CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

212839Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 212839
Submission date: 11/21/2018
Drug: cenobamate
Applicant: SK Life Science, Inc.
Indication: treatment of partial onset seizures in adults

Reviewing Division: Division of Neurology 2

Discussion:
The pharm/tox reviewer and supervisor found the nonclinical information for cenobamate adequate to support approval for the above indication. The primary toxicities were death and CNS related effects.

The embryofetal study in rats showed some possible teratogenic findings at the highest dose tested which provided exposure that was likely lower than the clinical exposure. In addition, deaths at the high dose reduced the adequacy of the study. The primary reviewer and supervisor recommend an additional embryofetal study in the rat to further assess the teratogenic potential.

A 26-week study in transgenic RasH2 mice and a 2-year study in Sprague-Dawley rats were conducted. The executive carcinogenicity assessment committee concluded that the studies were adequate and that there were no drug-related neoplasms in either study.

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that the information is adequate to support approval from a pharm/tox perspective.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

PAUL C BROWN
11/21/2019 08:32:25 AM

DEPARTMENT 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:	212839
Supporting document:	1
Applicant's letter date:	5/29/2018
CDER stamp date:	5/29/2018
Product:	Cenobamate (YKP3089)
Indication:	Partial onset seizures in adult patients
Applicant:	SK Life Sciences
Review Division:	DNP
Reviewer:	Ed Fisher
Supervisor:	Lois Freed
(acting) Division Director:	Nicholas Kozauer
Project Manager:	LaShawn Dianat

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of N212839 are owned by SK Life Sciences or are data for which SK Life Sciences 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 N212839.

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

TABLE OF CONTENTS

1 Executive Summary

1.1 Discussion of Nonclinical Findings

In primary pharmacology studies, cenobamate (CBM; YKP3089; R-enantiomer) was shown to modulate several properties of voltage-gated sodium channels and to preferentially inhibit persistent sodium currents. At higher concentrations it was also a positive allosteric modulator of GABAA ion channel receptors. CBM was shown to be active in various standard animal models of epilepsy thought to be predictive of efficacy against generalized and partial seizures.

In CNS safety pharmacology studies in rats, clinical signs of neurotoxicity (ataxia and decreased locomotor activity, muscle tone, and motor function) were observed at an oral dose of 100 mg/kg (Cmax 14.6 µg/mL, AUC 513 µg*h/mL), and mortality was seen at 300 mg/kg. CBM produced concentration-dependent inhibition of hERG-mediated potassium currents; the IC_{50} was estimated as 1869 µM, which is 11X higher than the clinical steady state Cmax after daily dosing at the MRHD of 400 mg (45.5 µg/mL; 169 µM). In isolated rabbit Purkinje fibers, QT interval shortening and changes in the normal action potential were seen at ≥100 µM (equivalent to 26.8 µg/mL). However, in the pivotal cardiovascular telemetry study in monkeys, there were no hemodynamic or electrophysiological effects following oral administration of doses up to 36 mg/kg (Cmax 31.5 µg/mL, AUC 533 µg*h/mL), and there were no drug-related ECG changes in the toxicology studies in monkeys.

Oral bioavailability was moderate to high in mice, rats, and monkeys, and very low (11%) in dogs. Clearance was low in mice, rats, and monkeys (1-7% of hepatic plasma flow) but high (60%) in dogs. Plasma protein binding was low to moderate (35-70%) in all species tested. With repeated dosing, systemic exposure generally increased dose proportionally, and exposures were generally somewhat higher in female rats and monkeys. In rats, exposures were lower after repeated dosing apparently due to auto-induction as indicated by increased CYP and UGT activities. In a rat tissue distribution study, the kidney had the highest tissue-to-blood ratios followed by the liver. CBM was extensively metabolized and the metabolite pattern was qualitatively similar across species. No major circulating metabolites were observed in humans. After single oral doses of labeled drug, most of the radioactivity (≥94.0% of the dose) was excreted within 168 hours for all nonclinical species, with 50-80% of the dose excreted in urine (88% in humans).

The toxicology package included repeat-dose toxicity (up to 13 weeks in mice, 26 weeks in rats, and 52 weeks in monkeys, in vitro and in vivo genotoxicity, carcinogenicity (26-week in transgenic rasH2 mice, 104-week in rats) and reproductive and developmental toxicity (including juvenile toxicity) studies. TK analyses were included in most of these studies. In all toxicology species, acute neurotoxicity was dose-limiting and resulted in minimal to no safety margins to clinical exposures (see Table 2 under section 5 below). Given this limitation, the studies are considered adequate. No unmonitorable serious toxicity was identified in the general toxicity studies, and there was no evidence of carcinogenic potential. A potential for adverse effects on development was identified in rat and rabbit studies.

In the 3-month mouse toxicity study, oral (gavage) doses ≥ 60 mg/kg/day resulted in mortality and CNS clinical signs (included tremors, ataxia, hypoactivity, myoclonic jerking, and recumbency). The NOAEL (30 mg/kg/day) was associated with a Cmax of approximately 60 µg/mL and AUC of ~440 µg·hr/mL (sexes combined). No other serious toxicity was observed.

In a 28-day toxicity study in rats, mortality was seen at an oral dose of 100 mg/kg/day, and CNS clinical signs (uncoordinated gait, decreased activity, decreased righting reflex) were seen at ≥ 60 mg/kg. In the chronic (26-week) oral rat study, mortality was seen at the HD of 48 mg/kg/day and CNS clinical signs (decreased activity, uncoordinated gait) at doses ≥ 24 mg/kg/day. There were no effects on BW, ophthalmology, hematology, or urinalysis parameters. Microscopic changes in the kidney (males) and liver (males and females) were attributed to drug, but renal changes were male rat specific, and the liver finding (centrilobular hepatocellular hypertrophy) is considered adaptive. The NOAEL (12 mg/kg/day) was associated with Week 26 Cmax and AUC0-t values of 9.3 ug/mL and 113.9 ug.h/mL in males and 17.6 ug/mL and 213.7 ug.h/mL in females, respectively.

In a 14-day monkey study at oral doses up to 60 mg/kg, there was no mortality, but clinical signs observed in both HD animals on Days 1 and 2 included crawling, lying on the cage floor, and clonic convulsions that ranged from slight to moderate in the male and slight to severe in the female. The dose level was subsequently decreased to 40 mg/kg/day starting on Day 3. Clonic convulsions in both animals and crawling in the female persisted on Day 3 at the reduced dose. Nystagmus was observed in the HD female on Days 3 and 4 along with severe hyperextension of the neck on Day 3, which had diminished to a slight hyperextension by Day 4. The female displayed slight tremors on Day 4. By Day 5 clinical signs at the HD consisted mostly of decreased activity, uncoordinated gait, and drowsiness in both animals. Clinical signs at the MD (30 mg/kg) included uncoordinated gait, drowsiness, and a slight decrease in activity throughout the treatment period, in both animals. The only other findings were increased WBC count in the HD male and dose-related increases in liver weights without macroscopic or microscopic changes. The NOAEL was 10 mg/kg.

In a neurotoxicity study carried out to evaluate the convulsions seen in the 14-day monkey toxicity study, animals were dosed orally for 4 days; 2 of 6 animals dosed with 60 mg/kg presented with abnormal, involuntary series of muscle contractions, which would have

been recorded as clonic convulsions according to clinical sign lexicon for the contract lab that conducted the 14-day toxicity study. However, when video recordings were reviewed by a veterinary neurologist these were interpreted as episodes of myoclonus or intentional tremors. Examinations of EEGs showed no epileptic spikes or paroxysmal depolarization shifts during these episodes. The muscle contractions appeared to increase upon stimulation of the animal and upon voluntary movements, and the animals appeared sedated but conscious. An increase in beta waves was noted in 1 of the 2 animals during the first episode of myoclonus. One of the 2 monkeys with myoclonus or intentional tremors was euthanized on Day 4 due to poor and deteriorating conditions. TK analysis showed that this animal had the highest Cmax of all monkeys treated with 60 mg/kg (127 ug/mL vs mean of 96 ug/mL in other monkeys). No spike trains were noted during muscular contractions suggesting that the clinical signs did not result from bursts of uncontrolled electrical activity in the brain and thus were not seizures. Animals dosed with 40 mg/kg presented ataxia, tremors, hypoglycemia, hypothermia, and evidences of sedation but did not have clonic convulsions (as defined in the contract lab's clinical sign lexicon). It was concluded that the HD dose of 60 mg/kg induced sedation, a vestibular syndrome (peripheral or central), and myoclonus or intentional tremors.

In the 28-day toxicity study in monkeys, severe clinical signs (including uncoordinated gait, drowsiness, decreased activity, hyperextension of the neck, ocular nystagmus, tremors, weakness) leading to pre-terminal sacrifice were seen at 36 mg/kg/day and CNS clinical signs were noted at all doses. Decreases in total bilirubin were noted at all doses and were considered drug-related, but there were no histopathological changes. Based on clinical signs and clinical chemistry changes, the LD (4 mg/kg/day) was a LOAEL. This dose was associated with Cmax values of 5.45 µg/mL and 6.47 µg/mL and AUC_{24h} values of 56.8 µg*h/mL and 84.9 µg*h/mL for males and females, respectively.

The 52-week chronic toxicity study in the monkey initially included oral doses up to 27 mg/kg/day, but the severity of clinical signs at the high dose (decreased activity, hunchback, crouching, and incoordination), which resulted in the moribund sacrifice of 1 HD female on Day 7, necessitated dose reduction to 22 mg/kg/day starting on Day 11. These signs were decreased after dose reduction, but incoordination was still noted at the reduced HD. Lymphoid hyperplasia of bone marrow was seen at the end of the treatment period in the MD and HD groups and was still present after the recovery period at the HD. The MD (18 mg/kg) was considered the NOAEL and was associated with Cmax values of 61.8 and 36.9 µg/mL and AUC_{24h} values of 1049 and 542 µg*h/mL in males and females, respectively.

CBM was negative for genotoxicity in in vitro (Ames, mouse lymphoma) and in vivo (rat bone marrow micronucleus) assays.

Administration of CBM to Tg.rasH2 mice at oral doses up to 35 mg/kg/day for 26 weeks did not result in an increase in tumors. Administration to rats for up to 90 weeks did not result in an increase in tumors at oral doses up to 20 mg/kg, which were associated with plasma AUC_{24h} of 170 and 230 µg.h/mL in males and females, respectively.

In the rat fertility study, administration of oral doses up to 44 mg/kg/day did not produce adverse effects on fertility, general reproductive performance, or early embryonic development. Plasma AUC_{24h} in females at the HD was approximately 400 µg.h/mL.

Oral administration of CBM to pregnant rats throughout organogenesis at doses of up to 60 mg/kg/day resulted in increased embryofetal mortality, reduced fetal body weights, and incomplete fetal skeletal ossification at the highest dose tested, which was associated with maternal toxicity. There was a small increase in visceral malformations at the HD; however, teratogenic potential could not be fully evaluated because of the high rate of embryofetal deaths, which resulted in an inadequate number of fetuses examined. Maternal plasma AUC_{24h} at the no-effect dose for adverse effects on embryofetal development (30 mg/kg/day) was approximately 300 µg.h/mL.

In the rabbit embryofetal development study, oral doses of up to 36 mg/kg/day throughout organogenesis resulted in increased embryofetal mortality at the HD, which was associated with maternal toxicity. Maternal plasma AUC_{24h} at the no-effect dose (12 mg/kg/day) for adverse effects on embryofetal development was 50 µg.h/mL.

Oral administration of up to 44 mg/kg/day to female rats throughout pregnancy and lactation produced neurobehavioral impairment (learning and memory deficit and increased auditory startle response in the offspring) at all doses, and decreased preweaning body weight gain and adverse effects on reproductive function (decreased numbers of corpora lutea, implantations, and live fetuses) in the offspring at the HD. Maternal plasma AUC_{24h} at the lowest effect dose (11 mg/kg/day) for adverse effects on pre- and postnatal development was approximately 100 µg*h/mL.

Although the current indication is for adults only, the sponsor conducted a juvenile animal toxicity study in rats in which oral doses of up to 120 and 80 mg/kg/day were administered to males and females, respectively, from postnatal day 7 to 70. Adverse effects included mortality, delayed sexual maturation, neurological (decreased grip strength) and neurobehavioral (learning and memory deficits) impairment, decreased sperm count, decreased brain weight, and ocular histopathology. Recovery from these effects was observed following discontinuation of dosing. Overall, a no-effect dose for adverse effects on postnatal development was not identified. At the LD, plasma AUC_{24h} ranged between approximately 100 and 250 µg*h/mL (sexes combined).

1.2 Recommendations

The application is approvable from a pharmacology/toxicology standpoint. A repeat embryofetal development study in rat should be conducted as a postmarketing requirement.

2 Drug Information

2.1 Drug

CAS Registry Number	913088-80-9
Generic Name	cenobamate (YKP3089)
Chemical Name	[(1R)-1-(2-chlorophenyl)-2-(tetrazol-2-yl) ethyl] carbamate]
Molecular Formula/Molecular Weight	$C_{10}H_{10}ClN_5O_2$/267.67
Pharmacologic Class	anticonvulsant (sodium channel blocker, GABAA positive allosteric modulator)

2.2 Relevant IND

76809

2.3 Proposed Clinical Population and Dosing Regimen

Proposed for treatment of partial-onset seizures in adult patients. The recommended initial dose is 12.5 mg once daily for two weeks; followed by 25 mg once daily for two weeks; followed by 50 mg once daily for two weeks. Doses should be increased in bi-weekly increments by no more than 50 mg once daily to a recommended maintenance dose of (b) (4) mg once daily. Maximum daily dose is 400 mg.

3 Studies Reviewed

Pharmacology

Primary, secondary, safety

Pharmacokinetics

ADME, TK

Repeat-Dose General Toxicity

Mouse

- 14-day
- 13-Week

Rat

- 28-day
- 26-week

Monkey

- 14-day
- 28-day
- 52-week

Genotoxicity

In vitro and in vivo assays

Carcinogenicity

26-week inTg.rasH2 mice
2-year in rat

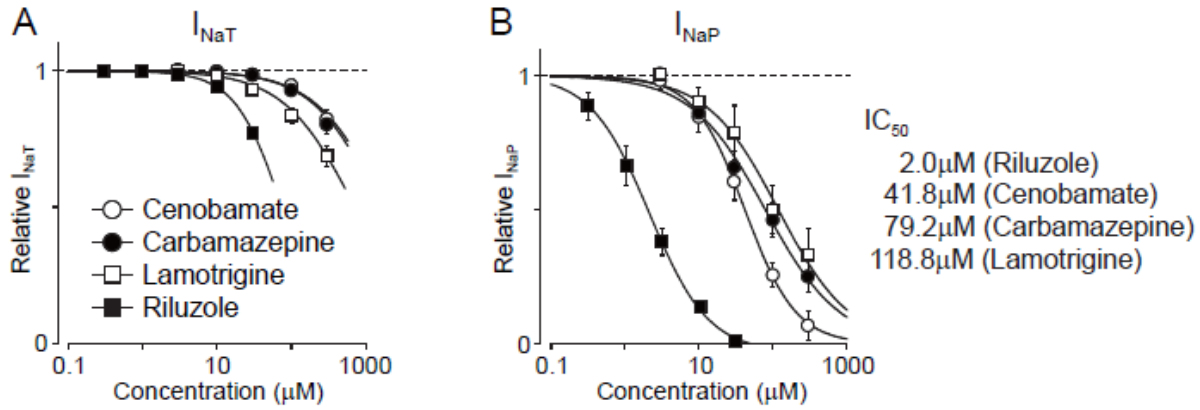
Reproductive and Developmental Toxicity

Fertility and early embryonic development (rat)
Embryofetal development (rat and rabbit)
Pre- and postnatal development (rat)
Juvenile development (rat)

4 Pharmacology

4.1 Primary Pharmacology

In an in vitro electrophysiology assay, cenobamate (CBM) produced state-dependent inhibition of the Nav1.7 current amplitude in HEK293 cells stably expressing hNav1.7with an IC50 value of 26 µM (equivalent to 6.96 µg/mL). The response was comparable to that seen with carbamazepine and lamotrigine (IC50s of 19 and 23 uM, respectively). In another electrophysiology study with sodium channels expressed in mammalian cells, CBM decreased the peak amplitude of hNav1.7 currents in a state- and concentration-dependent manner. The IC50 values of tonic, use-dependent, and inactivated-state blocks were 15670 µM, 478 µM, and 2.81 µM, respectively. When effects on voltage-gated Na+ channels were examined in rat hippocampal CA3 neurons using a whole-cell patch-clamp technique (below), CBM, like carbamazepine, was more potent in inhibiting the noninactivating (persistent) component of the tetrodotoxin-sensitive sodium current (INaP) compared to the transient component (INaT).



