, and 91. A total of 48 to 51/sex/group were included in the main study: 11-12/sex/group were necropsied at the end of the dosing period (PND 91); 7-8/sex/group were necropsied after the recovery period (PND 119); and 19-20/sex/group were selected for the reproductive phase and were necropsied following the completion of the breeding period (males; PND 137 to PND 141) or on GD 15 or earlier if presumed pregnant. A satellite group of 33-34/sex/group was included for TK on PND 7, while 18 main study rats/sex/group were used for TK on PND 36 and PND 91. From the main study, 12/sex/group in the primary necropsy and the 8/sex/group in the recovery necropsy were assigned to Subset A for behavioral evaluations near the end of dosing. 20/sex/group were assigned to Subset B and used for behavioral evaluations after recovery followed by reproductive assessments. Animals from this subset were also utilized for PND 36 and PND 91 TK. Endpoints evaluated included clinical signs and sedation observations, BW, food consumption, tibial lengths, estrous cycles, reproductive performance, developmental landmarks, neurobehavior, ophthalmology, TK parameters, clinical pathology, gross necropsy, reproductive and sperm parameters, organ weights, bone densitometry, and histopathologic examinations.

Table 1. Experimental design

Group Number	Test Article	Total Daily Dosage Level[a,b] (mg/kg/day)	BID Dosage Level[a] (mg/kg/dose)	Dose Concentration (mg/mL/dose)[a]	Dose Volume (mL/kg/dose)	Number of Animals[c]	
						Males	Females
1	Vehicle	0	0	0	5	48	50
2	Ganaxolone	20	10	2	5	48	48
3	Ganaxolone	45	22.5	4.5	5	48	49
4	Ganaxolone	90/150/ 250/500[d]	45/75/ 125/250[d]	9/15/ 25/50[d]	5	51	50

[a] Animals were dosed twice daily (BID) (12 hours ± 30 minutes apart based on the first dose time for each group by sex) at a volume of 5 mL/kg/dose.
[b] No correction factor was used at the Testing Facility.
[c] 18 animals/sex/group were utilized for PND 36 and PND 91 toxicokinetic collections. Up to 12 animals/sex/group were necropsied following the end of the dosing period on PND 92. Up to 8 animals/sex/group were necropsied following the completion of the recovery period on PND 119. Up to 20 females/group were necropsied on Gestation Day 15 or earlier if presumed pregnant, and 20 males/group were necropsied following the completion of the breeding period. The remaining animals (up to 8/sex/group) were euthanized on PND 21. All pups in the litter were assigned to the same dose group.
[d] Group 4 was dosed at 90 mg/kg/day during PND 7–27, at 150 mg/kg/day during PND 28–31, at 250 mg/kg/day during PND 32–35, and at 500 mg/kg/day during PND 36–91.

Group Number	Test Article	Total Daily Dosage Level[a,b] (mg/kg/day)	BID Dosage Level[a] (mg/kg/dose)	Dose Concentration (mg/mL/dose)[a]	Dose Volume (mL/kg/dose)	Number of Animals[c]	
						Males	Females
1	Vehicle	0	0	0	5	3	3
2	Ganaxolone	20	10	2	5	33	33
3	Ganaxolone	45	22.5	4.5	5	34	33
4	Ganaxolone	90	45	9	5	33	34

[a] Animals were dosed twice (BID) (12 hours ± 30 minutes apart based on the first dose time for each group by sex) on PND 7 at a volume of 5 mL/kg/dose. Blood was collected from each animal for TK assessment using a terminal bleeding scheme over a 24 hour period post the first daily dose. All animals were terminated without evaluation following completion of TK bleeds.
[b] No correction factor was used at the Testing Facility.
[c] All pups in the litter were assigned to the same dose group.

Dose selection was based on the results of a dose range-finding study (00398513) in which GNX (0, 15, 30, or 60 mg/kg/dose) was orally administered BID (0, 30, 60, or 120 mg/kg/day) from PND 7 to PND 28. Deaths (5 males and 4 females found dead between PND 7–16) and clinical signs of hypoactivity and shallow respiration were seen at the HD during the first week of dosing while only minor clinical signs and no changes in body weights were seen at the MD. It was shown that exposures had decreased significantly by PNDs 21 and 28. At the NOAEL (60 mg/kg/day), M/F AUC0-24h values declined from 7080/6360 ng*h/mL on PND 7 to 1066/1514 ng.h/mL on PND 28. Therefore, starting on PND 28 the HD in the current study was increased in a stepwise manner in an effort to compensate for the anticipated decrease in exposure. Based on previous rat PK data indicating that because of limited absorption, raising doses did not lead to proportional increases in exposures, the LD and MD were not escalated in order to avoid a ceiling effect.

Observations and Results

Mortality

Drug-related deaths were noted in the MD (1 female) and HD groups (6 males and 4 females) and occurred between PNDs 7 and 9 except for 1 HD male that was found dead on PND 14. Sedation was noted prior to death in these animals; 2 HD males were noted with sedation grade 3 (severe).

Clinical Signs

Sedative effects were noted with dose-dependent severity and incidence at all doses. Severe sedation (grade 3) was noted infrequently in the HD group at approximately 2 hours following the first dose administration. Sedation decreased in frequency following weaning on PND 21; however, increased incidences of hypoactivity at 1–2 hours following the first and/or second daily doses were noted throughout the dosing period at the MD and HD. In addition, single or infrequent instances of a cool body and/or extremities, shallow respiration, pale body, and/or prostrate posture were noted in all drug-treated groups at the daily examinations and/or 1–2 hours following dose administration.

Body Weights

BW gain was decreased at the MD and HD at different intervals periodically throughout the dosing period (approximately corresponding to dose increases at the HD); however, there were no SS differences among groups in overall BW gain (Table 2). There were no SS BW differences in males or females at the end of the dosing period (Figure 1).