Several other AEDs have been evaluated for effects on the INaP and been found to reduce the INaP at a dose lower than that which altered the transient sodium current (Stafstrom CE, Epilepsy Curr, 7(1):15-22, 2007).

CBM positively modulated the GABA-induced current of 6 receptor subtypes, with EC_{50} values ranging from 42 µM to 194 µM (equivalent to 11.2 to 51.9 µg/mL). However, CBM had limited agonist activity at these GABA receptor subtypes. A small modulation of GABA currents (GABAA receptor) was observed at 100 µM, and a small increase in GABA-mediated currents in mouse cortical neurons was also observed at 100 µM.

CBM showed broad spectrum activity in in vivo rat and mouse seizure models (including MES, SC Metrazol, 6 Hz psychomotor seizure, and hippocampal kindling), following oral, IP, or IV administration.

4.2 Secondary Pharmacology

In radioligand binding studies, only 5 binding sites resulted in any significant CBM-related effects, and these were at relatively high concentrations (below). However, the IC_{50} of 300 µM for the Cl^- channel is equivalent to 80 µg/mL, while CBM steady state clinical Cmax values at 200 and 400 mg/day were 24 and 45.5 µg/mL, respectively.

Assay	IC_{50}	Ki
β_1*(h)* (agonist radioligand)	370 µM	210 µM
Cl^- channel (GABA-gated) (antagonist radioligand)	300 µM	250 µM
Dopamine transporter*(h)* (antagonist radioligand)	710 µM	380 µM
κ (KOP) (agonist radioligand)	950 µM	640 µM
OX1*(h)* (agonist radioligand)	550 µM	540 µM

4.3 Safety Pharmacology

In a CNS safety pharmacology studies in S-D rats at oral (gavage) doses of up to 300 mg/kg, clinical signs of neurotoxicity (ataxia, decreased locomotor activity, muscle tone, and motor function) were observed at ≥100 mg/kg (Cmax 14.6 µg/mL, AUC 513 µg*h/mL) and mortality was seen at the HD.

A neurotoxicity safety pharmacology study ((b) (4) study #1007-2223) with EEG was carried out to evaluate the convulsions seen in the 14-day monkey toxicity study ((b) (4) Study No. 1004-1151). CBM was administered once daily by oral gavage to cynomolgus monkeys for 4 consecutive days. All animals presented with ataxia and evidence of sedation following administration of CBM (30, 40, or 60 mg/kg). Two of 6 animals dosed with the HD of 60 mg/kg presented with abnormal, involuntary series of muscle contractions, which would be recorded as clonic convulsions according to the (b) (4) (b) (4) clinical sign lexicon. However, when video recordings were reviewed by a veterinary neurologist these were interpreted as episodes of myoclonus or intentional tremors. Examinations of EEGs showed no epileptic spikes or paroxysmal depolarization shifts during these episodes. The muscle contractions appeared to increase upon stimulation of the animal and upon voluntary movements, and the animals appeared sedated but conscious. An increase in beta waves was noted in 1 of the 2 animals during the first episode of myoclonus. One of the 2 monkeys with myoclonus or intentional tremors was euthanized on Day 4 due to poor and deteriorating conditions. TK analysis showed this animal had the highest Cmax of all monkeys treated with 60 mg/kg (127 ug/mL vs mean of 96 ug/mL in other monkeys). No spike trains were noted during muscular contractions suggesting that the clinical signs did not result from bursts of uncontrolled electrical activity in the brain and thus were not seizures. Animals dosed with 40 mg/kg presented ataxia, tremors, hypoglycemia, hypothermia, and evidences of sedation but did not have clonic convulsions (as defined in the (b) (4) clinical sign

lexicon). It was concluded that the HD dose of 60 mg/kg induced sedation, a vestibular syndrome (peripheral or central), and myoclonus or intentional tremors.

In the definitive hERG assay, CBM produced concentration-dependent inhibition of hERG-mediated potassium currents, but the IC_{50} was estimated as 1869 µM, which is 11-fold higher than the clinical steady state Cmax observed after daily dosing at 400 mg (45.5 µg/mL; 169 µM). In isolated rabbit Purkinje fibers, effects included QT interval shortening and changes in the normal action potential, characterized as a lowering or depression of the plateau phase, at 100 µM (equivalent to 26.8 µg/mL) and a complete loss of the plateau at 1000 µM (equivalent to 268 µg/mL). In the pivotal cardiovascular telemetry study in monkeys, there were no effects on ECG parameters or circulatory function following oral administration of doses up to 36 mg/kg. There were no drug-related ECG changes in the chronic toxicology studies.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Oral bioavailability of cenobamate was moderate to high in mice, rats and monkeys (≥59%) but very low in dogs (11%), while clearance was low in mice, rats, and monkeys (1.2-7.3% of hepatic plasma flow (QL,P)) and higher in dogs (60% of QL,P). CBM PK parameters in various species after a single oral dose are shown in Table 1.

Table 1. Pharmacokinetic parameters of cenobamate following single PO doses

Species n/gender	Dose (mg/kg)	C_{max} (µg/mL)	t_{max} (h)	AUC_{24} (µg*h/mL)	AUC_{∞} (µg*h/mL)	$t_{½}$ (h)	F (%)	Section
Mouse 4/M	20	13.3	4.0	ND	114	2.1	59.6	3.1.1
Rat 2/M	15	16.9	5.0	135	137	1.98	119	3.1.4
Dog 4/M	15	2.39	0.63	ND	2.59	0.46	10.8	3.1.7
Monkey 3/M 3/F	 15 15	 31.5 26.7	 2.33 3.33	 436 359	 883 500	 22.7 13.4	 110 83.9	3.1.8

Note: ND = not determined; mg/kg = milligrams/kilograms; µg = micrograms, mL = milliliter, min = minute, L = liters, h = hour; F = oral bioavailability.

Distribution

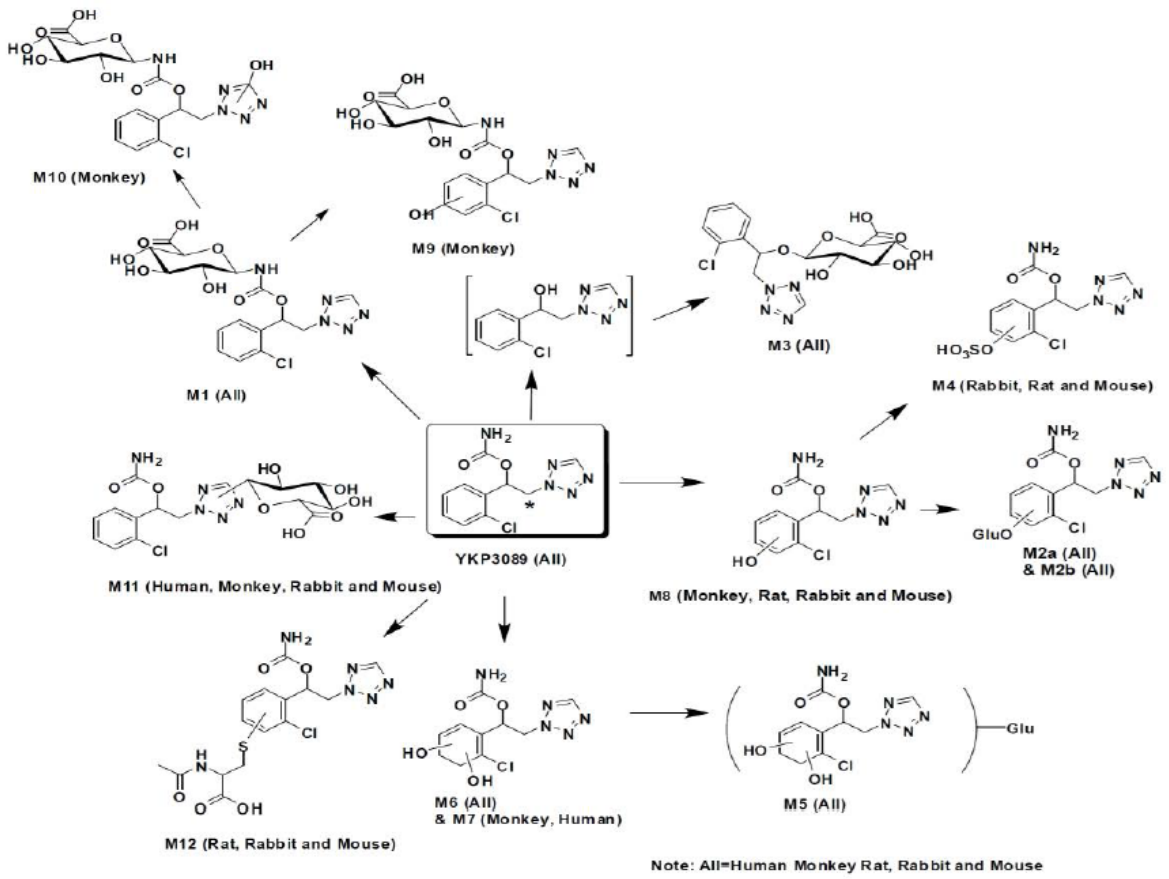
The protein binding of CBM was determined to be 43.2%, 54.9%, 35.4%, 65.0%, 60.7% to 70.7%, and 61.0% for mouse, rat, rabbit, dog, monkey, and human plasma, respectively. Tissue distribution of [14C]-CBM in male S-D rats following a single oral dose showed the highest tissue-to-blood ratios in kidney (4.7) and liver (2.7), with most ratios (including brain) around 1. Volume of distribution in toxicology species (mice, rats,

dogs, and monkeys) after IV dosing was similar or slightly lower than the total body water volume. Placental transfer was confirmed by the presence of drug in amniotic fluid and fetal blood samples from pregnant rats.

Metabolism

Metabolic profiles based on LC-MS analysis of plasma, urine, and feces samples collected from mice, rats, rabbits, monkeys, and humans following a single oral dose of [14C]-CBM are shown in Figure 1. N-glucuronide (M1) represented the major pathway for CBM in humans and monkeys (≥39%). In humans, 2 other metabolites accounted for >10% of the excreted dose: phenolic glucuronide (M2b) and the dihydrodiol metabolites combined (M6 and M7). In rats, oxidation of aromatic ring followed by the formation of dihydrodiol (M6) was the preferred route of elimination. In rabbits, aromatic ring oxidation followed by formation of a phenol derivative (M8), its glucuronide (M2a), and parent drug predominated in excreta. In mice, metabolites derived from aromatic ring oxidation such as dihydrodiol (M6) and phenolic glucuronide (M2b), and parent drug contributed to most of the elimination of CBM. Overall, metabolism was qualitatively similar between the toxicology species and humans. There were no unique human metabolites.

Figure 1. Proposed CBM biotransformation pathways



M1 = N-glucuronide; M2a = O-glucuronide; M2b = O-glucuronide (regio-isomer); M3 = Side chain O-glucuronide; M4 = Sulfate conjugate; M5 = Glucuronide of dihydrodiol; M6 = Dihydrodiol diastereomer; M7 = Dihydrodiol diastereomer; M8 = Cenobamate-OH; M9 = Hydroxylated N-glucuronide; M10 = N-oxide of M1; M11 = N-glucuronide-tetrazole; M12 = Mercapturic acid adduct; *location of ^{14}C radiolabel

Parent drug was the major circulating radioactive component in plasma samples from all species (i.e. mouse, rat, rabbit, monkey, and human). In an ex vivo human metabolite study, only the parent drug (>98%) and the M1 (N-glucuronide) metabolite were found in plasma. Exposure (AUC) to M1 was found to be 1.2% of the parent drug. No in vivo chiral inversion of CBM (R-enantiomer) to its S-enantiomer, YKP3090, was apparent in plasma from rats, monkeys or humans. Since there were no major circulating metabolites, additional testing of metabolites was not necessary.

Excretion

Following a single oral dose of [14C]-CBM, most of the radioactivity (≥94.0% of the dose) was excreted in urine and feces within 168 hours for all nonclinical species examined. Mice, rabbits, and monkeys excreted 70.0 to 78.8% of the dose in urine; whereas rats excreted 52.6% to 74.4% into urine, similar to humans in which 87.8% of the dose was eliminated within 312 hours.

TK

TK data are included under toxicology. Plasma level comparisons are shown in Table 2.

Table 2 CBM exposures at NOAEL and safety margins to human values

Species	Study Duration	Study Number	Nonclinical No Observed Adverse Effect Level				Safety Margins (Nonclinical Species / Human) [Male/Female at 200 mg/day and 400 mg/day]		
			Dose (mg/kg/day)	Dose (mg/m^2) [f]	C_{max} (µg/mL)	AUC_{24} (µg*h/mL)	C_{max}	AUC_{24}	Dose (200 & 400 mg)
Human[a]	14 days	AA24143	200 (mg/day)	123.3	23.9	482	NA	NA	NA
Human[a]	14 days	YKP3089C018	400 (mg/day)	246.7	45.5	861	NA	NA	NA
Mouse	Carcinogenicity[b]	SK13035	35	105	M: 59.5 F: 54.5	NA	M/F: 2.49 / 2.28 M/F: 1.31 / 1.20	NA	0.85 0.43
Rat	Segment II[c]	AA26092	30	180	23.6 (22 mg/kg) 30.4 (44 mg/kg)	401 544	0.99 / 1.27 0.52 / 0.67	0.83 / 1.13 0.47 / 0.63	1.46 0.73
Rat	1-month	1004-1151	30	180	M: 13.2 F: 27.3	M: 137 F: 324	M/F: 0.55 / 1.14 M/F: 0.29 / 0.60	M/F: 0.28 / 0.67 M/F: 0.16 / 0.38	1.46 0.73
Rat	6-month	SK07/038	12	72	M: 9.3 F: 17.6	M: 114 F: 214	M/F: 0.39 / 0.74 M/F: 0.20 / 0.39	M/F: 0.24 / 0.44 M/F: 0.13 / 0.25	0.58 0.29
Rat	Carcinogenicity[d]	SK13015	20	120	M: 13.0 F: 20.5	M: 173 F: 232	M/F: 0.54 / 0.86 M/F: 0.29 / 0.45	M/F: 0.36 / 0.48 M/F: 0.20 / 0.27	0.97 0.49
Rabbit	Segment II[e]	30/020	12	144	5.1	51.3	0.21 0.11	0.11 0.06	1.17 0.58
Monkey	1-month	1004-0743	4	48	M: 5.45 F: 6.47	M: 56.8 F: 84.9	M/F: 0.23 / 0.27 M/F: 0.12 / 0.14	M/F: 0.12 / 0.18 M/F: 0.07 / 0.10	0.39 0.19
Monkey	3-month interim	SK07/037	18	216	M: 32.9 F: 34.9	M: 493 F: 574	M/F: 1.38 / 1.46 M/F: 0.72 / 0.77	M/F: 1.02 / 1.19 M/F: 0.57 / 0.67	1.75 0.88
Monkey	6-month interim	SK07/037	18	216	M: 22.7 F: 26.9	M: 393 F: 516	M/F: 0.95 / 1.13 M/F: 0.50 / 0.59	M/F: 0.82 / 1.07 M/F: 0.46 / 0.60	1.75 0.88
Monkey	12-month	SK07/037	18	216	M: 61.8 F: 36.9	M: 1049 F: 542	M/F: 2.59 / 1.54 M/F: 1.36 / 0.81	M/F: 2.18 / 1.12 M/F: 1.22 / 0.63	1.75 0.88

AUC_{tau} = area under the plasma concentration-time curve over a dosing interval tau; F = female; M = male; NA = not applicable

[a] Steady state C_{max} and AUC_{tau} after 14 days of 200 mg/day from a healthy volunteer multiple ascending dose study; Steady state C_{max} and AUC_{tau} after 14 days of 400 mg/day from a healthy volunteer multiple ascending dose study; [b] Exposure at 2 h on Day 177 (6 months); [c] Taken from Segment III SK13019 on GD 18; [d] Exposure at Week 26; [e] Taken from separate Pregnant Rabbit TK study SK15005; [f] Dose were converted to mg/m^2 using FDA Guidance for Industry (2005): Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers; body weight for human was assumed to be 60 kg

6 General Toxicology

6.1 Repeat-Dose Toxicity

Study title: 13-Week Oral Gavage Toxicity and Toxicokinetic Study in Mice in Support of YKP3089

Study no.:	SK10009 ((b) (4) # 8224426)
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 April 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Batch No.: AA-022087-Batch-04-2009

Key Study Findings

Daily oral (gavage) administration of CBM (0 (vehicle), 10, 30, 60, or 120/90 mg/day; 5 mL/kg) to CD1 mice for 91 days resulted in mortality and clinical signs at ≥ 60 mg/kg/day, and centrilobular hepatocellular hypertrophy (non-adverse) at all but the LD. The HD was terminated on Day 7 due to the excessive toxicity (seen after first dose) at 120 and 90 mg/kg/day. The NOAEL (30 mg/kg/day) was associated with Cmax values of 64100 and 62667 ng/mL and AUC0-24 values of 471211 and 412057 ng·hr/mL in males and females, respectively.