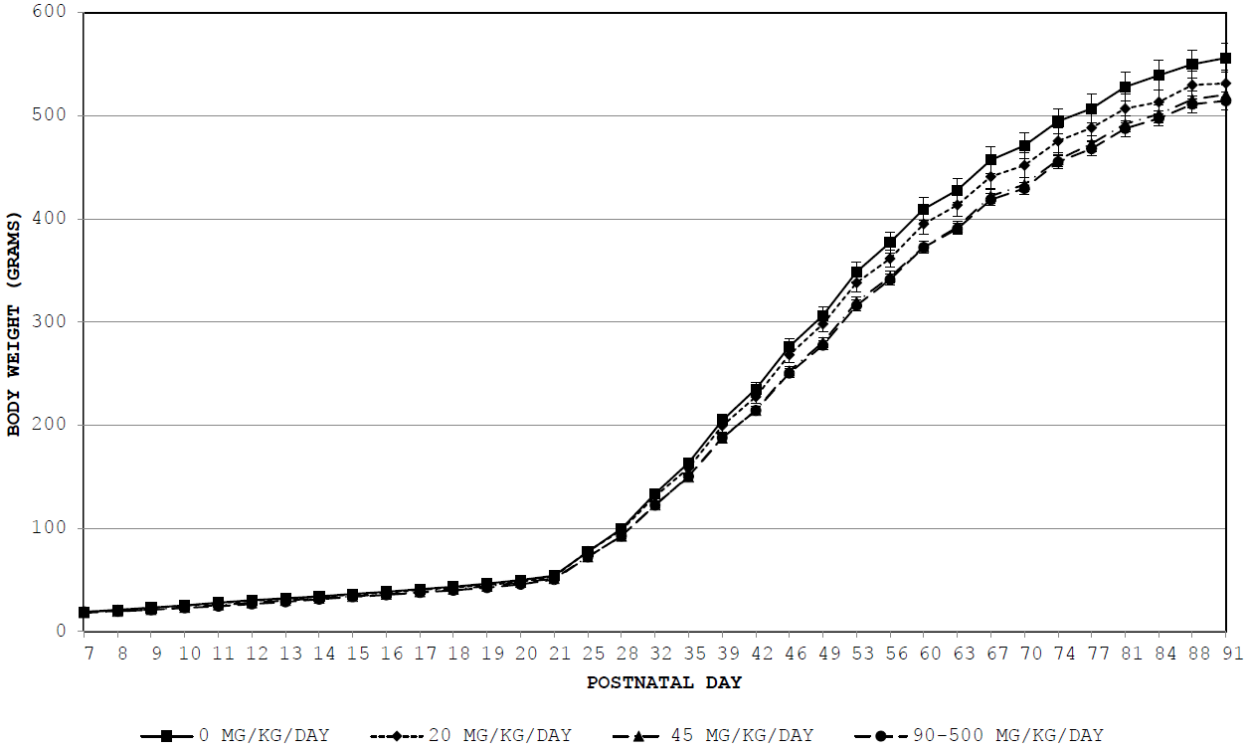
Table 2 Body weight changes (gm)

	MALES			
GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
PND 7- 91				
MEAN	537.3	513.1	502.5	495.7
S.D.	48.37	44.01	32.58	31.03
N	12	12	12	13

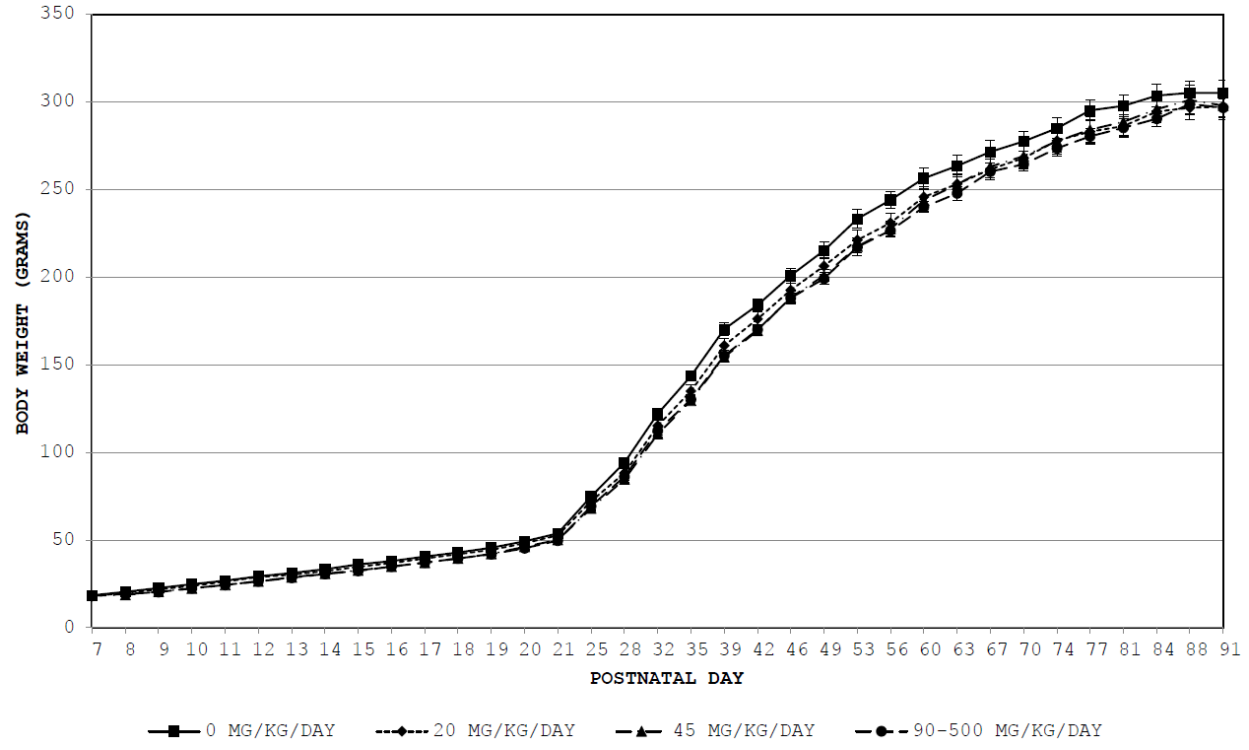
	FEMALES			
GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
PND 7- 91				
MEAN	286.7	279.7	279.7	278.8
S.D.	27.93	21.52	28.35	18.68
N	13	12	12	12

Figure 1. Body weights (gm)

Males



Females



Food Consumption

There were no effects on food consumption.

Bone growth

Tibial lengths were decreased dose-dependently (NS) in males and females at the end of the treatment period (Table 3). After the recovery period, there were no differences among groups in males, but dose-dependent differences (NS) remained in females.

Table 3. Tibial length

PND 91

				MALES
GROUP:	1	2	3	4
NUMBER OF ANIMALS TESTED	12	12	12	13
TIBIAL LENGTH MM				
MEAN	46.73	45.93	45.95	45.46
S.D.	2.135	1.608	1.928	2.238
N	12	12	12	13

				FEMALES
GROUP:	1	2	3	4
NUMBER OF ANIMALS TESTED	13	12	12	12
TIBIAL LENGTH MM				
MEAN	41.52	40.76	40.02	39.89
S.D.	1.415	1.976	1.632	1.721
N	13	12	12	12

1- 0 MG/KG/DAY 2- 20 MG/KG/DAY 3- 45 MG/KG/DAY 4-90-500 MG/KG/DAY
None significantly different from control group
DATA PRESENTED ARE NUMBER OF ANIMALS EXHIBITING RESPONSE, UNLESS OTHERWISE NOTED
PND= POSTNATAL DAY

PND 119

				FEMALES
GROUP:	1	2	3	4
NUMBER OF ANIMALS TESTED	11	11	11	11
TIBIAL LENGTH MM				
MEAN	44.04	42.96	42.59	42.18
S.D.	1.891	1.464	2.512	2.105
N	11	11	11	11

1- 0 MG/KG/DAY 2- 20 MG/KG/DAY 3- 45 MG/KG/DAY 4-90-500 MG/KG/DAY
None significantly different from control group
DATA PRESENTED ARE NUMBER OF ANIMALS EXHIBITING RESPONSE, UNLESS OTHERWISE NOTED
PND= POSTNATAL DAY

Sexual maturation

Age of attainment of vaginal patency was dose-dependently increased (SS) at all doses, reaching an approximately 4-day delay in the MD and HD groups (Table 4). BWs at the age of attainment were increased (4.2%, 8.1%, and 9.9% compared to C), indicating that the delay was not attributable to a growth delay. In males, age of attainment of balanopreputial separation and body weights at the age of attainment were unaffected by treatment.

Table 4. Sexual maturation

MALES
BALANOPREPUTIAL SEPARATION

GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
BALANOPREPUTIAL SEPARATION (PND)				
MEAN	45.5	45.5	46.2	46.2
S.D.	2.10	1.81	2.48	2.45
N	12	12	12	13
BODY WEIGHT (GRAMS)				
MEAN	268.6	260.8	252.3	250.8
% DIFFERENCE		-2.9	-6.1	-6.6
S.D.	24.12	17.76	20.79	22.76
N	12	12	12	13

FEMALES
VAGINAL PATENCY

GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
VAGINAL PATENCY (PND)				
MEAN	32.4	34.3*	36.2**	36.1**
S.D.	1.67	0.98	2.43	2.19
N	13	12	12	12
BODY WEIGHT (GRAMS)				
MEAN	125.2	130.5	135.3	137.6
% DIFFERENCE		4.2	8.1	9.9
S.D.	11.87	8.89	10.88	19.33
N	13	12	12	12

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test
N= NUMBER OF LITTERS, MEANS EXPRESSED AS MEAN OF INDIVIDUAL LITTER MEANS
PND= POSTNATAL DAY

Neurobehavioral assessment

Locomotor activity

Locomotor activity (total) appeared to be dose-dependently increased (particularly during the first interval and in the combined intervals) in treated males at the end of treatment (Subset A), and a similar trend was seen after the recovery period (Subset B); however, SS was not reached at either time (Table 5).

Table 5. Locomotor activity

Male - PND 83

Total

Group	Statistics	Interval No. 1	2	3	4	5	6	Combined Intervals
1 - Vehicle Control	LSMean	1446.70	609.40	289.50	56.00	60.40	90.60	425.43
0 mg/kg/day	SELSM	74.869	76.386	62.879	14.938	15.383	31.777	27.980
	N	20	20	20	20	20	20	
2 - ganaxolone	LSMean	1493.55	776.35	378.15	146.80	89.20	76.25	493.38
20 mg/kg/day	SELSM	61.101	68.653	68.139	41.151	38.014	34.755	33.989
	N	20	20	20	20	20	20	
3 - ganaxolone	LSMean	1570.37	682.21	223.89	102.32	206.47	227.00	502.04
45 mg/kg/day	SELSM	100.827	80.166	58.963	34.544	67.904	81.678	40.047
	N	19	19	19	19	19	19	
4 - ganaxolone	LSMean	1586.21	756.79	369.79	144.68	146.63	105.42	518.25
90-500 mg/kg/day	SELSM	64.589	70.581	78.036	45.171	60.576	30.840	35.966
	N	19	19	19	19	19	19	

No significant difference between Group 1 and any of the other groups was detected at the 5% level.

Male - PND 110

Total

Group	Statistics	Interval No. 1	2	3	4	5	6	Combined Intervals
1 - Vehicle Control	LSMean	1509.75	745.60	229.75	62.75	61.80	96.00	450.94
0 mg/kg/day	SELSM	86.213	92.345	39.214	22.091	22.447	37.854	29.613
	N	20	20	20	20	20	20	
2 - ganaxolone	LSMean	1490.40	669.50	405.45	137.45	92.55	81.35	479.45
20 mg/kg/day	SELSM	59.751	72.251	70.612	35.889	30.498	28.721	30.820
	N	20	20	20	20	20	20	
3 - ganaxolone	LSMean	1520.90	727.85	235.30	95.50	95.85	178.90	475.72
45 mg/kg/day	SELSM	64.173	63.747	73.302	31.117	29.614	46.602	35.007
	N	20	20	20	20	20	20	
4 - ganaxolone	LSMean	1624.95	838.05	320.40	128.90	47.65	111.00	511.82
90-500 mg/kg/day	SELSM	79.898	90.887	54.738	38.334	22.766	34.458	34.527
	N	20	20	20	20	20	20	

No significant difference between Group 1 and any of the other groups was detected at the 5% level.

Auditory startle habituation

The auditory startle response (maximum startle) appeared to be increased (generally dose-dependently) in (Subset A) females at the end of treatment. Although SS was not reached (Table 6), it is unusual to see no evidence of habituation across trial intervals in controls. No effects were apparent in males (Subsets A and B) or in recovery females (Subset B).

Table 6. Auditory startle response

Female - PND 82

PEAK

Group	Statistics	Trial Interval 1	2	3	4	5	Combined Trials
1 - Vehicle Control	LSMean	840.52	886.45	862.15	838.16	898.38	865.13
0 mg/kg/day	SELSM	105.779	105.779	105.779	105.779	105.779	88.233
	N	20	20	20	20	20	
2 - ganaxolone	LSMean	1139.95	957.83	929.78	824.97	886.14	947.73
20 mg/kg/day	SELSM	105.779	105.779	105.779	105.779	105.779	88.233
	N	20	20	20	20	20	
3 - ganaxolone	LSMean	1267.78	1113.32	1065.63	1007.80	1022.79	1095.47
45 mg/kg/day	SELSM	105.779	105.779	105.779	105.779	105.779	88.233
	N	20	20	20	20	20	
4 - ganaxolone	LSMean	1176.90	1081.77	1085.39	1177.77	1091.82	1122.73
90-500 mg/kg/day	SELSM	105.779	105.779	105.779	105.779	105.779	88.233
	N	20	20	20	20	20	

No significant difference between Group 1 and any of the other groups was detected at the 5% level.