Methods

Doses:	0 (vehicle), 10, 30, 60, or 120/90 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% Methylcellulose
Species/Strain:	Mice / Crl:CD1(ICR)
Number/Sex/Group:	10/sex/group
Age:	8 to 10 Weeks
Weight:	M: 28.9-38.9 gm, F: 23.2 to 29.5 g
Satellite groups:	39/sex/grp TK
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Dose selection was based on a 14-day study in CD-1 mice (Study No. SK09004) with doses of 0, 30, 60, and 120 mg/kg. Due to mortality and severe clinical signs including loss of righting reflex, uncoordinated gait, and decreased activity, the HD was lowered to 100 mg/kg on Day 3. There was significant BW loss (~10% from Day 1) for 2 to 3 days following administration of 120 mg/kg, but BWs recovered when the dose was lowered. Dose-related increases in liver weights were observed at ≥60 mg/kg. Based on minimal clinical signs and liver weight increases, the NOAEL for this study was considered to be 60 mg/kg.

Group	Subgroup	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)[a]	1 (Toxicity)	10	10	0	0
	2 (Toxicokinetic)	6	6	0	0
2 (Low)	1 (Toxicity)	10	10	10	2
	2 (Toxicokinetic)[b]	39	39	10	2
3 (Low-Mid)	1 (Toxicity)	10	10	30	6
	2 (Toxicokinetic)[b]	39	39	30	6
4 (Mid)	1 (Toxicity)	10	10	60	12
	2 (Toxicokinetic)[b]	39	39	60	12
5 (High)[c]	1 (Toxicity)	10/10	10/10	120/90	24/18
	2 (Toxicokinetic)[b]	39/15	39/18	120/90	24/18

a Group 1 received vehicle control article only.

b Three animals/sex/group in Groups 2 through 5 (Subgroup 2) were available as possible replacement animals for blood collections (dependent on survival).

c Due to the declining health of Group 5 animals, dosing was suspended after a single dose at 120 mg/kg/day on Day 1 of the dosing phase, and all Group 5 animals were sacrificed on Day 2 of the dosing phase. A replacement Group 5 was formed from all remaining extra animals and dosing commenced at a lowered dose level of 90 mg/kg/day on Day 6 of the dosing phase. However, due to pronounced clinical observations noted after a single dose on Day 6 of the dosing phase, replacement Group 5 animals were sacrificed on Day 7 of the dosing phase.

Observations and Results

Mortality

Dosing was suspended after a single dose at 120 mg/kg/day due to the poor condition of the animals, and the group was sacrificed on Day 2. A replacement group was created from all remaining extra animals and dosing commenced at 90 mg/kg/day on Day 6. However, due to pronounced clinical observations after a single dose, this group was also terminated on Day 7. All animals in Groups 1 through 4 survived to scheduled necropsy, with the exception of 1 Group 3 female found dead on Day 9, 1 Group 4 male sacrificed on Day 20, and 1 Group 4 female sacrificed on Day 70. These deaths were considered of uncertain relationship to drug. In addition, 1 Group 3 TK female was sacrificed on Day 24 due to gavage-related trauma.

Clinical Signs

Dose-dependent clinical observations in animals given ≥ 60 mg/kg/day included tremors, ataxia, hypoactivity, myoclonic jerking, recumbency, squinting eyes, irregular and/or labored respiration, skin and pelage abnormalities (cold to touch, discolored haircoat or skin, pale ears, and rough haircoat), and recumbency. Clinical observations were noted 4 hours postdose and, except for Group 5, resolved prior to the subsequent dose.

Body Weights

No drug-related effects on body weight (BW) or and BW gain were seen at ≤ 60 mg/kg/day. BW was not evaluated at the HD.

Food Consumption

No drug-related alteration in food consumption was noted at ≤ 60 mg/kg/day.

Hematology

No drug-related effects were observed in the hematology results.

Clinical Chemistry

Increased globulin and decreased albumin-to-globulin ratio were seen at 30 and 60 mg/kg/day. Slight increases (NS) in ALT were seen in females at 60 mg/kg/day.

Gross Pathology

There were no drug-related macroscopic observations.

Organ Weights

A drug-related increase in liver/gallbladder weights was seen in males at all doses and in females at 30 or 60 mg/kg/day. The increased liver/gallbladder weight was dose-related and correlated with microscopic centrilobular hepatocyte hypertrophy at these doses.

Histopathology

Centrilobular hepatocellular hypertrophy observed in males and females given 30 or 60 mg/kg/day was considered drug-related but not adverse.

Toxicokinetic

TK results are shown in Table 1. Values for Cmax and AUC0-24 were slighter higher on Day 1 than during Week 13 of dosing, indicating no accumulation after repeated dosing. Increases in Cmax and AUC0-24 were not consistently dose-proportional.

Table 1 Toxicokinetic Parameters for YKP3089 in Mouse Plasma

Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)		AUC_{0-24} (ng·hr/mL)	
		Day 1	Week 13	Day 1	Week 13
10	M	19900	17933	181195	144232
	F	26067	17367	220833	119317
30	M	58067	64100	745870	471211
	F	73100	62667	857147	412057
60	M	101900	85067	1665183	1033550
	F	114600	85600	2017500	976841
120	M	166667	-	3659067	-
	F	208000	-	4432333	-

Study title: YKP3089: a 26-week oral toxicity study with an 8-week recovery period, including a 13-week Interim, followed by a 4-week recovery period, in Sprague Dawley rats

Study no.:	SK07/038 (1007-0781)
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1 May 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot no. 1401-1401-07-001, 99.7%

Key Study Findings

CBM (0 (vehicle), 12, 24, 48 mg/day) was administered to Sprague-Dawley (S-D) rats orally (by gavage) once daily for 13 or 26 weeks, followed by a 4- or 8-week recovery period. A total of 8 HD animals (6 Main, 2 TK) were euthanized due to deteriorating condition attributed to drug. Drug-related (D-R) clinical signs at the MD and HD included decreased activity, uncoordinated gait, and increased salivation. There were no D-R effects on BW, ophthalmology, hematology, or urinalysis parameters. Microscopic changes observed in the kidney (males) and liver (males and females) were attributed to drug, but renal changes were associated with either the male rat alpha 2U globulin mechanism or early (spontaneous) chronic progressive nephropathy (CPN, tubular deposition of proteinaceous casts), and centrilobular hepatocellular hypertrophy was considered adaptive. The NOAEL (12 mg/kg/day) was associated with Week 26 Cmax and AUC_{0-t} values of 9.3 ug/mL and 113.9 ug.h/mL in males and 17.6 ug/mL and 213.7 ug.h/mL in females, respectively.

Methods

Doses:	0 (vehicle), 12, 24, 48 mg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% w/v Aqueous Methylcellulose
Species/Strain:	Rat, Sprague-Dawley
Number/Sex/Group:	15-20/sex/group main, 5-10/sex/C&HD recovery, 6-9/sex/grp TK (see table below)
Age:	8-9 weeks

Groups	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Number of Animals									
				13-Week Main		13-Week Recovery		26-Week Main		26-Week Recovery		Toxicokinetics	
				M	F	M	F	M	F	M	F	M	F
1. Control *	0	0	5	15	15	5	5	20	20	10	10	6	6
2. Low dose	12	2.4	5	15	15	-	-	20	20	-	-	9	9
3. Mid dose	24	4.8	5	15	15	-	-	20	20	-	-	9	9
4. High dose	48	9.6	5	15	15	5	5	20	20	10	10	9	9

* Control animals received the control/vehicle formulation only (0.5% w/v Methylcellulose)

Dose selection was based on the results of a 28-day toxicity study in SD rats ((b) (4) (b) (4) Study No. 1004-1151) in which oral (gavage) doses of 0 (0.5% methylcellulose vehicle), 10, 30, or 100 mg/kg/day were administered. Mortality was seen at the HD on Days 4 and 5 (2 females found dead) and HD animals were sacrificed due to poor and deteriorating condition on Days 3 (1 M, 2 F), 4 (1 F), 5 (2 F), and 22 (1 F). Clinical signs preceding mortality and moribund sacrifice included: uncoordinated gait, decreased activity, decreased righting reflex, hunched back, weakness, lying on cage floor, gasping, and shallow respiration. Similar but less severe clinical signs were observed in surviving HD animals following lowering of the dose to 60 mg/kg on Day 6. Dose-related hepatocellular hypertrophy was noted at the MD and HD and was associated with minimal hepatocellular necrosis in 2 HDF.

Observations and Results

Mortality

Six main study HD females (4521G, 4522G, 4531J, 4530J, 4525H, and 4549O) were sacrificed on Days 8, 7, 14, 21, 93, and 140, respectively. Clinical signs noted prior to sacrifice generally included uncoordinated gait, decreased activity, cold to touch, recumbency, and slow skin turgor. For animals 4525H and 4549O, sacrificed later during the study, similar clinical signs were noted throughout the treatment period. Histopathological examinations did not identify a clear cause of death. TK females 4556Q and 4555Q were sacrificed in deteriorating condition on Days 175 and 182, respectively. A necropsy examination (no histopathology) was performed on female 4556Q based on clinical observations at the time, and bilateral, pale discoloration (green) of the kidneys was noted.

Clinical Signs

Dose-related clinical signs noted for many MD animals, most HD males, and all HD females included decreased activity, uncoordinated gait, and increased salivation. Decreased activity and uncoordinated gait were noted as early as Day 1 and were, in general, more frequent during the first 2 weeks of dosing, but continued to be noted thereafter in some animals. In some cases, these observations continued throughout the treatment period. These clinical signs were not noted during the recovery period.

Body Weights

A slight reduction in BW gain was noted in HD males and females during approximately the first 2 weeks of dosing. Thereafter, BW gain was comparable among groups. There were no significant differences in BWs during the study.

Food Consumption

D-R increases (~10% compared to C, SS) in mean food intake were noted in HD males (starting at Week 20) and females (beginning at Week 16).

Ophthalmoscopy

There were no D-R ophthalmoscopic effects.

Hematology

There were no D-R alterations in hematology and coagulation parameters.

Clinical Chemistry

Increases in total protein (up to 9%, SS) and albumin (up to 8%, SS) were noted at the MD and HD. Recovery group values were comparable to C.

Urinalysis

There were no D-R differences in urinalysis parameters.

Gross Pathology

There were no D-R findings.

Organ Weights

Increased liver and kidney weights (absolute and/or relative) were noted, primarily at the HD.

Histopathology

D-R microscopic changes were observed in the kidney (males only) and liver (males and females) of Main study animals. All renal changes were associated with either the male rat alpha 2U globulin mechanism (hyaline droplets, tubular/granular casts, tubular necrosis) or early (spontaneous) chronic progressive nephropathy (CPN, tubular deposition of proteinaceous casts. In the liver, dose-related centrilobular hepatocellular hypertrophy was seen at all doses. Vacuolated hepatocytes with vacuoles of different sizes and hepatocellular necrosis or degeneration/necrosis were observed at all doses (including controls) with no indication of dose-dependence and so were not considered related to drug. Following an 8-week recovery period, the incidence and/or severity of the renal and liver changes had decreased, indicating a partial recovery.

Toxicokinetic

TK parameters after the first dose and at 13 and 26 weeks are shown in Table 1.

Table 1. Toxicokinetic Parameters for YKP3089 in Rat Plasma

Males

Study Week	Group Number	Dose level (mg/kg)	T_{max} (hr)	C_{max} (µg/mL)	$AUC_{(0-t)}$ (hr*µg/mL)	$AUC_{(0-\infty)}$ (hr*µg/mL)	% Diff. of $AUC_{(0-\infty)}$ and $AUC_{(0-t)}$	T ½ (hr)	V_d[4] (mL/kg)	CL/F (mL/hr/kg)
1	2[1]	12	4	9.8	165.9	NA	NA	NA	NA	NA
	3[1]	24	6	19.4	362.0	NA	NA	NA	NA	NA
	4[1]	48	6	33.1	653.8	NA	NA	NA	NA	NA
13	2[2]	12	NC	NC	NC	NC	NC	NC	NC	NC
	3[3]	24	2	11.2	83.1	171.0	105.8	12.1	2454.6	140.4
	4	48	4	28.0	260.3	266.9	2.5	4.3	1107.7	179.8
26	2	12	2	9.3	113.9	117.3	3.0	4.1	607.5	102.3
	3	24	2	14.3	117.1	155.6	24.7	4.9	1086.3	154.2
	4	48	0.5	37.0	525.3	530.1	0.9	3.2	421.0	90.6

Females

1	2[1]	12	2	10.7	185.5	NA	NA	NA	NA	NA
	3[1]	24	8	19.5	419.6	NA	NA	NA	NA	NA
	4[3]	48	4	27.7	556.4	1074.7	93.2	21.2	1364.3	44.7
13	2[2]	12	NC	NC	NC	NC	NC	NC	NC	NC
	3	24	2	28.9	253.6	255.2	0.6	2.8	384.4	94.1
	4[3]	48	4	43.8	627.5	649.2	3.5	5.4	572.9	73.9
26	2	12	2	17.6	213.7	226.5	6.0	5.3	403.9	53.0
	3[5]	24	8	34.2	397.9	415.5	4.4	4.5	375.0	57.8
	4	48	2	39.6	704.2	737.8	4.8	5.0	467.2	65.1

1. No elimination phase, therefore no regression analysis was performed and values related to K_{el} ($T_{½}$, $AUC_{(0-\infty)}$, Vd, CL/F and % Diff. of $AUC_{(0-\infty)}$ and $AUC_{(0-t)}$) could not be estimated.
2: Not calculated due to insufficient data.
3: The AUC(0-∞), T½ and CL results for this animal are presented for information only since the R^2 was below 0.90 or the extrapolated AUC was >30% of the $AUC_{(0-\infty)}$.
4: Reported for information only as this is an oral administration.
5: C_{max} used to calculate TK parameters.
NA: Not applicable
NC: Not calculated.

Study title: YKP3089: a 28-day oral toxicity study in cynomolgus monkeys

Study no.:	(b) (4) Study No.: 1004-0743
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study report:	5/5/05
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	YKP3089, Lot # DIT040503, 99.6%

Key Study Findings

Oral (gavage) administration of CBM (0, 4, 12, or 36 mg/kg) to cynomolgus monkeys (3/sex/grp) resulted in severe clinical signs leading to pre-terminal sacrifice of 1 HD female and HD reduction from 36 to 24 mg/kg starting on Day 7. Drug-related clinical signs were noted at all doses, with dose-related increasing severity. Decreases in total bilirubin were noted at all doses and were considered drug-related, although the changes were not strictly dose-related in females. There were no histopathological changes. Based on clinical signs and possible clinical chemistry changes, the LD was a LOAEL. This dose was associated with Cmax values of 5.45 µg/mL and 6.47 µg/mL and AUC24 values of 56.8 µg*h/mL and 84.9 µg*h/mL for males and females, respectively.

Methods

Doses:	0 (vehicle), 4, 12, 36/24 mg/day
Frequency of dosing:	QD
Route of administration:	oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose
Species/Strain:	cynomolgus
Number/Sex/Group:	3/sex/group
Age:	2-3 years
Weight:	1.7-2 kg males,1.8-2.2 kg females
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	none that impacted study quality or integrity

CBM was administered once daily by oral gavage to cynomolgus monkeys (3/sex/group) for 28 consecutive days. The HD was reduced to 24 mg/kg starting on Day 7 due to the severity of clinical signs.

Treatment Group	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
				Males	Females
1. Control *	0	0	5	3	3
2. Low Dose	4	0.8	5	3	3
3. Mid Dose	12	2.4	5	3	3
4. High Dose (Days 1 to 6)	**36**	**7.2**	5	3	3
4. High Dose (As of Day 7)	**24**	**4.8**			

*The Control animals received the Vehicle, 0.5% (w/v) methylcellulose in Sterile Water for Injection USP, alone.

Doses were based on the results of a 14-day study ((b) (4) Study Nos.: 2004-0143) in which cynomolgus monkeys (1/sex/group) were administered oral (gavage) doses of 0 (0.5% (w/v) methylcellulose), 10, 30, or 60 mg/kg. There was no mortality during the study. Clinical signs observed in both HD animals on Days 1 and 2 included slight vomiting, crawling, lying on the cage floor, and clonic convulsions that ranged from slight to moderate in the male and slight to severe in the female (see neurotoxicity safety pharmacology study above). The dose level was subsequently decreased to 40 mg/kg/day as of Day 3. Slight clonic convulsions in both animals and crawling in the female persisted on Day 3 at the reduced dose which also resulted in decreased appetite, decreased activity, vomiting, and drowsiness. Nystagmus was observed in the HD female on Days 3 and 4 along with severe hyperextension of the neck on Day 3, which had diminished to a slight hyperextension by Day 4. The female was also cold to touch and displayed slight tremors on Day 4, accompanied by labored respiration, which lasted from Day 4 to Day 5. By Day 5 clinical signs at the HD consisted mostly of decreased activity, uncoordinated gait, and drowsiness in both animals, with decreased appetite and vomiting also seen in the female. Clinical signs at the MD consisted of decreased appetite, vomiting, uncoordinated gait, drowsiness, and a slight decrease in activity throughout the treatment period, in both animals. The only other findings were increased WBC count in the HD male and dose-related increases in liver weights without macroscopic or microscopic changes. The NOAEL was 10 mg/kg.

Observations and Results

Mortality

No mortality occurred, but severe clinical signs and deteriorating condition led to the sacrifice of 1 HD female (4503C) on Day 4. This animal displayed severe clinical signs (decreased appetite, uncoordinated gait, drowsiness, decreased activity, hyperextension of the neck, ocular nystagmus, tremors, weakness, dehydration, and decreased corporal temperature), decreases in RBC parameters, and increases in WBCs, neutrophils, glucose, and AST. There were no macroscopic findings at necropsy.

Clinical Signs

Drug-related clinical signs were seen at all doses with dose-related increases in severity.

Clinical signs included decreased appetite, uncoordinated gait, decreased activity, weakness, drowsiness, changes in the consistency of the feces, and ptosis. The HD female that was sacrificed also showed sporadic hyperextension of the neck.

Body Weights

There were no drug-related (D-R) changes in body weight during the study.

Ophthalmoscopy

No adverse ocular effects were noted during the study.

Electrocardiography

There were no changes in the electrocardiograms that could be attributed to drug.

Hematology

There were no D-R alterations in hematology and coagulation parameters.

Clinical Chemistry

Decreases in total bilirubin were noted in all drug-treated groups (SS in MD and HD males and LD and MD females), but the toxicological significance of this change was unclear and not discussed.

Urinalysis

There were no D-R differences in urinalysis parameters.

Gross Pathology

There were no D-R macroscopic findings.

Organ Weights

Increased liver weights were seen in HD males and MD and HD females, and spleen weights were increased in MD and HD males and HD females.

Histopathology

No D-R microscopic findings were noted.

Toxicokinetic

There was no indication of accumulation during the 28 day-treatment period based on Cmax and AUC values, which increased linearly with dose (Table 1). Exposures were higher in females.

Table 1. TK Parameters in Monkeys on Days 1 and 28 after Oral Administration of Cenobamate

Dose Level (mg/kg/day)	Gender	C_{max} (µg/mL)	t_{max} (h)	AUC_{24} (µg*h/mL)
Day 1				
4	Male	5.44	1.33	45.1
	Female	5.52	2.33	63.9
12	Male	17.8	1.67	245
	Female	19.1	2.33	287
36	Male	31.5	3.00	533
	Female	55.9	5.00	995
Day 28				
4	Male	5.45	1.00	56.8
	Female	6.47	1.67	84.9
12	Male	17.0	2.33	226
	Female	21.4	2.00	318
24	Male	30.7	3.33	465
	Female	38.5	2.00	592

Study title: YKP3089: a 52-week oral toxicity study, with 13-week and 26-week interim portions, including 4-week recovery (following 13 weeks of administration) or a 3-month recovery (following 52 weeks of administration), in cynomolgus monkeys

Study no.: (b) (4) Study No.: 1007-0793
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/09/07
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Lot no. 1401-1401-07-001, 99.3%

Key Study Findings

Oral (gavage) administration of CBM (0, 9, 18, or 27/22 mg/kg/day) to cynomolgus monkeys for 52 weeks resulted in severe clinical signs (decreased activity, hunchback, crouching, and incoordination), sacrifice of 1 HD female (on Day 7), and dose reduction (on Day 11) at the HD. Minimal hepatocellular hypertrophy in the liver (associated with dose-related increases in liver weight) and minimal to mild lymphoid hyperplasia of bone marrow were seen at the MD and HD. Following 3 months of recovery the bone marrow lymphoid hyperplasia was still present at the HD. The MD (18 mg/kg) was considered the NOAEL and was associated with Cmax values of 61.8 and 36.9 µg/mL and AUC24 values of 1049 and 542 µg*h/mL in males and females, respectively.

Methods

Doses:	0 (vehicle), 9, 18, 27/22 mg/day
Frequency of dosing:	QD
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% w/v methylcellulose in sterile water for injection
Species/Strain:	cynomolgus
Number/Sex/Group:	4/sex/group main + 2 recovery/sex for C & HD
Age:	3-4 years
Weight:	2.4 - 3.4 kg for males, 2.2 - 2.8 kg for females
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	None that impacted study quality or integrity

CBM was administered once daily for 52 consecutive weeks to cynomolgus monkeys followed by a 3-month recovery phase as indicated below:

Treatment Groups	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Number of Animals			
				52 Week Main		52 Week Recovery	
				M	F	M	F
1. Control *	0	0	5	4	4	2	2
2. Low dose	9	1.8	5	4	4	0	0
3. Mid dose	18	3.6	5	4	4	0	0
4. High dose	27/22**	5.4/4.4	5	4	4	2	2

* Control animals received the control/vehicle formulation only (0.5% w/v Methylcellulose)
** Dose level of Group 4 was reduced to 22 mg/kg on Day 11 due to clinical signs in this group.

Dose selection was based on the results of the 28-day monkey study (LAB No. 1004-0743) in which severe clinical signs (decreased appetite, uncoordinated gait, drowsiness, decreased activity, hyperextension of the neck, ocular nystagmus, tremors, weakness, dehydration and decreased body temperature) and deteriorating condition led to preterminal sacrifice on Day 4 of 1 HD female animal and HD reduction from 36 to 24 mg/kg starting on Day 7.

Observations and Results

Mortality

One HD female monkey (4513) was euthanized on Day 7 with clinical signs which included cold to touch, moderate tremors, and slightly uncoordinated. There was no cause of moribundity established by macroscopic and microscopic examination.

Clinical Signs

Clinical signs noted during the treatment period at a dose of 27 mg/kg included decreased activity, hunchback, crouching, and incoordination. These clinical signs were reduced after Day 11 once the dose was reduced to 22 mg/kg. Neurological assessments (cranial nerve function, spinal segmental reflexes, general observations and attitudinal and postural reactions) were found to be abnormal in some HD animals: incoordination was noted following dosing on Days 6 and 13.

Body Weights

Body weights were unaffected by drug administration.

Ophthalmoscopy

No D-R ophthalmic lesions were observed.

Hematology

There were no D-R alterations in hematology and coagulation parameters.

Clinical Chemistry

There were no drug-related changes in serum chemistry parameters.

ECG

There were no ECG changes attributed to drug administration.

Gross Pathology

There were no findings considered to be associated with drug.

Organ Weights

Increases in liver weights (absolute and relative to body weight) were seen at the MD and HD in both sexes and correlated with microscopic hepatocellular hypertrophy.

Histopathology

Minimal hepatocellular hypertrophy was noted in 2/4 MD males and in 4/4 males and 3/4 females at the HD. Increased incidences and severity of lymphoid hyperplasia was noted in the femur and sternal bone marrow at the MD and HD (Table 1). The hyperplasia was characterized by random discrete clusters of small to medium sized lymphocytes, occasionally forming a germinal center. Following 3 months of recovery, the bone marrow lymphoid hyperplasia was still present at the HD (1 HD male), while hepatocellular hypertrophy had completely reversed.

Table 1. Microscopic findings in femur and sternum bone marrow at Week 52

Tissue/Finding	Sex	Male				Female			
Dose Level (mg/kg/day)		0	9	18	27/22	0	9	18	27/22
Group		1	2	3	4	1	2	3	4
Number examined		4	4	4	4	4	4	4	4
Femur Bone Marrow									
Hyperplasia: lymphoid	**Total**	**0**	**0**	**1**	**0**	**0**	**0**	**2**	**4**
	Minimal	—	—	1	—	—	—	2	3
	Mild	—	—	—	—	—	—	—	1
Sternum Bone Marrow									
Hyperplasia: lymphoid	**Total**	**1**	**2**	**3**	**3**	**0**	**0**	**1**	**4**
	Minimal	1	2	3	3	—	—	1	3
	Mild	—	—	—	—	—	—	—	1

Toxicokinetics

TK parameters are shown in Table 2.

Table 2. Toxicokinetic parameters in monkeys after oral administration of CBM

Dose Level (mg/kg/day)	Gender	C_{max} (μg/mL)	t_{max} (h)	AUC_{24} (μg*h/mL)
Day 1				
9	Male	14.3	3.0	184
	Female	16.9	2.6	209
18	Male	28.6	2.3	481
	Female	25.7	3.0	427
27	Male	43.5	3.7	708
	Female	40.2	6.0	706
Week 13				
9	Male	15.6	2.5	214
	Female	18.2	2.0	235
18	Male	46.4	3.0	770
	Female	32.2	2.5	441
22	Male	46.8	3.0	655
	Female	56.3	2.4	755
Week 26				
9	Male	13.4	2.3	177
	Female	14.7	3.5	197
18	Male	41.1	2.5	694
	Female	39.6	1.8	477
22	Male	55.0	2.5	602
	Female	55.5	3.0	796
Week 52				
9	Male	14.1	5.5	231
	Female	22.0	2.8	285
18	Male	61.8	3.5	1,049
	Female	36.9	4.0	542
22	Male	55.6	2.1	781
	Female	66.9	4.8	1,159

7 Genetic Toxicology

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

Study title: YKP3089 (Batch: 1401-1401-05-501) - Bacterial reverse mutation test (Plate incorporation and Pre-incubation methods)

Study no.:	AA30488
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/18/2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	YKP3089, 1401-1401-05-501, 99.6%

Methods and Results

CBM (DMSO vehicle) was negative when evaluated for mutagenic potential in Salmonella typhimurium (tester strains TA98, TA100, TA1535, TA1537 and TA102) using the plate incorporation (52, 164, 512, 1600, and 5000 ug/plate with and without rat liver S9) and preincubation (492, 878, 1568, 2800 and 5000 ug/plate with and without S9) methods. No precipitate was noted in any of the strains used. Signs of cytotoxicity (reduction in bacterial lawn and/or number of revertants) were noted in some strains. No statistically or biologically significant increases in the number of revertants were noted in any of the other strains used, with or without activation metabolic. Positive controls demonstrated the expected responses.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: YKP3089 (Batch: 1401-140 1-05-501) - In vitro Mammalian Cell Gene Mutation Test on L5178Y Mouse Lymphoma Cells TK+/- (Microwell method).

Study no.:	AA30489
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/25/2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	YKP3089, 1401-1401-05-501, 99.6%

Methods and Results

CBM (in DMSO) was negative when evaluated for mutagenicity in L5178Y mouse lymphoma cells, under short (approximately 4 hours- either with or without metabolic activation) and long (approximately 24 hours -without metabolic activation) exposure conditions. With short treatment (87, 155, 276, 492, 878, 1568, 2800, or 5000 ug/mL), precipitate was noted at ≥1568 ug/mL, and cytotoxicity was seen at ≥ 492 ug/mL in the

absence of metabolic activation and at ≥ 878 ug/mL in the presence of metabolic activation. No statistically or biologically significant increases in the mutant frequency were noted. With the long treatment (1.7, 5.4, 17, 52, 164, 512, 1600, or 5000 ug/mL) precipitate and marked signs of cytotoxicity were noted at 1600 ug/mL and above, but no statistically or biologically significant increases in the mutant frequency were noted. In an additional experiment conducted at the concentrations of 276, 492, 878, 1568 and 2800 ug/mL (quarter-log progression), precipitate was noted at ≥1568 ug/mL, and signs of cytotoxicity were noted at ≥ 878 ug/mL. A statistically significant increase in mutant frequency was seen at 878 ug/mL, but because this increase (1.74X vehicle control) did not meet the lab's criteria (>2-fold increase and 125 induced mutants) it was not considered biologically significant. The positive controls (methylmethanesulfonate and cyclophosphamide) showed the expected responses.

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

Study title: YKP3089- Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow

Study no:	30/027
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/6/2003
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	YKP3089, SI-02-092, 100%

Methods and Results

CBM (oral gavage doses of 0(1% CMC), 53, 94, or 168 mg/kg) was negative when tested for the potential to induce micronuclei in rat (S-D) bone marrow erythrocytes samples 24 or 48 hours after dosing. In the preliminary toxicity screen (doses up to 2000 mg/kg), mortality was seen at 168 mg/kg (1/6) or greater, and dose-related clinical signs (subdued behavior, piloerection, tremor, ataxia, watering and/or half-closed eyes, decubitus, lethargy or coma) were observed in all groups. Dose-related decreases in the PCE/NCE ratios were seen at doses from 53 to 168 mg/kg.