Learning and memory (Biel maze)

When testing was conducted at the end of the dosing period (Subset A) or after the recovery period (Subset B), there were no apparent effects on Biel maze performance. An isolated increase (SS) in errors on the first trial in Path B was seen in HD males on PND 111 (Table 7), but in the absence of a dose-response, a corresponding increase in escape latency, or any other SS group differences, this could not be considered a clear drug-related effect.

Table 7. Biel maze performance

Male - PND 111 - Learning Path B

No. of Errors

Group	Statistics	Trial Interval 9	10	11	12	13	14	Combined Trials
1 - Vehicle Control	LSMean	11.60	6.45	7.45	2.85	3.85	2.65	5.81
0 mg/kg/day	SELSM	1.125	1.188	1.508	1.011	1.166	0.791	0.559
	N	20	20	20	20	20	20	
2 - ganaxolone	LSMean	14.95	5.55	3.60	1.50	3.25	1.45	5.05
20 mg/kg/day	SELSM	1.320	1.031	1.179	0.492	1.088	0.463	0.505
	N	20	20	20	20	20	20	
3 - ganaxolone	LSMean	13.85	8.10	5.50	1.70	1.00	0.75	5.15
45 mg/kg/day	SELSM	1.765	1.471	1.414	0.533	0.391	0.281	0.582
	N	20	20	20	20	20	20	
4 - ganaxolone	LSMean	16.26 A	10.14	5.73	3.37	3.69	1.36	6.76
90-500 mg/kg/day	SELSM	1.132	1.842	1.797	1.333	1.237	0.328	0.709
	N	19	19	19	19	19	19	

Significantly different from Group 1: A - p-value <= 0.05 (Dunnett's test)

Reproductive Performance

When reproductive subset (Subset B) animals were mated on PND 119, there were no clearly drug-related effects on male or female reproductive parameters (Table 8).

Table 8.

Results of Reproductive Performance

Parameter	Dose Level (mg/kg/day)				(b) (4) HC[a]
	0	20	45	90–500	Mean (Range)
Male Mating Index (%)	100.0	90.0	84.2	100.0	96.8 (83.3–100.0)
Female Mating Index (%)	100.0	100.0	100.0	100.0	98.1 (83.3–100.0)
Male Fertility Index (%)	89.5	90.0	78.9	85.0	91.9 (60.0–100.0)
Female Fertility Index (%)	89.5	85.0	94.7	85.0	93.4 (60.0–100.0)
Male Copulation Index (%)	89.5	88.9	93.8	85.0	95.1 (70.0–100.0)
Female Conception Index (%)	89.5	85.0	94.7	85.0	95.3 (65.2–100.0)
Estrous Cycle Length (days)	4.4	4.2	5.3	5.1	4.3 (3.2–7.6)
Pre-Coital Interval	2.1	1.9	1.8	2.8	2.9 (1.8–4.8)

[a] (b) (4) historical control data.

Ovarian and Uterine Examinations

In pregnant females Subset B, there were no drug-related effects on mean numbers of corpora lutea, implantation sites, or intrauterine survival (postimplantation loss and viable fetuses).

Sperm Analysis

There was a dose-related decrease (SS at MD and HD for left, HD for right testis) in testis weights in treated males from Subset B (Table 9). Testicular sperm concentration and sperm production rate were slightly decreased (NS), but not in dose-related manner.

Table 9.

SUMMARY OF SPERM MOTILITY AND CONCENTRATION

GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
MOTILITY (%)				
MEAN	87.	88.	90.	87.
S.D.	8.1	5.2	5.8	8.1
N	20	20	20	20
CAUDA EPID, LT WEIGHT (GRAMS)				
MEAN	0.3435	0.3302	0.3384	0.3424
S.D.	0.04158	0.02687	0.03142	0.02886
N	20	20	20	20
CAUDA EPID, LT CONCENTRATION (MILLIONS/GRAM)				
MEAN	557.1	637.1	548.8	630.0
S.D.	117.13	125.02	82.20	112.76
N	20	20	20	20
TESTIS, LT WEIGHT (GRAMS)				
MEAN	2.05	2.00	1.92*	1.87**
S.D.	0.117	0.163	0.191	0.176
N	20	20	20	20
TESTIS, LT CONCENTRATION (MILLIONS/GRAM)				
MEAN	83.1	77.3	78.1	78.5
S.D.	14.53	15.89	19.46	10.45
N	20	20	20	20
SPERM PRODUCTION RATE (MILLIONS/GRAM/DAY)				
MEAN	13.6	12.7	12.8	12.9
S.D.	2.38	2.60	3.19	1.71
N	20	20	20	20

MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
* = Significantly different from the control group at 0.05
** = Significantly different from the control group at 0.01

Clinical Pathology

Hematology

Decreases (SS) in absolute monocytes were seen at all doses in treated males. A decrease (16%) was still seen at the HD after the recovery period, but the difference was NS. There were no group differences in coagulation parameters.

Clinical chemistry

At the primary necropsy on PND 92, decreases (SS) in total bilirubin concentrations were noted for all drug-treated males and females compared to C. These differences did not persist to the recovery necropsy (PND 119).

Urinalysis

There were no drug-related changes in urinalysis parameters.

Ophthalmic examinations

When animals were examined near the end of the dosing period (PNDs 87–90), there were no drug-related ophthalmic changes.

Necropsy

Organ weights

At the end of the dosing period (PND 92), drug-related increases (SS) in absolute and relative thyroid and liver weights were seen in HD Subset A females. Microscopically, these appeared to correlate with increased thyroid follicular cell height and decreased colloid area and hepatocellular hypertrophy, respectively, at the end of treatment. No drug-related organ weight changes were seen in the Subset A recovery group.

Following the reproductive assessment in Subset B rats, absolute brain weights were decreased at all doses in both sexes (Table 10). Since relative weights were not SS different and the effect was not seen at the end of the dosing period in Subset A animals or at the end of the 28-day recovery period in Subset A animals this finding was not considered drug-related. However, the group sizes were greater at the Subset B necropsy (20 vs 8/sex/grp), relative weights were not calculated for females because final BW was not available due to pregnancy, and brain weight is generally spared from BW effects, all supporting a drug-related effect.