In the main test, there was no mortality, but dose-related clinical signs were noted at all doses. An approximately 50% decrease in the PCE/NCE ratio was seen at the high dose (168 mg/kg) compared to C. There were no statistically significant increases in the number of MNPCEs compared to negative controls at either the 24-or 48-hour sampling time. The positive control (cyclophosphamide) induced the expected statistically significant increases in MNPCEs.

8 Carcinogenicity

Study title: YKP3089: 26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice

Study no.:	AD68SY.7G8R (b) (4)
Study report location:	4.2.3.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/6 and 1/8/14, in males and females
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	AA-022087-Batch-04-2009
CAC concurrence:	Yes, minutes dated 11/5/13

Key Study Findings

Oral (gavage) administration of CBM (0, 5, 15, or 35 mg/kg/day) to Tg.rasH2 mice for 26 weeks did not increase the incidence of neoplastic lesions. There were no drug-related deaths or clinical signs. BW gain was +0.3, -3.8, and -7.2% of C in males and -14.1, -7.9, and -14.9% of C in females, at the LD, MD, and HD, respectively. SS increases in liver weights at the MD and HD correlated with increased incidences of centrilobular hypertrophy. Incidences of pulmonary tumors, splenic hemangiosarcomas, and other tumor types were comparable across drug- and vehicle-treated groups (there were no SS group differences in sponsor's or FDA statistical analysis). The expected SS increases in incidences of pulmonary tumors and splenic hemangiosarcomas were seen in positive controls.

Adequacy of Carcinogenicity Study

Adequate

Appropriateness of Test Models

Appropriate

Evaluation of Tumor Findings

Negative

Methods

Doses:	0, 5, 15, or 35 mg/kg/day
Frequency of dosing:	QD
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% w/v methylcellulose in DI water
Basis of dose selection:	28-day oral (gavage) study in CByB6F1 mice
Species/Strain:	Tg.rasH2 mice [CByB6F1-Tg(HRAS)2Jic (+/- hemizygous c-Ha-ras)]
Number/Sex/Group:	25
Age:	8 weeks at initiation of dosing
Satellite groups:	no
Deviation from study protocol:	none

Tg.rasH2 mice (25/sex/group + 5-8 CByB6F1/sex/grp TK) were administered CBM (0 (vehicle: 5% w/v methylcellulose), 5, 15, or 35 mg/kg/day) once daily by oral gavage (10 mL/kg) for 26 weeks. Positive controls (10/sex/grp) received 3 IP injections of 1000 mg/kg/day of urethane in saline on Days 1, 3 and 5 and were sacrificed on Day 72 (females) or Day 74 (males). Main study animals were sacrificed on Days 183 or 184. Blood was collected from TK animals at 2 and 4 hours post-dose on Day 177.

Group	Dose Levels (mg/kg/day)	Concentration (mg/mL)	Number of Animals			
			Main Cohort (Tg.rasH2)		TK Cohort** (CByB6F1)	
			Male	Female	Male	Female
Group 1	0	0	25	25	5	5
Group 2	5	0.5	25	25	8	8
Group 3	15	1.5	25	25	8	8
Group 4	35	3.5	25	25	8	8
Group 5*	1000 (urethane)	100	10	10	-	-
Total			110	110	29	29

*The positive control animals were administered a total of 3 intraperitoneal (i.p.) injections, once each day on Days 1, 3, and 5.
**Extra TK animals (2/sex) were used to ensure adequate animals for TK bleeding
- Not Applicable

Dose selection was based on the results of a 28-day oral (gavage) study in CByB6F1 mice at doses of 0 (0.5% w/v methylcellulose), 15, 35, or 75 mg/kg. The HD of 35 mg/kg for the 26-week study was based on mortality and microscopic pathology observations (focal necrosis of hepatocytes) at the HD in the 28-day study. The ECAC concurred with

the sponsor's proposed doses of 0, 5, 15, or 35 mg/kg/day based on mortality in the 28-day study.

Observations and Results

Mortality

There was no apparent drug-related increase in mortality (Table 1).

Table 1. Mortality in Tg.rasH2 mouse carcinogenicity study

Group	YKP3089 Dose Levels (mg/kg/day)	Animal Number	Day of Removal	Mode of Death	Cause of Death
Males					
1	0	1006	177	Natural Death	Lung Carcinoma and Lung Hemangiosarcoma
		1017	35	Moribund Sacrifice	Stomach Squamous Cell Carcinoma
2	5	1041	169	Moribund Sacrifice	Multicentric Histiocytic Sarcoma and Nasal Cavity Hemangiosarcoma
		1279	133	Natural Death	Undetermined
3	15	1053	146	Natural Death	Skin Hemangiosarcoma
4	35	1089	156	Moribund Sacrifice	Skin Hemangiosarcoma
		1100	151	Moribund Sacrifice	Multicentric Hemangiosarcoma
Females					
2	5	1140	157	Moribund Sacrifice	Mediastinum Hemangiosarcoma
3	15	1162	110	Natural Death	Multicentric Hemangiosarcoma
		1185	176	Moribund Sacrifice	Skin Hemangiosarcoma and Nasal Cavity Adenocarcinoma

Clinical Signs

There were no drug-related effects on clinical signs.

Body Weights

BW gain was +0.3, -3.8, and -7.2% of C in males and -14.1, -7.9, and -14.9% of C in females at the LD, MD, and HD, respectively. SS decreases in BW were observed in HD males on Day 127 and in HD females on Day 183 (both 5.6% compared to C).

Feed Consumption

The mean daily food consumption between Days 1 and 183 was similar between the vehicle control and drug-treated males and females.

Gross Pathology

There were no drug-induced gross lesions. The pulmonary and splenic gross findings in the positive control group were expected based on the carcinogenic effect of urethane.

Histopathology

Neoplastic

Incidences of pulmonary tumors were comparable in vehicle and drug-treated mice and fell within the historical control range (Table 2). The positive controls showed the expected response.

Table 2. Incidences of lung tumors

Males						
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**HCR (%)**
Number Examined	25	25	25	25	10	
Lung, adenoma, single	3 (12%)	0 (0%)	3 (12%)	2 (8%)	0 (0%)	0-24
Lung, adenoma, multiple	0 (0%)	0 (0%)	0 (0%)	0 (0%)	10* (100%)	0-8
Lung, carcinoma	1 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0-8
Total Number of Animals with Lung tumors	4 (16%)	0 (0%)	3 (12%)	2 (8%)	10* (100%)	NA

Females						
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**HCR (%)**
Number Examined	25	25	25	25	10	
Lung, adenoma, single	4 (16%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0-24
Lung, adenoma, multiple	0 (0%)	0 (0%)	0 (0%)	0 (0%)	10* (100%)	0-4
Lung, carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0-4
Total Number of Animals with Lung tumors	4 (16%)	0 (0%)	0 (0%)	1 (4%)	10* (100%)	NA

Nominal Dose:
Group 1 – Vehicle Control (0.5% w/v methylcellulose in DI water)
Group 2 – YKP3089 5 mg/kg/day
Group 3 – YKP3089 15 mg/kg/day
Group 4 – YKP3089 35 mg/kg/day
Group 5 – Urethane
HCR: (b) (4) Historical Control Range % in control mice.
NA: Not applicable
* Statistically significant when compared to vehicle control (see Pathology report, Appendix C).

There were no SS differences in incidences of hemangiosarcomas or hemangiomas (either alone or combined) in individual organs or across all organs between the vehicle and drug-treated mice of either sex, although the total incidence of hemangiosarcoma and hemangioma appeared to be increased somewhat in HD males (sponsor's Table 3). There were SS increases in incidences of splenic hemangiosarcomas in positive controls (60 and 100% in males and females; not shown).

Table 3. Incidences of hemangiosarcomas and hemangiomas

Males					
	Group 1	**Group 2**	**Group 3**	**Group 4**	**HCR (%)**
Number Examined	25	25	25	25	
Spleen, Hemangiosarcoma	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0-16
Multicentric, Hemangiosarcoma	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0-4
Nasal Cavity, Hemangioma	0 (0%)	0 (0%)	1 (4%)	1 (4%)	NPO
Nasal cavity, Hemangiosarcoma	0 (0%)	1 (4%)	0 (0%)	1 (4%)	0-4
Lungs, Hemangiosarcoma	1 (4%)	0 (0%)	0 (0%)	1 (4%)	0-4
Skin, Hemangiosarcoma	0 (0%)	0 (0%)	1 (4%)	1 (4%)	0-4
Urinary Bladder, Hemangiosarcoma	1 (4%)	0 (0%)	0 (0%)	0 (0%)	NPO
Total animals with hemangioma/hemangiosarcoma	2 (8%)	1 (4%)	2 (8%)	6 (24%)	NA
Females					
	Group 1	**Group 2**	**Group 3**	**Group 4**	**HCR (%)**
Number Examined	25	25	25	25	
Spleen, Hemangiosarcoma	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0-16
Multicentric, Hemangiosarcoma	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0-4
Nasal Cavity, Hemangiosarcoma	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0-4
Mediastinum, Hemangiosarcoma	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0-4
Skin, Hemangiosarcoma	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0-4
Total animals with hemangiosarcoma	2 (8%)	1 (4%)	3 (12%)	0 (0%)	NA

Nominal Dose:
Group 1 – Vehicle Control Group 2 – YKP3089 5 mg/kg/day
Group 3 – YKP3089 15 mg/kg/day Group 4 – YKP3089 35 mg/kg/day
HCR: (b) (4) Historical Control Range % in control mice (Paranjpe, et al., 2013 a) (See Appendix 2).
NA: Not applicable.
NPO: Not Previously Observed in the BioReliance Historical Control Database.

The FDA reviewer's analysis showed a SS dose-response relationship in males across the vehicle control and the treated groups for the incidence of hemangiosarcoma in the whole body (p-value = 0.0474). However, the pairwise comparisons showed no tumor types with a SS increase in tumor incidences in CBM-treated groups, when compare to the vehicle control group in either male or female mice (see Table 4 below from review by Malick Mbodj, PhD).

Table 4: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or the pairwise Comparisons
Treated Groups and Control Group in Mice

sex	Organ Name	Tumor Name	0 mg Cont (N=65) P - Trend	5 mg Low (N=65) P - C vs. L	15 mg Med (N=65) P - C vs. M	35 mg High (N=65) P - C vs. H
Male	WHOLE_BODY	hemangiosarcoma	2/25 (24) 0.0474*	1/25 (24) 0.8830	1/25 (25) 0.8901	5/25 (25) 0.2258

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
*: Statistically significant at 0.05 for common or rare tumor in dose response relationship.

There were no SS differences in incidences of other tumors (non-pulmonary and non-vascular) between the vehicle and drug-treated groups (sponsor's Table 4).

Table 4. Incidences of other tumors

Males					
	Group 1	**Group 2**	**Group 3**	**Group 4**	**HCR (%)**
Number Examined	25	25	25	25	
Multicentric, Histiocytic Sarcoma	0 (0%)	1 (4%)	0 (0%)	0 (0%)	NPO
Nasal Cavity, Adenocarcinoma	0 (0%)	0 (0%)	0 (0%)	1 (4%)	NPO[1]
Liver, hepatocellular adenoma	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0-4
Harderian gland, adenoma	0 (0%)	1 (4%)	0 (0%)	1 (4%)	0-8
Harderian gland, carcinoma	1 (4%)	1 (4%)	0 (0%)	0 (0%)	0-4
Stomach, squamous cell carcinoma	1 (4%)	0 (0%)	0 (0%)	0 (0%)	0-4
Spleen, Histiocytic Sarcoma	0 (0%)	1 (4%)	0 (0%)	0 (0%)	NPO
Females					
	Group 1	**Group 2**	**Group 3**	**Group 4**	**HCR (%)**
Number Examined	25	25	25	25	
Nasal Cavity, Adenocarcinoma	1 (4%)	0 (0%)	1 (4%)	1 (4%)	0-4
Cervix, Sarcoma	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0-4
Harderian gland, adenoma	0 (0%)	2 (8%)	0 (0%)	2 (8%)	0-16
Mammary gland, adenocarcinoma	1 (4%)	0 (0%)	0 (0%)	0 (0%)	NPO
Thymus, Thymoma	1 (4%)	2 (8%)	1 (4%)	0 (0%)	0-12

Nominal Dose:
Group 1 – Vehicle Control Group 2 – YKP3089 5 mg/kg/day
Group 3 – YKP3089 15 mg/kg/day Group 4 – YKP3089 35 mg/kg/day
HCR: (b) (4) Historical Control Range % in control mice (Paranjpe, et al., 2013 a) (See Appendix 2).
1 = Adenoma reported in HCR

Non Neoplastic

Drug-induced non-neoplastic lesions were limited to centrilobular hypertrophy of the liver in MD and HD males and HD females.

Toxicokinetics

Mean concentrations (from up to 3 animals/sex/group/time point) of CBM in CByB6F1 mouse plasma, 2 and 4 hours after dosing on Day 177 is shown in Table 6.

Table 6. Plasma levels in CByB6F1 mice

YKP3089 Dose (mg/kg/day)	Sex	Time (h)	YKP3089 Mean Plasma Concentration (µg/mL)
5	Females	2	9.370
		4	7.325*
	Males	2	10.737
		4	8.223
15	Females	2	29.300
		4	27.467
	Males	2	30.900
		4	27.467
35	Females	2	54.467
		4	54.267
	Males	2	59.500
		4	56.300

*calculated without the outlier animal #1259; with this animal the mean plasma concentration is 5.041 µg/mL

Study title: 104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with YKP3089 in Rats

Study no.:	(b) (4) Study Number 8286441
Study report location:	4.2.3.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/1/13
GLP compliance:	Yes/
QA statement:	Yes
Drug, lot #, and % purity:	AA-022087-Batch-04-2009, 99.9%
CAC concurrence:	Yes (minutes dated 5/21/13)

Key Study Findings

CBM (0, 4, 8, or 20 mg/kg/day) was administered to SD rats by oral gavage. Dosing was terminated during Weeks 87 and 90 for males and females, respectively, due to survival reaching 20 or fewer in the respective control groups. Mortality was not increased by CBM. Administration of CBM had no effects on body weight or body weight gain in either sex. There were no drug-related clinical signs. No drug-related neoplastic findings were observed. Week 26 exposures (AUC) were 173000 and 232000 ng·hr/mL in HD males and females, respectively.

Adequacy of Carcinogenicity Study

Adequate

Evaluation of Tumor Findings

Negative

Methods

Doses:	0, 4, 8, 20 mg/kg
Frequency of dosing:	QD
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose
Basis of dose selection:	13/26 week toxicity study in SD rat
Species/Strain:	Crl:CD(SD)
Number/Sex/Group:	65
Age:	5-6 weeks
Weight:	118 to 221 g for males,122 to 186 g for females
Paradigm for dietary restriction:	no
Dual control employed:	no
Interim sacrifice:	no
Satellite groups:	TK groups, 6-9/sex/grp
Deviation from study protocol:	Early termination

CBM (0 (0.5% w/v methylcellulose), 4, 8, or 20 mg/kg) was administered daily via oral gavage (5 mL/kg) to Crl:CD(SD) rats (65/sex/grp + 6-9/sex/grp TK) for 87 (males) or 90 weeks (females). The terminal sacrifice was carried out before 104 weeks when the criterion of 20 survivors in controls was reached. Endpoints were mortality, clinical observations, mass observations, body weights, food consumption, and anatomic pathology. Blood samples were collected for TK evaluations at 13 and 26 weeks.

Group	Subgroup	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)[a]	1 (Carcinogenicity)	65	65	0	0
	2 (Toxicokinetic)	6	6	0	0
2 (Low)	1 (Carcinogenicity)	65	65	4	0.8
	2 (Toxicokinetic)	9	9	4	0.8
3 (Mid)	1 (Carcinogenicity)	65	65	8	1.6
	2 (Toxicokinetic)	9	9	8	1.6
4 (High)	1 (Carcinogenicity)	65	65	20	4
	2 (Toxicokinetic)	9	9	20	4

a Group 1 received vehicle control article only.

Doses were based on the results of a 13/26-week toxicity study in S-D rats, with oral (gavage) doses of 0 (0.5% methylcellulose vehicle), 12, 24, or 48 mg/kg, in which mortality, CNS clinical signs, and renal histopathology were seen at the HD. The ECAC concurred with the sponsor's proposed doses of 0, 4, 8, or 20 mg/kg/day, by oral gavage, in both sexes based on the findings of CNS clinical signs and mortality in females and CNS clinical signs and kidney toxicity in males at the high dose of 48 mg/kg in the 13/26-week study

Observations and Results

Mortality

There was no drug-related effect on survival (Table 1). Dosing was stopped during Weeks 87 and 90 for males and females, respectively, due to survival reaching 20 or fewer in the respective control groups. LD and MD males had higher unadjusted survival than the control group and LD females had higher unadjusted survival than the control group.

Table 1. Mortality in rat carcinogenicity study

Males

Group	Dose Level (mg/kg/day)	Surviving Animals	% Survival
1	0	20/65	30.8
2	4	27/65 (63)[a]	41.5 (42.8)[b]
3	8	28/65 (64)[a]	43.1 (43.8)[b]
4	20	25/65 (64)[a]	38.5 (39.1)[b]
Summary		100/260 (256)[a]	38.5 (39.1)[b]

a Total number of animals adjusted for accidental deaths
b Percentage survival adjusted for accidental deaths

Females

Group	Dose Level (mg/kg/day)	Surviving Females	% Survival
1	0	19/65	29.2
2	4	29/65	44.6
3	8	19/65	29.2
4	20	22/65	33.8
Summary		89/260	34.2

Clinical Signs

There were no drug-related increases in clinical signs.

Body Weights and Food Consumption

There were no clearly drug-related effects on food consumption, BW gain (Table 2), or BW (Figure 1).

Table 2. Body weight gain in rat carcinogenicity study

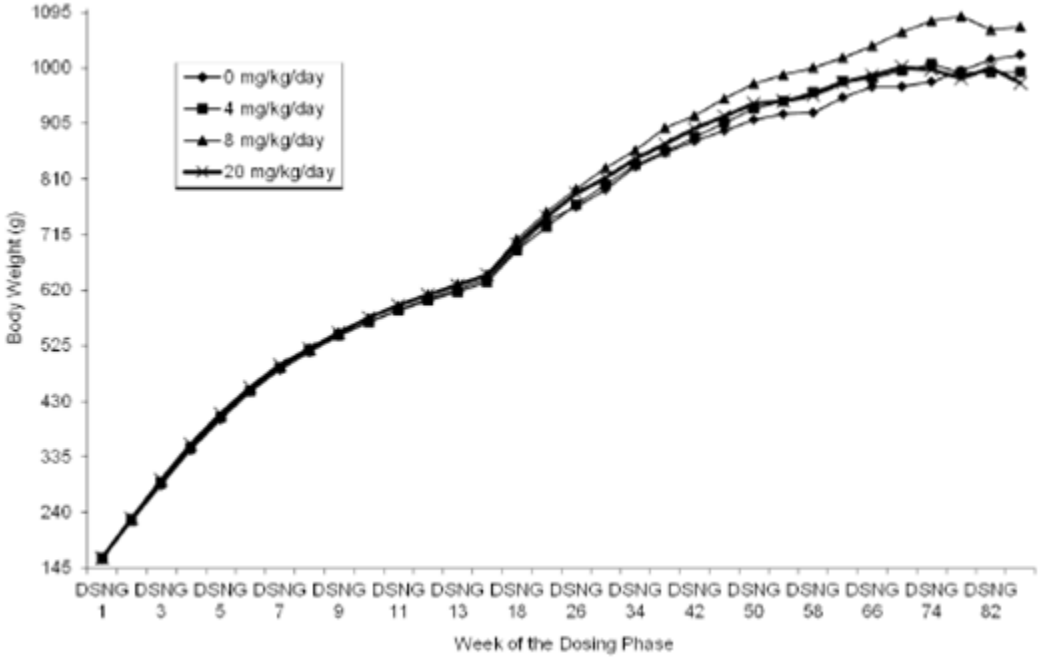
Test Article (dosage) 1 2 3 4
YKP3089 mg/kg/day 0 4 8 20

Group/ Subgroup/ Sex	Phase Wk	Data Presented in "g" Interval X through X DSNG 1 - 14	14 - 26	26 - 54	54 - 86	1 - 86
1/1/M	Mean	478	126	167	124	864
	SD	60.1	38.1	73.4	81.9	156.8
	N	64	62	55	21	21
	P(overall)	0.5089	0.0195	0.0969	0.0056	0.0974
2/1/M	Mean	471	132	175	81	831
	SD	52.6	30.3	78.4	147.8	193.5
	N	64	63	59	31	31
	P(v1)	-	0.6461	-	0.9730	-
3/1/M	Mean	486	145*	195	136	913
	SD	67.3	40.0	73.5	77.3	183.8
	N	65	65	57	31	31
	P(v1)	-	0.0069	-	0.7217	-
4/1/M	Mean	484	137	165	71*	812
	SD	64.9	32.9	54.0	71.6	106.0
	N	64	62	57	29	29
	P(v1)	-	0.1955	-	0.0482	-
	Statistics	A	A	A	AT	A
1/1/F	Mean	189	59	148	135	511
	SD	32.1	30.6	59.1	69.0	126.2
	N	65	65	58	24	22
	P(overall)	0.7405	0.4161	0.0665	0.4761	0.8164
2/1/F	Mean	194	57	130	121	493
	SD	30.7	33.0	55.5	67.8	139.3
	N	65	65	64	35	30
3/1/F	Mean	188	53	130	111	482
	SD	31.2	30.5	53.3	76.9	131.2
	N	65	64	62	24	20
4/1/F	Mean	189	62	151	110	475
	SD	35.0	31.5	56.2	42.4	127.4
	N	65	64	59	29	22
	Statistics	A	A	A	A	A

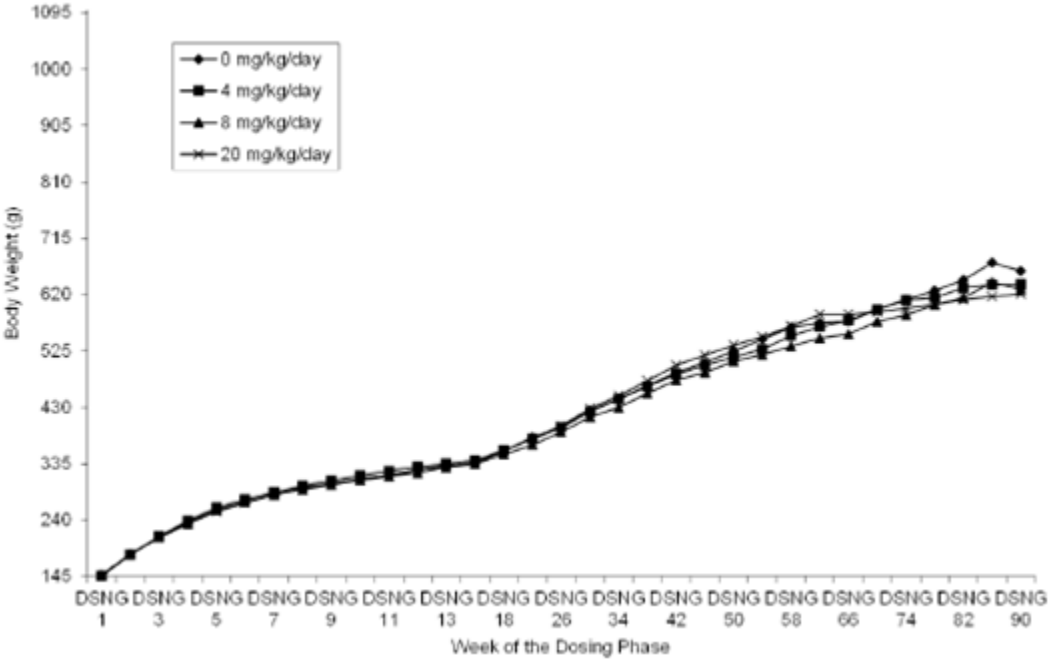
* P<=0.05
A = ANOVA and Dunnett's
T = Rank-transformed data

Figure 1. Body weight in rat carcinogenicity study

Males



Females



Gross Pathology

No drug-related macroscopic findings were observed.

Histopathology

Neoplastic

No neoplasms showed a SS dose-response or incidence in males. In females, mammary adenoma had a SS dose-response and increased incidence at the HD compared to C in the sponsor's analysis (Table 3). However, the incidences were low and preneoplastic lesions (hyperplasia) and mammary gland fibroadenomas and carcinomas did not occur with a dose-dependent incidence or severity; the incidence and severity of hyperplasia were actually decreased in HD females. Therefore, the increase in mammary gland adenomas was not considered drug-related.

Table 3. Mammary tumors in 2-year rat study

			Unadjusted Lifetime Incidence Rate			
	Group	Trend	1	2	3	4
Tissue and Lesion	Dose Level (mg/kg/day)	(1,2,3,4)	0	4	8	20
Mammary Gland, Female B-Fibroadenoma						
	No. Examined		65	65	65	65
	Fatal Tumor		4	4	4	5
	Incidental Tumor		14	29	15	20
	Total Tumors		18	33	19	25
	Log-Rank P-value (v1)	0.4756	N/A	0.0232*	N/A	0.1998
	Wilcoxon P-value (v1)	0.6033	N/A	0.0714	N/A	0.3704
Mammary Gland, Female B-Adenoma						
	No. Examined		65	65	65	65
	Fatal Tumor		0	0	0	0
	Incidental Tumor		0	0	2	3
	Total Tumors		0	0	2	3
	Log-Rank P-value (v1)	0.0130*	N/A	N/A	0.0914	0.0391*
	Wilcoxon P-value (v1)	0.0825	N/A	N/A	0.0983	0.0397*
Mammary Gland, Female B-Adenoma/M-Carcinoma/B-Fibroadenoma						
	No. Examined		65	65	65	65
	Fatal Tumor		15	13	12	10
	Incidental Tumor		20	31	22	24
	Total Tumors		35	44	34	34
	Log-Rank P-value (v1)	0.8703	N/A	0.2312	N/A	N/A
	Wilcoxon P-value (v1)	0.9353	N/A	0.3029	N/A	N/A
Mammary Gland, Female B-Adenoma/B-Fibroadenoma						
	No. Examined		65	65	65	65
	Fatal Tumor		4	4	4	5
	Incidental Tumor		14	29	16	22
	Total Tumors		18	33	20	27
	Log-Rank P-value (v1)	0.3432	N/A	0.0232*	0.3462	0.1200
	Wilcoxon P-value (v1)	0.5544	N/A	0.0714	0.3093	0.3189

* = Significant at 5% level; ** = Significant at 1% level;

The FDA reviewer's also analysis showed a SS dose-related increase in the incidence of benign adenoma in the mammary gland in females (p-value = 0.0249), since this tumor type is considered a rare tumor (Table 2 from review by Malick Mbodj, PhD); however, the pairwise comparisons showed no SS increase in mammary gland tumors in females or other tumor types in YKP3089 treated groups, when compared to the vehicle control group, in either males or females.

Table 2: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or the pairwise Comparisons
Treated Groups and Control Group in Rats

sex	Organ Name	Tumor Name	0 mg Cont (N=65) P - Trend	4 mg Low (N=65) P - C vs. L	8 mg Med (N=65) P - C vs. M	20 mg High (N=65) P - C vs. H
Female	Mammary Gland, Female	B-Adenoma	0/65 (27) 0.0249*	0/65 (31) NC	2/65 (29) 0.2636	3/65 (30) 0.1388
		B-Fibroadenoma	18/65 (37) 0.3576	33/65 (47) 0.0372@	19/65 (37) 0.5000	25/65 (41) 0.1936
		B-Adenoma/ B-Fibroadenoma	18/65 (37) 0.2359	33/65 (47) 0.0372@	20/65 (38) 0.4547	27/65 (42) 0.1204

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.
*: Statistically significant at 0.025 for rare tumor in dose response relationship,
@: not Statistically significant at 0.01 for common tumor in pairwise comparison.

Non Neoplastic

Centrilobular hepatocellular hypertrophy and vacuolation were dose-dependently increased at all doses. A possible increase in retinal degeneration was seen in drug-treated groups (sponsor's Table 4).

Table 4. Retinal degeneration in 2-year rat study

	YKP3089							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	4	8	20	0	4	8	20
Number Examined	20	27	28	25	19	29	19	22
Eye								
Degeneration/atrophy, retina								
Total Finding Incidence	1	3	3	3	1	5	2	8
Percent Incidence	5%	11%	11%	12%	5%	17%	11%	36%

Toxicokinetics

The increases in Cmax and AUC0-24 were generally dose-proportional and values were higher in females than males (Table 5).

Table 5. TK parameters in rat carcinogenicity study

Interval	Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
Week 26	2	4	M	3290	2.00	38,200
			F	5590	4.00	76,400
	3	8	M	7020	2.00	83,300
			F	8950	4.00	118,000
	4	20	M	13,000	4.00	173,000
			F	20,500	4.00	232,000

F = Female; M = Male

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: YKP3089: study of female and male fertility and early embryonic development to implantation period in rats

Study no.:	(b) (4) Study No.: 11-4381
Study report location:	4.2.3.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/3/11
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot # AA-022087, 99.9%

Key Study Findings

No drug-related mortality, clinical signs, BW changes, or adverse effects on male or female mating or fertility indices, estrous cyclicity, C-section parameters, or sperm parameters observed. At the end of the dosing period all drug-treated males had dose-related centrilobular hepatocellular hypertrophy that correlated with increased liver weights and increases in CYP2B1/2, 2E1, 3A1/2, and CYP2A1. The NOAEL for reproductive toxicity was 44 mg/kg/day.

Methods

Doses:	0 (vehicle), 11, 22, 44 mg/kg
Frequency of dosing:	QD
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% w/v aqueous methylcellulose
Species/Strain:	S-D rat
Number/Sex/Group:	22/sex/group
Age:	10 weeks at initiation of dosing
Weight:	Male: 306.7 to 384.8 g Female: 191.3 to 256.9 g
Satellite groups:	none
Deviation from study protocol:	None that impacted study quality or integrity

S-D rats (22/sex/group) were administered oral (gavage) doses of CBM (0 (0.5% w/v aqueous methylcellulose), 11, 22, or 44 mg/kg/day) for 2 weeks prior to mating in females and 4 weeks prior mating in males and continuing throughout the mating period to GD 6 in females and 2 days after the end of pairing in males. Females were euthanized on GD 14 and C-sections performed. Parameters evaluated were: viability, clinical observations, body weights, food consumption, mating performance and fertility, organ weights, and macroscopic observations in males and females; and microscopic pathology, sperm analysis, and Cytochrome P450 in males only.

Dose selection was based on the results of the 28-day rat study in which mortality and severe clinical signs were seen at 100 mg/kg and the 13-week portion of the 26-week rat study in which animals were euthanized due to drug-related clinical signs at the HD of 48 mg/kg.

Observations and Results

Mortality:

There were no drug-related deaths.

Clinical signs:

No drug-related clinical signs were reported.

Body weight and food consumption:

BW and food consumption did not appear to be affected.

Fertility and reproductive performance:

There were no effects on male or female mating or fertility indices (100% in all drug-treated groups, Table 1) or on male or female mating performance, as indicated by the number of days to mating. There were no drug-related effects on estrous cycling. C-section parameters were similar among groups (Table 2).