Table 10. Brain weights - Subset B

MALES - SCHEDULED NECROPSY - RECOVERY PERIOD - SUBSET B

PROJECT NO.: 00398514A PAGE 1

SUMMARY OF ORGAN WEIGHTS AND RELATIVE ORGAN WEIGHTS

	MALES			
GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
FINAL BODY WT (G)				
MEAN	683.	642.	653.	647.
% DIFFERENCE		-6.0	-4.4	-5.3
S.D.	67.6	57.7	48.0	69.8
N	20	20	20	20
BRAIN (G)				
MEAN	2.30	2.19**	2.21**	2.17**
% DIFFERENCE		-4.8	-3.9	-5.7
S.D.	0.109	0.072	0.095	0.083
N	20	20	20	20
BRAIN (G/100 G FINAL BODY WEIGHT)				
MEAN	0.340	0.344	0.339	0.338
S.D.	0.0305	0.0307	0.0265	0.0327
N	20	20	20	20

** = Significantly different from the control group at 0.01 using Dunnett's test

FEMALES - SCHEDULED NECROPSY - RECOVERY PERIOD - SUBSET B

PROJECT NO.: 00398514D PAGE 1

SUMMARY OF ORGAN WTS. AND ORGAN WTS. RELATIVE TO BRAIN WTS.

	FEMALES			
GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
BRAIN (G)				
MEAN	2.16	2.06**	2.04**	2.02**
% DIFFERENCE		-4.6	-5.6	-6.5
S.D.	0.095	0.104	0.094	0.099
N	19	18	16	20

** = Significantly different from the control group at 0.01 using Dunnett's test

Dose-related decreases (SS at MD and/or HD) in epididymis and testis weights were seen in Subset B males (Tables 11 and 12).

Table 11. Epididymis weights – Subset B

MALES - SCHEDULED NECROPSY - RECOVERY PERIOD - SUBSET B

PROJECT NO.: 00398514A PAGE 3

SUMMARY OF ORGAN WEIGHTS AND RELATIVE ORGAN WEIGHTS

	MALES			
GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
EPIDIDYMIS, LT (G/100 G FINAL BODY WEIGHT)				
MEAN	0.111	0.114	0.112	0.117
S.D.	0.0206	0.0220	0.0137	0.0182
N	20	20	20	20
EPIDIDYMIS, LT (G/100 G BRAIN)				
MEAN	32.607	33.054	33.071	34.758
S.D.	5.4238	4.9507	3.8525	5.1082
N	20	20	20	20
EPIDIDYMIS, RT (G)				
MEAN	0.88	0.85	0.84	0.82**
% DIFFERENCE		-3.4	-4.5	-6.8
S.D.	0.062	0.039	0.053	0.068
N	20	20	20	20
EPIDIDYMIS, RT (G/100 G FINAL BODY WEIGHT)				
MEAN	0.129	0.134	0.129	0.128
S.D.	0.0115	0.0105	0.0101	0.0154
N	20	20	20	20
EPIDIDYMIS, RT (G/100 G BRAIN)				
MEAN	38.075	38.954	37.971	37.837
S.D.	2.7684	1.7029	2.8627	3.4165
N	20	20	20	20

** = Significantly different from the control group at 0.01 using Dunnett's test

Table 12. Testis weights – Subset B

MALES - SCHEDULED NECROPSY - RECOVERY PERIOD - SUBSET B

PROJECT NO.: 00398514A PAGE 4

SUMMARY OF ORGAN WEIGHTS AND RELATIVE ORGAN WEIGHTS

	MALES			
GROUP:	0 MG/KG/DAY	20 MG/KG/DAY	45 MG/KG/DAY	90-500 MG/KG/DAY
TESTIS, LT (G)				
MEAN	2.05	2.00	1.92*	1.87**
% DIFFERENCE		-2.4	-6.3	-8.8
S.D.	0.117	0.163	0.191	0.176
N	20	20	20	20
TESTIS, LT (G/100 G FINAL BODY WEIGHT)				
MEAN	0.302	0.313	0.295	0.290
S.D.	0.0339	0.0331	0.0369	0.0319
N	20	20	20	20
TESTIS, LT (G/100 G BRAIN)				
MEAN	89.024	91.008	87.247	85.980
S.D.	5.8564	6.1442	10.6472	7.9856
N	20	20	20	20
TESTIS, RT (G)				
MEAN	2.04	2.01	1.93	1.88**
% DIFFERENCE		-1.5	-5.4	-7.8
S.D.	0.118	0.165	0.183	0.167
N	20	20	20	20
TESTIS, RT (G/100 G FINAL BODY WEIGHT)				
MEAN	0.301	0.315	0.297	0.293
S.D.	0.0306	0.0337	0.0358	0.0336
N	20	20	20	20
TESTIS, RT (G/100 G BRAIN)				
MEAN	88.836	91.654	87.636	86.781
S.D.	5.6694	6.3500	10.2644	7.8136
N	20	20	20	20

** = Significantly different from the control group at 0.01 using Dunnett's test

Macroscopic

There were no macroscopic findings considered drug-related at the end of the dosing period or after the recovery period.

Histopathology

At the primary necropsy (PND 92), dose-related increased thyroid follicular cell height and decreased colloid area were seen at all doses in both sexes. These corresponded to increased thyroid gland weights in HD females and were considered non-adverse findings. Hepatocellular hypertrophy was seen in HD males and females and correlated to increased liver weights in this group. At the recovery necropsy (PND 119), thyroid changes were still present in all dose groups. There were no drug-related microscopic changes in the male reproductive tract (right testis, right epididymis, and left epididymis) of C or HD males in Subset B (stage aware testicular microscopic examination performed) and no brain abnormalities at either necropsy (expanded histopathology examination conducted according to Bolon et al, Toxicologic Path, 41:1028-1048, 2013).

Bone Evaluations

No drug-related effects on bone measurements or densitometry parameters at the femur (distal metaphysis and diaphysis) and lumbar vertebral bodies were observed at the end of treatment or following the recovery period.

Toxicokinetics

TK parameters for GNX at on PNDs 7, 36, and 91 are shown in Table 13. Despite dose escalation in the HD group, the effect of autoinduction could not be compensated for, presumably due to limited absorption; exposures declined by about 1/3 between PND 7 and PND 36 at the HD. On PNDs 36 and 91 exposures increased considerably less than dose-proportionally; a 25-fold increase in dose resulted in a 3.3/2.2-fold increase in male/female AUC on PND 91.

Table 13. Ganaxolone TK Parameters on PND 7, 36, and 91

Parameters		Group					
		2		3		4	
		M	F	M	F	M	F
PND 7		**20 mg/kg/day**		**45 mg/kg/day**		**90 mg/kg/day**	
t_{last}	h	24	24	24	24	24	24
t_{max1}	h	1	2	2	2	2	12
C_{max1}	ng/mL	155	119	770	674	726	1480
C_{max1}/Dose*	kg·ng/mL/mg	15.5	11.9	34.2	30.0	16.1	32.9
t_{max2}	h	14	13	14	14	16	13
C_{max2}	ng/mL	146	140	620	518	844	605
C_{max2}/Dose*	kg·ng/mL/mg	14.6	14.0	27.6	23.0	18.8	13.4
AUC_{last}	h·ng/mL	1050	961	5520	3930	9630	11400
AUC_{last}/Dose^	h·kg·ng/mL/mg	52.4	48.1	123	87.3	107	127
$AUC_{0\text{-}24h}$	h·ng/mL	1050	961	5520	3930	9630	11400
$AUC_{0\text{-}24h}$/Dose^	h·kg·ng/mL/mg	52.4	48.1	123	87.3	107	127
PND 36		**20 mg/kg/day**		**45 mg/kg/day**		**500 mg/kg/day**	
t_{last}	h	20	24	24	24	24	24
t_{max1}	h	2	1	1	1	4	2
C_{max1}	ng/mL	65.3	185	131	218	227	180
C_{max1}/Dose*	kg·ng/mL/mg	6.53	18.5	5.84	9.69	0.907	0.719
t_{max2}	h	14	14	14	14	16	14
C_{max2}	ng/mL	102	209	213	485	326	354
C_{max2}/Dose*	kg·ng/mL/mg	10.2	20.9	9.48	21.6	1.31	1.41
AUC_{last}	h·ng/mL	518	1500	1110	2370	3570	3630
AUC_{last}/Dose^	h·kg·ng/mL/mg	25.9	74.9	24.7	52.7	7.13	7.26
$AUC_{0\text{-}24h}$	h·ng/mL	524	1500	1110	2370	3570	3630
$AUC_{0\text{-}24h}$/Dose^	h·kg·ng/mL/mg	26.2	74.9	24.7	52.7	7.13	7.26
PND 91		**20 mg/kg/day**		**45 mg/kg/day**		**500 mg/kg/day**	
t_{last}	h	24	24	24	24	24	24
t_{max1}	h	2	1	1	1	2	2
C_{max1}	ng/mL	76.9	324	55.7	348	103	286
C_{max1}/Dose*	kg·ng/mL/mg	7.69	32.4	2.48	15.5	0.412	1.15
t_{max2}	h	13	13	13	14	16	16
C_{max2}	ng/mL	115	267	108	420	129	358
C_{max2}/Dose*	kg·ng/mL/mg	11.5	26.7	4.81	18.7	0.517	1.43
AUC_{last}	h·ng/mL	510	2020	723	3120	1660	4420
AUC_{last}/Dose^	h·kg·ng/mL/mg	25.5	101	16.1	69.4	3.32	8.85
$AUC_{0\text{-}24h}$	h·ng/mL	510	2020	723	3120	1660	4420
$AUC_{0\text{-}24h}$/Dose^	h·kg·ng/mL/mg	25.5	101	16.1	69.4	3.32	8.85

*: Cmax values were divided by the BID dose level (10, 22.5 and 45 or 250 mg/kg/dose for Group 2, 3 and 4, respectively);

^: AUC values were divided by the daily dose level.

Study title: An Oral (Gavage) Acute Neuropathology Study of Ganaxolone in Neonatal Sprague Dawley Rats

Study no.:	00398515
Study report location:	4.2.3.5.4
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	10 Nov 2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	17JM-052/ 99%

Key Study Findings

Neonatal SD rats (10/sex/grp) were administered GNX (0 (vehicle), 10, 22.5, or 45 mg/kg/dose) by oral gavage BID (12 hrs between doses) on PND 7. A positive control group was administered a single dose of MK-801 (1 mg/kg IP) on PND 7. All animals survived to the scheduled necropsy on PND 8; no drug-related clinical observations were noted in any group. Decreased BW gain (or loss) was seen in the MD and HD GNX and MK-801 groups during PND 7–8, resulting in decreased BWs (7 and 16%, respectively) compared to C on PND 8. Dose-related increases in the incidence and/or severity of neuronal necrosis (visible by H&E staining and confirmed by Fluoro-Jade B staining), most notably in the retrosplenial cortex, thalamic nuclei, and dorsal subiculum of the hippocampus, was seen at all GNX doses in both sexes. The necrosis in the GNX HD group generally had the same severity and distribution pattern of neuronal necrosis as that associated with the MK-801 positive control group.

Methods

Doses:	0 (vehicle), 10, 45, 90 mg/kg/day
Frequency of dosing:	BID
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	hypromellose, polyvinyl alcohol, sodium lauryl sulfate, methylparaben, propylparaben, sodium benzoate, (b) (4) citric acid, sodium citrate (b) (4), sucralose, (b) (4) simethicone emulsion, purified water, cherry artificial flavor (b) (4)
Species/Strain:	Rat/ Crl:CD(SD)
Number/Sex/Group:	10/sex/grp/
Age:	Postnatal Day 7

Table 1.

Experimental Design

Group No.	Test Article	Total Dosage Level[a] (mg/kg)	Dose Level (mg/kg/dose)	Dose Concentration[b] (mg/mL)	Dose Volume (mL/kg/dose)	Number of Animals[c]	
						Male	Female
1	Vehicle	0	0	0	5	10	10
2	Ganaxolone	20	10	2	5	10	10
3	Ganaxolone	45	22.5	4.5	5	10	10
4	Ganaxolone	90	45	9	5	10	10
5	MK-801[d]	1.0	NA	0.2	5	10	10

NA – not applicable.

[a] Animals in Groups 1–4 were dosed twice (12 hours ± 30 minutes apart based on the first dose time for each group/sex) on PND 7.

[b] Not corrected for salt, purity and water content at the Testing Facility.

[c] 10 litters were used for this study, with 1/sex/litter assigned to each group.

[d] Positive control article group.

Neonatal SD rats (10/sex/grp) were administered GNX (0 (vehicle), 10, 22.5, or 45 mg/kg) by oral gavage BID (12 hrs between doses) on PND 7 (Table 1). A positive control group was administered a single IP dose of MK-801 (1 mg/kg) on PND 7. On PND 8, pups were sacrificed and CNS tissues (brain, eyes with optic nerve, sciatic nerve, and cervical thoracic, and lumbar spinal cord) were collected from all pups, immersion fixed, paraffin embedded, sectioned, and stained with H&E (for routine histopathology), luxol fast blue-cresyl violet (to evaluate myelin and neuronal cellular architecture), or immunostained with Fluoro-Jade B (FJB) or Caspase 9 (to detect neuronal degeneration and necrosis).

Dose selection was based on the results of an acute PK study in PND 7 pups (00398512) in which doses of 200 (100 mg/kg/dose BID) and 300 mg/kg/day (150 mg/kg/dose BID), resulted in mortality while there were no clinical signs at a dose of 100 mg/kg (50 mg/kg/dose BID). The positive control (MK-801) has been shown to induce neurotoxicity following a single dose at 1 mg/kg IP (Kuroda et al, 2015; Ikonomidou et al, 1999).