Table 1. Mating and fertility indices in rat FEED study

	Dose Level (mg/kg/day)			
	0	**11**	**22**	**44**
Mating Index (%) (male and female)	100	100	100	100
Fertility Index (%) (male and female)	90.9	100	100	100

Table 2.

Females	Summary of Cesarean Section Data								
	Corpora Lutea	Implant-ations	Resorptions Early	Resorptions Late	Resorptions Total	Dead Fetuses	Live Fetuses	Pre Impl. Loss (%)	Post Impl. Loss (%)
Group 1 - 0.0 mg/kg/day									
Mean	16.4	15.8	0.9	0.2	1.1	0.0	14.7	4.0	7.1
SD	2.48	2.84	1.17	0.89	1.97	0.00	3.28	10.13	11.74
N	20	20	20	20	20	20	20	20	20
Group 2 - 11.0 mg/kg/day									
Mean	16.7	16.0	1.1	0.0	1.1	0.0	14.9	4.0	6.6
SD	2.21	2.00	1.02	0.00	1.02	0.00	1.82	6.26	6.11
N	22	22	22	22	22	22	22	22	22
Group 3 - 22.0 mg/kg/day									
Mean	17.2	16.8	0.5	0.0	0.5	0.0	16.2	2.5	3.4
SD	2.20	1.93	0.86	0.00	0.86	0.00	2.14	3.53	5.32
N	22	22	22	22	22	22	22	22	22
Group 4 - 44.0 mg/kg/day									
Mean	15.7	15.0	0.9	0.0	0.9	0.0	14.1	3.8	6.0
SD	2.35	2.25	1.58	0.00	1.58	0.00	2.67	6.04	10.55
N	21	21	21	21	21	21	21	21	21

Necropsy:

No drug-related differences in male reproductive organ weights (not examined microscopically) or sperm motility, count, or morphology were observed. In males, increased liver weights correlated with a dose-related incidence and severity of centrilobular hypertrophy and increases in CYP2B1/2, 2E1, 3A1/2, and CYP2A1.

9.2 Embryofetal Development

Study title: YKP 3089 - Embryotoxicity study by the oral route (gavage) in the rat (Segment II)

Study no.:	(b) (4) Study No.: AA26092
Study report location:	4.2.3.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	24 May 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot # 1401-1401-05-501, 99.6%

Key Study Findings

Oral (gavage) administration of CBM (0, 10, 30, or 60 mg/kg) to pregnant S-D rats (25/group) throughout organogenesis (GD 6-17) resulted in developmental toxicity at the HD. Maternal toxicity was excessive at the HD (3 deaths, 2 moribund sacrifices, marked clinical signs, 54% decrease in BW gain from GD 6-18), and there was a decrease in the number of females with viable fetuses at term (n=13). Embryofetal toxicity at the HD included increased postimplantation loss (4X), a reduction in fetal weight (-13%), and incomplete ossification. Malformations (anophthalmia and enlarged ventricle) were seen in 3(2) HD fetuses(litters), but as noted in the report, "Marked maternal toxicity at 60 mg/kg/day (far exceeding the minimal maternal toxicity expected in the high dose) confounded the interpretation of any teratogenic potential at this dose level." The MD produced a 14% reduction (SS) in maternal BW gain over the dosing period, but GD 20 BW was only 3.5% below C (NS). There was no clear evidence of embryofetal toxicity at the MD.

Methods

Doses:	0 (vehicle), 10, 30, 60 mg/kg
Frequency of dosing:	QD
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% w/v aqueous methylcellulose
Species/Strain:	S-D rat
Number/Sex/Group:	25/sex/group
Age:	10-13 weeks
Weight:	191 to 270 g
Satellite groups:	none
Deviation from study protocol:	None that impacted study quality or integrity

CBM (0 (0.5% MC), 10, 30, 60 mg/kg/day) was administered by daily oral gavage to time-mated female S-D rats (25/group) from GD 6 to 17. Clinical condition, body weights and food consumption were monitored. C-sections were performed and litter parameters were recorded on GD20. At necropsy, females were examined macroscopically, and fetuses

were weighed, sexed and examined for external abnormalities. Approximately half of the fetuses were examined internally prior to processing for skeletal examination. The remaining fetuses were preserved for visceral examination by the modified Wilson-Barrow technique.

Dose selection was based on the results of a dose range-finding study (# AA26091, conducted in the same facility as the definitive study, formerly (b) (4) (b) (4) in which CBM (0, 20, 40, or 60 mg/kg/day) was administered by oral gavage to S-D rats (6/group) from GDs 6 to 17. There were no maternal deaths or animals sacrificed. Drug-related clinical signs (subdued behavior, loss of balance, decubitus/prostration, piloerection and hypersalivation) were observed at the HD. Maternal BW gain during the dosing period was decreased 20 and 52% and GD 20 BW was decreased 5 and 10%, compared to C, at the MD and HD, respectively. There were dose-related increases in early resorptions (total number: 1, 4, 5, and 7) and decreases in fetal BWs (4.1, 4.0, 3.8, and 3.8 g in C, LD, MD, and HD). There were no dead fetuses and no external malformations.

Observations and Results

Mortality:

Five HD females (nos. 77, 88, 94, 97 and 98) were found dead or sacrificed in a moribund condition (between GDs 10 and 19). Clinical signs prior to death or sacrifice of these females included a thin appearance, pallor, subdued behavior, prostration, loss of balance and raised hair. The death/sacrifice of these females was clearly attributed to the maternal toxicity observed at this dose. There was no mortality in the control or lower dose groups.

Clinical signs:

Drug-related clinical changes consistent with a poor general condition such as a thin appearance, pallor, subdued behavior, lacrimation, loss of balance, raised hair and colored (pale) feces were observed in the majority of HD group females.

Body weight and food consumption:

A marked (SS) drug-related BW loss occurred during the first 5 days of treatment (days 6 to 11 of gestation) and was 54% below C from GD 6-18 at the HD (Table 1, Figure 1). Absolute BW was decreased (SS) between GDs 11 and 20 (15% below C on GD20, Table 2). There was a transient but SS reduction in BW gain at the MD, and absolute BW decreases were SS on GD 11 and 15.

Table 1.

SUMMARY OF GESTATION BODY WEIGHT CHANGES (GRAMS)

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 30 mg/kg/day	Group 4 High dose 60 mg/kg/day
DAYS 0 TO 6	MEAN	43.1 d	43.5	40.5	40.7
	S.D.	9.8	7.4	7.7	7.9
	N	25	25	24	13
DAYS 6 TO 11	MEAN	28.4 k	28.2	11.4**	-21.4**
	S.D.	3.4	6.4	13.6	23.2
	N	25	25	24	13
DAYS 11 TO 15	MEAN	25.8 d	24.5	25.6	30.3
	S.D.	7.8	6.8	8.7	12.3
	N	25	25	24	13
DAYS 15 TO 18	MEAN	35.7 d	36.7	40.3	32.8
	S.D.	10.8	9.7	9.6	10.7
	N	25	25	24	13
DAYS 18 TO 20	MEAN	27.8 d	25.6	28.7	27.8
	S.D.	9.8	7.0	7.8	11.8
	N	25	25	24	13
DAYS 6 TO 18	MEAN	90.0 k	89.4	77.4*	41.7**
	S.D.	18.0	16.4	15.6	36.0
	N	25	25	24	13

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test * = p<0.05 ** = p<0.01

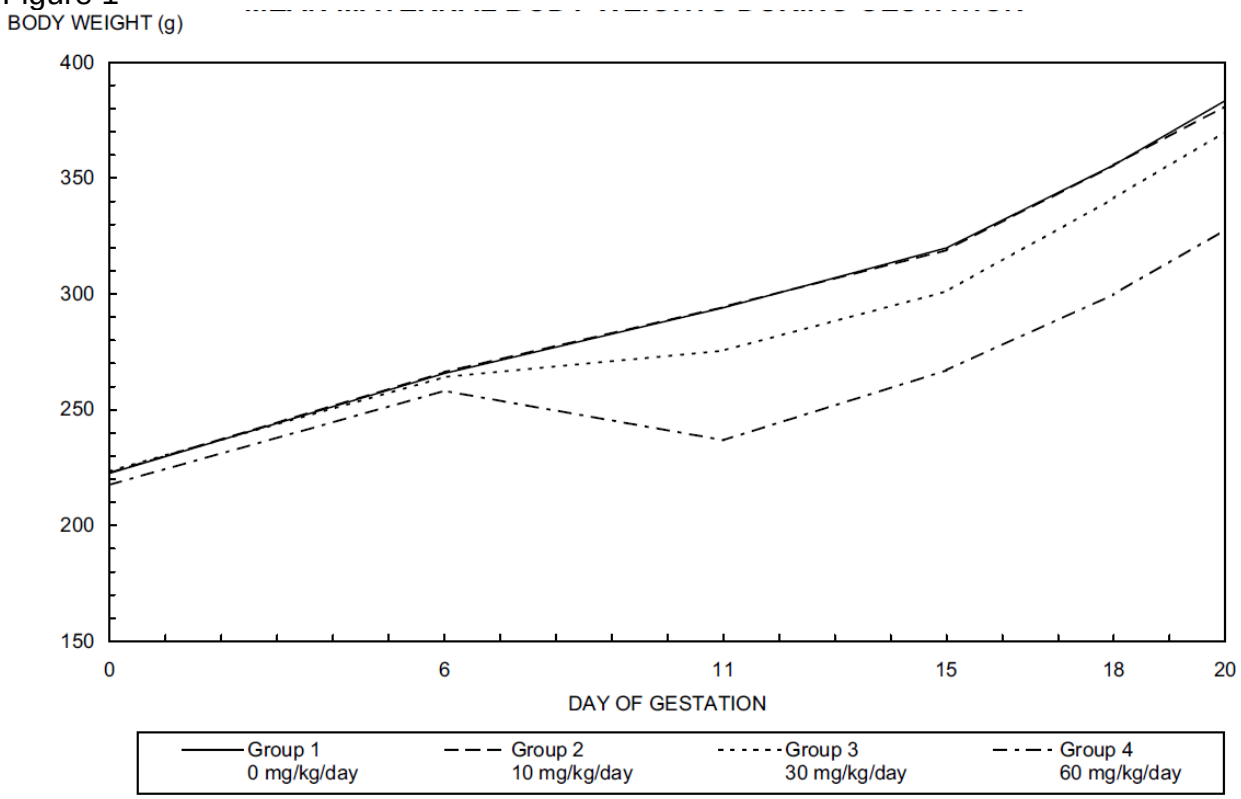
Table 2

SUMMARY OF GESTATION BODY WEIGHTS (GRAMS)

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 30 mg/kg/day	Group 4 High dose 60 mg/kg/day
DAY 0	MEAN	222.6 d	222.7	223.5	217.5
	S.D.	16.5	15.9	13.0	16.8
	N	25	25	24	13
DAY 6	MEAN	265.7 d	266.2	264.1	258.2
	S.D.	16.7	16.2	14.0	15.4
	N	25	25	24	13
DAY 11	MEAN	294.1 d	294.4	275.5**	236.8**
	S.D.	17.5	19.4	15.8	28.0
	N	25	25	24	13
DAY 15	MEAN	319.9 k	318.9	301.1*	267.1**
	S.D.	19.8	22.1	18.4	38.3
	N	25	25	24	13
DAY 18	MEAN	355.7 d	355.6	341.4	299.9**
	S.D.	24.3	26.3	21.7	41.3
	N	25	25	24	13
DAY 20	MEAN	383.5 d	381.2	370.2	327.8**
	S.D.	29.8	31.0	24.2	47.9
	N	25	25	24	13

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test * = p<0.05 ** = p<0.01

Figure 1



Toxicokinetics

TK analysis was not conducted in this study.

Necropsy

There were no drug-related macroscopic findings at the terminal necropsy examination of the adult females.

Pregnancy and Litter Data:

There 25, 25, 24, and 19 pregnant females at the terminal caesarean sections in the respective groups (Table 3). Two HD females were not pregnant and there were 4 deaths. In addition, 6/19 HD dams had no viable fetuses at term due to complete litter loss. Post-implantation loss was increased (4 fold) and the number of live fetuses decreased (-25 %) in the HD group. This was primarily due to early resorptions in the 6 HD females with no viable fetuses. BWs were decreased (SS) in surviving fetuses at the HD. There were no apparent litter effects at the LD and MD.

Table 3

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 30 mg/kg/day	Group 4 High dose 60 mg/kg/day
Pregnant	N	25	25	24	19
Dams with no Viable Fetuses	N	0	0	0	6
Dams with Viable Fetuses	N	25	25	24	13
Corpora Lutea	TOTAL	343	323	344	269
No. per animal	MEAN	13.7 d	12.9	14.3	14.2
	S.D.	3.6	3.5	3.4	4.2
Implantation Sites	TOTAL	297	283	319	234
No. per animal	MEAN	11.9 d	11.3	13.3	12.3
	S.D.	4.4	3.8	3.1	3.6
Preimplantation Loss	TOTAL	46	40	25	35
No. per animal	MEAN	1.8 d	1.6	1.0	1.8
	S.D.	2.7	2.4	1.3	2.2
% per animal	MEAN%	15.5 k	13.0	6.7	11.8
	S.D.	22.8	20.7	7.8	12.6
Live Fetuses	TOTAL	269	259	291	154
No. per animal	MEAN	10.8 k	10.4	12.1	8.1
	S.D.	4.3	3.6	3.3	6.4
Males	TOTAL	127	122	141	67
	MEAN%	48.1 k	43.9	46.5	45.2
	S.D.	21.3	17.2	19.8	15.4
Females	TOTAL	141	136	150	87
	MEAN%	51.9 k	56.1	53.5	54.8
	S.D.	21.3	17.2	19.8	15.4
Postimplantation Loss	TOTAL	28	24	28	80
No. per animal	MEAN	1.1 k	1.0	1.2	4.2
	S.D.	1.0	0.8	1.1	5.1
% of implants per animal	MEAN%	10.7 k	8.0	9.5	39.1
	S.D.	11.1	6.6	9.6	44.1
Dead Fetuses	TOTAL	0	0	0	0
No. per animal	MEAN	0.0 k	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
% of implants per animal	MEAN%	0.0 k	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Resorptions: Early	TOTAL	28	23	27	79
No. per animal	MEAN	1.1 k	0.9	1.1	4.2
	S.D.	1.0	0.8	1.1	5.1
% of implants per animal	MEAN%	10.7 k	7.7	9.2	38.7
	S.D.	11.1	6.8	9.3	44.2
Resorptions: Late	TOTAL	0	1	1	1
No. per animal	MEAN	0.0 k	0.0	0.0	0.1
	S.D.	0.0	0.2	0.2	0.2
% of implants per animal	MEAN%	0.0 k	0.3	0.3	0.4
	S.D.	0.0	1.5	1.4	1.8
Fetal Body Weight (g)	MEAN	3.9 d	4.0	3.9	3.4*
	S.D.	0.4	0.5	0.5	0.7
	N	25	25	24	13
Male Fetuses	MEAN	4.1 k	4.2	4.1	3.6*
	S.D.	0.5	0.4	0.3	0.7
Female Fetuses	MEAN	3.8 d	3.9	3.7	3.3**
	S.D.	0.5	0.5	0.5	0.7

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test * = p<0.05 ** = p<0.01

Fetal Evaluations:

There were 1 and 3 (2) malformed fetuses (litters) in groups 3 and 4, respectively, compared with none in the control and LD groups (Table 4). Two fetuses from different high dose litters (dam nos. 79 and 90) had anophthalmia. Another fetus from HD dam no. 79 together with 1 from the MD group (dam no. 52) had enlarged ventricular chambers. The frequencies were above the historical background for the lab. There were also 2 fetuses from the same HD litter with dilated cerebral ventricles.

Table 4.