Observations and Results

Mortality

There was no unscheduled mortality.

Clinical Signs

There were no clinical observations

Body Weights

Decreased BW gain (or loss) was seen at the MD and HD during PND 7–8, resulting in decreased (SS) BWs on PND 8 (7 and 16% compared to C). BW losses similar to that noted at the HD were seen in the positive control and resulted in a similar BW decrease (15%).

Necropsy

Macroscopic

There were no drug-related gross necropsy observations.

Histopathology

GNX-related neuronal necrosis was seen in all dose groups, with a dose-dependent increase in incidence and/or severity (Table 1). In the GNX-treated animals, neuronal necrosis was present in the cerebral cortex (frontal, cingulate, retrosplenial, piriform, and parietal cortices), thalamus, hippocampus (subiculum, rarely CA1), midbrain (superior and inferior colliculi and medial geniculate), and/or basal ganglia. On H&E, necrosis was characterized by neurons with pyknotic to karyorrhectic nuclei, dense eosinophilic cytoplasm, and scattered nuclear debris. In FJB stained slides, the necrosis appeared as clusters of fluorescent-positive cells and cellular debris. Neuronal necrosis seen in the positive control group confirmed the validity of the study procedure. The incidence and severity and regions of the brain affected by neuronal necrosis were generally similar between the HD GNX and MK-801 groups.

Table 1. Incidence of Selected Histopathologic Findings

Sex	**Males**					**Females**				
Test Article	**Control Vehicle**	**Ganaxolone**			**MK-801**	**Control vehicle**	**Ganaxolone**			**MK-801**
Dose Level (mg/kg) [b]	**0**	**20**	**45**	**90**	**1.0**	**0**	**20**	**45**	**90**	**1.0**
Brain [a,d]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	1	6	10	10	0	1	5	9	10
Minimal	0	1	4	2	1	0	1	4	0	0
Mild	0	0	1	2	4	0	0	1	4	5
Moderate	0	0	1	6	5	0	0	0	5	5
Basal Ganglia[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	0	0	3	3	0	0	0	4	4
Minimal	0	0	0	2	3	0	0	0	4	2
Mild	0	0	0	1	0	0	0	0	0	2
Cerebral Cortex[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	0	3	10	9	0	2	4	9	10
Minimal	0	0	2	4	5	0	1	4	1	1
Mild	0	0	1	4	2	0	0	0	5	4
Moderate	0	0	0	2	2	0	1	0	3	5
Hippocampus[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	0	1	6	3	0	0	1	4	6
Minimal	0	0	1	1	1	0	0	1	1	2
Mild	0	0	0	3	1	0	0	0	1	4
Moderate	0	0	0	2	1	0	0	0	2	0
Hypothalamus[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	0	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	0	0	1	0

Medulla Oblongata[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	0	0	0	1	0	0	0	0	0
Minimal	0	0	0	0	1	0	0	0	0	0
Midbrain[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	0	2	5	6	0	0	0	7	9
Minimal	0	0	2	4	3	0	0	0	5	6
Mild	0	0	0	1	2	0	0	0	2	3
Moderate	0	0	0	0	1	0	0	0	0	0
Pons[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	0	0	0	1	0	0	0	0	0
Minimal	0	0	0	0	1	0	0	0	0	0
Thalamus[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Neuronal Necrosis[c]	0	1	4	8	8	0	0	2	8	10
Minimal	0	1	2	0	0	0	0	1	1	6
Mild	0	0	1	4	5	0	0	1	5	2
Moderate	0	0	1	4	3	0	0	0	2	2

[a] Number of tissues examined from each group.

[b] Animals given vehicle control and ganaxolone (Groups 1–4) were dosed twice daily (BID) (12 hours ± 30 minutes apart based on the first dose time for each group/sex during the first daily dose) at a volume of 5 mL/kg/dose

[c] The diagnoses and severity scores were based on the evaluation of both the H&E- and the FJB-stained slides. Other subanatomic sites were evaluated, but only those subanatomic sites with findings are listed in this table.

[d] The brain tissue is the overall score based on the evaluation of the different subanatomic sites of the brain.

10 Integrated Summary and Safety Evaluation

Ganaxolone (GNX, 3α-hydroxy-3β-methyl-5α-pregnan-20-one) is the 3β-methyl analog of the neurosteroid allopregnanolone, a metabolite of progesterone. GNX exhibited an in vitro pharmacological profile consistent with activity as a positive allosteric modulator of the GABAA receptor and potent anticonvulsant activity in several animal models of epilepsy (Carter et al, JPET 280:1284–1295, 1997). Based on binding and functional assay results, GNX did not appear to have nuclear hormone activity. However, alterations in estrous cyclicity were seen in the rat fertility study and there were effects on reproductive system development in the juvenile rat toxicity study.

The primary metabolite observed during in vitro incubations across all species was 16α-hydroxyganaxolone; however, recent in vivo metabolism studies identified a major human-specific, non-conjugated plasma metabolite, designated M2 (oxy-dehydro-ganaxolone, (b) (4) designation M60b). This metabolite has been synthesized and evaluated for pharmacological activity and genotoxicity. M2 demonstrated no functional activity at GABAA receptors and was negative for mutagenicity in the Ames test; however, it produced a positive response (increased the incidence of aberrant metaphases) in an in vitro chromosome aberration assay. Another metabolite, the hydroxy-ganaxolone sulfate conjugate M47 ((b) (4) designation), was present in pooled human plasma in a similar range as that of M2 in terms of percentage of total radioactivity (percent of metabolite radioactivity from the 0-30 day-pool data and relative exposure to GNX). Given its abundance in plasma and published literature reporting that sulfate conjugates of neuroactive steroids may have pharmacological activity (Harteneck C, Molecules 18:12012-12028, 2013; Ratner et al, Front Endocrinol 10:169, 2019), safety concerns cannot be dismissed. While a hydrophilic conjugate would not be expected to readily cross the BBB to reach the brain, facilitated transport cannot be ruled out according to the clinical pharmacology reviewer.

(b) (4) suspension was developed and replaced earlier cyclodextrin-based formulations in the most recent nonclinical studies. GNX administration resulted in autoinduction in rodents and this and apparent saturable absorption limited the plasma drug exposures that could be achieved in the rat toxicity studies. In addition, GNX-induced sedation was dose-limiting in both rats and dogs. However, effects on hepatic metabolizing enzymes were not observed in dogs; therefore, adequate exposure margins were achieved in the general toxicity studies in this species.

The most common effect observed in the pivotal repeat-dose toxicology studies in adult rats and dogs was dose-related, reversible sedation, an expected pharmacological action of a positive modulator of GABAA receptors. Otherwise, there was little evidence of target organ or systemic toxicity in either species. One death at the HD in the 6-month SD rat study and convulsions in a single animal in the chronic dog study (in Weeks 33 and 36) represented the only significant adverse effects observed. The rat death, on Day 2 of the study, appeared to be secondary to sedation. The convulsions appeared in an apparent outlier animal with plasma levels greater than those in other dogs receiving the same

dose and which were approximately 10-20-fold greater than those in human adults receiving the MRHD of 1800 mg (262 ng/mL and 3000 ng.h/mL at a dose of 2000 mg). Testicular atrophy observed in 1 HD animal in the 1-month dog study was considered possibly drug-related, but no testicular toxicity was seen at the same dose in the 12-month dog study.

Of most concern were the developmental effects observed in the pre- and postnatal, neonatal, and juvenile rat studies. In a mouse embryofetal development (EFD) study, fetal malformations were increased in all drug-treated groups compared to controls. Because there was no clear dose relationship (1, 4(4), 5(4), and 5(3) fetuses (litters) malformed in groups dosed with 0, 50, 175, or 300 mg/kg/day, respectively), these were not considered drug-related in the study report. However, in separate mouse study, plasma levels plateaued at doses ≥125 mg/kg, apparently due to saturation of absorption; therefore, a dose-response may not be expected (maternal plasma drug levels were not determined in the EFD study). In addition, there was a pattern of CNS defects in the EFD study, which lends biological plausibility. GABA has been shown to influence multiple aspects of brain development, including the proliferation, migration, and morphological development of neurons (Antonopoulos et al, Eur J Neurosci 9:291-8, 1997; Wu and Sun, Metab Brain Dis 30: 367–379, 2015). GABAA receptor ligand administration during organogenesis has been reported to disrupt neural tube formation in rats and it is thought that GABAergic activity may contribute to the teratogenic effects of valproic acid, which induces neural tube defects in mice (Briner, BMC Pharmacol 1:2, 2001). Maternal plasma drug exposures (AUC) at the low-effect dose (50 mg/kg/day) for embryofetal developmental toxicity in the mouse were approximately 10-fold lower than that in humans at the MRHD.

In a combination EFD and pre- and postnatal development study in rats, there were no effects on litter parameters or fetal abnormalities at C-section on GD20, but in offspring from dams allowed to litter, BW gain was decreased in HD males during lactation. This growth impairment persisted throughout postweaning study period (PNW 4-19). This was associated with delayed reflex development during the pre-weaning period and a slight delay in attainment of sexual maturation of HD male offspring. Decreased (SS) locomotor activity was seen in HD male offspring when assessed at PNW 5 and in MD and HD males when retested at PNW 11. There were no effects on learning and memory; however, this was assessed by a relatively insensitive test (passive avoidance). In offspring, mating performance was unaffected by treatment. The no-effect dose (10 mg/kg/day) for pre- and postnatal developmental toxicity in rats was associated with maternal plasma drug exposures approximately less than that in human adults at the MRHD.

In the juvenile rat study, drug-related deaths were noted at the MD (1 female) and HD (6 males and 4 females) and generally occurred between PNDs 7–9. For these animals, sedation (grade 3 in 2 animals) was noted prior to death and sedative effects were seen in surviving animals at all doses, primarily during the pre-weaning period. BW gain and BWs were decreased (SS) sporadically at the MD and HD, but there were no SS differences among groups in BW gain over the entire treatment period or on final BWs. Age of attainment of vaginal patency was dose-dependently increased (SS) at all doses,

reaching an approximately 4-day delay in the MD and HD groups. Surprisingly, given the evidence of possible GNX effects on brain development, there were no clearly drug-related effects on neurobehavioral assessments (motor activity, auditory startle response, and learning and memory in Biel maze), during the dosing period or after the recovery period. Apoptotic neurodegeneration induced in rats by other agents that potentiate GABAA receptors has reportedly been associated with persistent learning and memory impairments (Jevtovic-Todorovic et al, J Neurosci 23:876–882, 2003). There were also no drug-related effects on reproductive performance or spermatogenic parameters. In animals sacrificed following the reproductive assessment, absolute brain weights were decreased at all doses in both sexes. Since relative weights were not SS different and the effect was not seen at the end of the dosing period or at the end of the 28-day recovery period in another subset of animals, this finding was not considered drug-related by the sponsor. However, the group sizes were greater in the reproductive subset (20 vs 8/sex/grp), relative weights were not calculated for females because final BW was not available due to pregnancy, and brain weight is generally spared from BW effects. Dose-related decreases (SS at MD and/or HD) in epididymis and testis weights were seen in the reproductive subset males, but there were no apparent drug-related microscopic changes and no microscopic brain abnormalities. GNX exposures (AUC) at the LOAEL for juvenile developmental toxicity were lower than those expected in pediatric patients receiving therapeutic doses.

In an acute neurotoxicity study in which neonatal SD rats (10/sex/grp) were administered single doses of GNX on PND 7, dose-related increases in the incidence and/or severity of neuronal necrosis (visible by H&E staining and confirmed by Fluoro-Jade B staining), most notably in the retrosplenial cortex, thalamic nuclei, and dorsal subiculum of the hippocampus, was seen doses associated with plasma drug concentrations lower than that in pediatric patients receiving therapeutic doses. The necrosis in the GNX HD group generally had the same severity and distribution pattern of neuronal necrosis as that associated with the MK-801 positive control group. This is a known effect of drugs that enhance GABAergic neurotransmission as well as those, like MK-801, that block NMDA receptors. The window of vulnerability to these changes in rats (postnatal days 0-14) coincides with a period of brain development that begins during the third trimester of pregnancy in humans and continues postnatally for an undetermined period of time. Peak susceptibility is thought to coincide with the period of synaptogenesis, which reaches its maximum in humans by 2 years of age, the youngest age for which GNX is indicated (Maksimovic et al, Int J Mol Sci 23:1889, 2022).

Conclusions

The safety assessment is considered adequate given the testing limitations resulting from the pharmacokinetic and pharmacologic properties of GNX. Developmental risks for use in pregnancy and during the neonatal and juvenile periods have been identified which should be described in labeling. The carcinogenic potential of GNX has not been evaluated, but it was previously agreed (Type C meeting minutes dated 01/11/2018) that a 26-week carcinogenicity study in the CB6F1-Tg rasH2 transgenic mouse and a 104-week carcinogenicity study in SD rats could be conducted postmarketing. The toxic

potential of 2 major human-specific metabolites (M2 and M47) has not been fully evaluated; however, it was also previously decided that if clinical efficacy were demonstrated the additional nonclinical safety information needed for any major human metabolites not adequately covered in animals could be submitted post-approval. The application is considered approvable from a pharmacology/toxicology standpoint.

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