SUMMARY OF FETAL VISCERAL OBSERVATIONS

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 10 mg/kg/day	Group 3 Intermed. dose 30 mg/kg/day	Group 4 High dose 60 mg/kg/day
Litters Evaluated	N	23	24	24	13
Fetuses Evaluated	N	128	123	137	73
Live	N	128	123	137	73
Dead	N	0	0	0	0
EYES					
Litter Incidence	N	0	0	0	2
Fetal Incidence	N	0	0	0	2
M ANOPHTHALMIA					
Fetal Incidence	N	0	0	0	2
	%	0.0	0.0	0.0	2.7
Litter Incidence	N	0 f	0	0	2
	%	0.0	0.0	0.0	15.4
BRAIN					
Litter Incidence	N	0	0	0	1
Fetal Incidence	N	0	0	0	2
A CEREBRAL VENTRICLE : DILATED					
Fetal Incidence	N	0	0	0	2
	%	0.0	0.0	0.0	2.7
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	7.7
HEART					
Litter Incidence	N	0	0	1	1
Fetal Incidence	N	0	0	1	1
M VENTRICULAR CHAMBER : ENLARGED					
Fetal Incidence	N	0	0	1	1
	%	0.0	0.0	0.7	1.4
Litter Incidence	N	0 f	0	1	1
	%	0.0	0.0	4.2	7.7

Statistical key: f=Chi2/Fisher Exact test
OBSERVATION CODE: M-MALFORMATION V-VARIATION A-ANOMALY

Increased incidences of reduced ossification (primarily sternum and caudal vertebrae) and unilateral rudimentary 14th ribs were seen in HD fetuses, consistent with generalized incomplete ossification in association with the reduced fetal weight in the HD group.

Study title: YKP 3089 - Embryotoxicity study by the oral route (gavage) in the rabbit (Segment II)

Study no.:	30/020
Study report location:	4.2.3.5
Conducting laboratory and location:	(b) (4)
Date of study report:	9/27/04
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	DIT030602, 100%

Key Study Findings

Oral (gavage) administration of CBM (0, 4, 12, or 36 mg/kg) to pregnant NZW rabbits throughout organogenesis (GD 6-19) resulted in developmental and maternal toxicity. Embryofetal mortality was increased (postimplantation loss 2X C) at the HD, which was also associated with decreased maternal BW gain. There were no apparent drug-related increases in fetal abnormalities. Maternal plasma exposures (AUC) at the NOAEL for developmental toxicity (12 mg/kg) were 63 and 51 ug.h/mL on GD 6 and 19, respectively.

Methods

Doses:	0, 4, 12, 36 mg/kg/day
Frequency of dosing:	QD
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose in water
Species/Strain:	New Zealand White
Number/Sex/Group:	20/sex/group
Satellite groups:	no
Study design:	Dosing GD 6-19, C-section GD 29
Deviation from study protocol:	Due to low pregnancy rate in HD group (14/20; 70%), 16 HD and 8 C females added to study

Each fetus was examined for external defects then killed by an intraperitoneal injection of sodium pentobarbitone. All fetuses were examined viscerally and sexed at the time of caesarean section. Following this, the head was removed from approximately half of the fetuses in each litter and placed in Harrison's fluid to be examined by serial sectioning. The eviscerated fetal carcasses were processed for skeletal examination. The skeletal examinations were performed following maceration of the soft tissues with aqueous potassium hydroxide, staining of the skeleton with Alizarin red, then passage into glycerol.

Doses were based on a preliminary study ((b) (4) 30/021) in which oral (gavage) administration of CBM (0, 20, 30, or 40 mg/kg) to time-mated female NZW rabbits (6/group) from GD 6 to 19 resulted in transient maternal BW loss (GDs 6-13) and decreased fetal BW (8% compared to C) at the HD. There were no effects on embryofetal mortality or external structural abnormalities (fetuses not examined for visceral or skeletal abnormalities).

Observations and Results

Mortality

One HD female was sacrificed after aborting on GD 21 of gestation following a prolonged period of treatment-related reduced food consumption and body weight loss. One C female was sacrificed under similar circumstances on GD 27.

Clinical Signs

The only drug-related clinical sign was an increased incidence of persistent reduction in fecal output during the treatment period in the HD group compared to C.

Body Weight

BW gain over the course of treatment (GDs 6-20) was decreased (64% compared to C, SS) at the HD, primarily due to BW loss during the first 3 days of treatment (Table 1). The effect on BW did not appear (Figure 1) to be excessive for a rabbit EFD study.

Table 1.

SUMMARY OF GESTATION BODY WEIGHT CHANGES (GRAMS)

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 4 mg/kg/day	Group 3 Intermed. dose 12 mg/kg/day	Group 4 High dose 36 mg/kg/day
DAYS 0 TO 6	MEAN	107 d	103	95	93
	S.D.	84	69	78	77
	N	23	19	19	26
DAYS 6 TO 9	MEAN	19 k	21	1	-64**
	S.D.	36	31	72	77
	N	23	19	19	26
DAYS 9 TO 13	MEAN	61 k	63	73	14*
	S.D.	52	40	43	86
	N	23	19	19	26
DAYS 13 TO 16	MEAN	40 d	66	62	59
	S.D.	51	54	62	89
	N	23	19	19	26
DAYS 16 TO 20	MEAN	21 d	11	16	42
	S.D.	41	55	50	73
	N	23	19	19	26
DAYS 20 TO 24	MEAN	45 d	42	42	43
	S.D.	54	46	46	71
	N	23	19	19	26
DAYS 24 TO 29	MEAN	93 d	46	79	82
	S.D.	67	64	68	64
	N	23	19	19	26
DAYS 6 TO 20	MEAN	141 d	161	152	51*
	S.D.	90	104	107	168
	N	23	19	19	26

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test * = p<0.05 ** = p<0.01

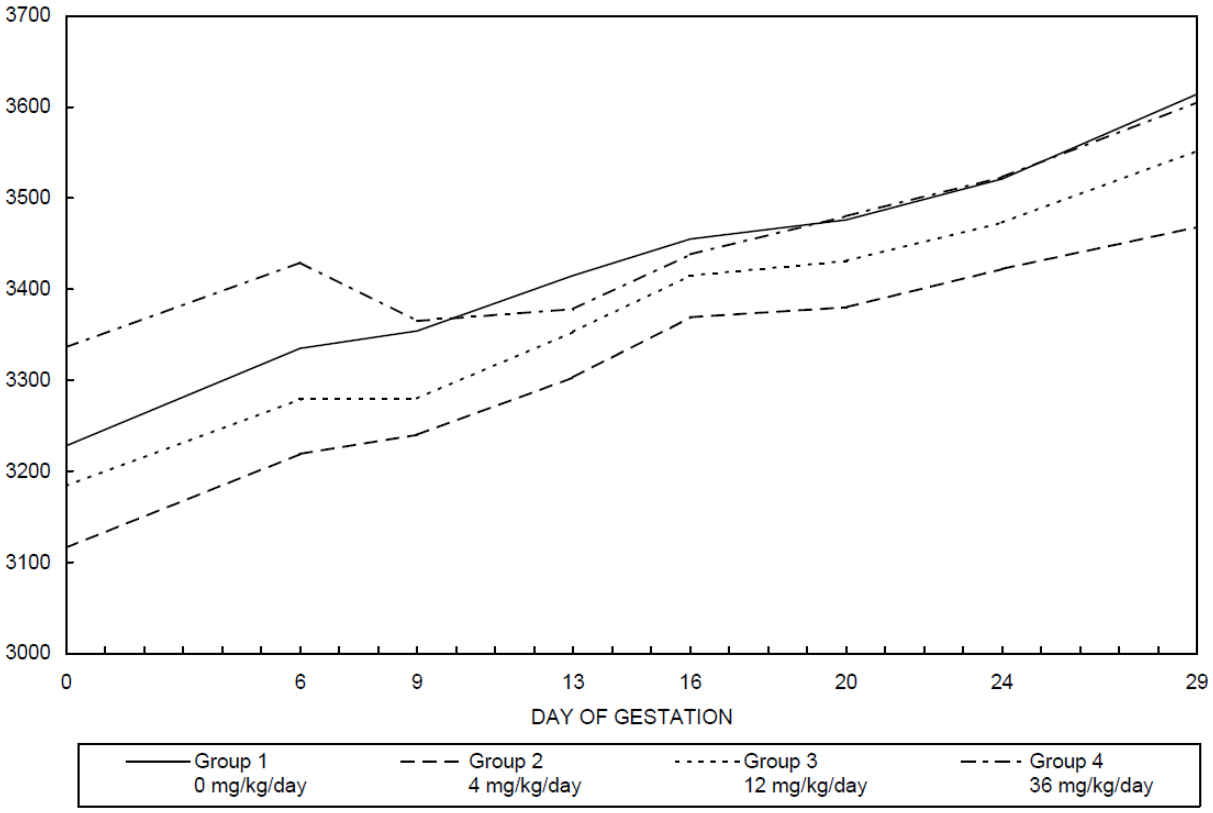


Figure 1. Body weight in rabbit embryofetal development study

Toxicokinetics

TK analysis was not conducted in this study, but in a separate TK study ((b) (4) Study No. 15-4438, report dated 7/25/16) conducted in pregnant NZW rabbits with the same doses, the following values were determined.

Table 2. TK parameters in rabbits

Dose level (mg/kg/day)	C_{max} (ng/mL)		AUC_{0-24} (ng.h/mL)	
	GD 6	GD 19	GD 6	GD 19
4	1540 (240)	1640 (250)	15100 (3500)	12800 (3000)
12	4970 (1080)	5080 (1170)	63100 (25000)	51300 (16800)
36	21100 (3800)	19500 (3800)	311000 (62000)	240000 (100000)

Necropsy

There were no drug-related macroscopic changes noted among adult females in any group.

Cesarean Section Data

From the initial group sizes of 20, there were 18, 19, 19, and 12 pregnant females at the terminal caesarean sections in C, LD, MD, and HD groups, respectively. All of these does had viable fetuses with the exception of 1 each in the C and HD groups (nos. 4648 and 4708 respectively). In addition, 1 HD female (no. 4691) aborted on day 21 of gestation. Because of the low pregnancy rate, which was considered incidental, an additional 16 presumed pregnant females were added to the HD group and 8 females were added to the C group to run concurrently with the additional HD females. Of these, 6/8 and 15/16 were pregnant, 1 C female (no. 4844) aborted on day 27 of gestation, and 1 HD female (no. 4861) delivered early on day 29 of gestation. So, there were a total of 23, 19, 19 and 26 females with viable fetuses available for examination in the respective groups.

Embryofetal death (early and late resorptions) was increased (NS) at the HD compared to C (Table 3). The number of live fetuses was decreased (NS) at the LD and HD, but the LD effect was due to decreased implants compared to C. One HD female (no. 4691) aborted and another (no. 4708) had no viable fetuses. Each of these does had a prolonged period of drug-related reduced food consumption and body weight loss.

Table 3.

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 4 mg/kg/day	Group 3 Intermed. dose 12 mg/kg/day	Group 4 High dose 36 mg/kg/day
Pregnant	N	24	19	19	27
Dams with no Viable Fetuses	N	1	0	0	1
Dams with Viable Fetuses	N	23	19	19	26
Corpora Lutea	TOTAL	267	188	209	305
No. per animal	MEAN	11.1 d	9.9	11.0	11.3
	S.D.	2.6	1.8	2.7	2.5
Implantation Sites	TOTAL	241	165	192	270
No. per animal	MEAN	10.0 d	8.7	10.1	10.0
	S.D.	2.6	2.7	3.0	2.2
Preimplantation Loss	TOTAL	26	23	17	35
No. per animal	MEAN	1.1 d	1.2	0.9	1.3
	S.D.	1.3	1.4	1.9	1.4
% per animal	MEAN%	9.9 k	13.9	7.6	11.0
	S.D.	13.1	19.3	15.5	11.9
Live Fetuses	TOTAL	224	151	178	231
No. per animal	MEAN	9.3 d	7.9	9.4	8.6
	S.D.	2.5	2.7	2.8	2.9
Males	TOTAL	98	56	66	102
	MEAN%	44.4 k	33.1	36.0	44.8
	S.D.	14.1	20.3	19.7	15.5
Females	TOTAL	126	95	112	129
	MEAN%	55.6 k	66.9	64.0	55.2
	S.D.	14.1	20.3	19.7	15.5

Postimplantation Loss	TOTAL	17	14	14	39
No. per animal	MEAN	0.7 k	0.7	0.7	1.4
	S.D.	1.2	0.9	1.0	2.4
% implants per animal	MEAN%	8.8 k	9.1	7.6	13.8
	S.D.	21.0	12.6	10.6	22.3
Dead Fetuses	TOTAL	0	0	0	0
No. per animal	MEAN	0.0 k	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
% of implants per animal	MEAN%	0.0 k	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Resorptions: Early	TOTAL	12	8	6	21
No. per animal	MEAN	0.5 k	0.4	0.3	0.8
	S.D.	0.9	0.8	0.6	2.0
% of implants per animal	MEAN%	7.1 k	6.0	4.4	8.0
	S.D.	20.6	12.8	9.7	19.7
Resorptions: Late	TOTAL	5	6	8	18
No. per animal	MEAN	0.2 k	0.3	0.4	0.7
	S.D.	0.5	0.5	1.0	1.4
% of implants per animal	MEAN%	1.7 k	3.1	3.2	5.8
	S.D.	4.3	4.8	6.0	11.2
Fetal Body Weight (g)	MEAN	35.5 k	37.5	36.8	35.8
	S.D.	3.5	7.1	5.7	4.2
	N	23	19	19	26
Male Fetuses	MEAN	36.0 d	35.9	37.0	36.3
	S.D.	3.7	5.0	7.3	4.1
Female Fetuses	MEAN	35.2 d	37.2	36.2	35.8
	S.D.	3.7	7.6	6.4	5.1

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test

Fetal evaluations

No external or visceral malformations were reported. Skeletal malformations were observed in 0, 1, 3 and 2 fetuses from as many litters in the C, LD, MD, and HD groups, respectively (female nos. 4670, 4674, 4677, 4690, 4695 and 4856; Table 4). All had thoracic or lumber vertebral abnormalities that resulted in scoliosis for all fetuses but one (female no. 4856). The overall incidence of malformed fetuses (6/560: 1.1%) was less than in the historical control data (1.8% in 2002). There were no clear drug effects on incidences of anomalies or variations.

Table 4.

SUMMARY OF FETAL SKELETAL OBSERVATIONS

		Group 1 Control 0 mg/kg/day	Group 2 Low dose 4 mg/kg/day	Group 3 Intermed. dose 12 mg/kg/day	Group 4 High dose 36 mg/kg/day
Litters Evaluated	N	23	19	19	26
Fetuses Evaluated	N	224	151	178	231
Live	N	224	151	178	231
Dead	N	0	0	0	0
A VERTEBRA, THORACIC : MISSHAPEN CENTRUM					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.4
Litter Incidence	N	0 f	0	0	1
	%	0.0	0.0	0.0	3.8
M VERTEBRA, THORACIC : MALFORMED					
Fetal Incidence	N	0	1	2	1
	%	0.0	0.7	1.1	0.4
Litter Incidence	N	0 f	1	2	1
	%	0.0	5.3	10.5	3.8
LUMBAR VERTEB.					
Litter Incidence	N	0	2	1	2
Fetal Incidence	N	0	2	1	2
A VERTEBRA, LUMBAR : NUMBER = 5					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.7	0.0	0.4
Litter Incidence	N	0 f	1	0	1
	%	0.0	5.3	0.0	3.8
M VERTEBRA, LUMBAR : MALFORMED					
Fetal Incidence	N	0	0	1	1
	%	0.0	0.0	0.6	0.4
Litter Incidence	N	0 f	0	1	1
	%	0.0	0.0	5.3	3.8
A VERTEBRA, LUMBAR : UNOSSIFIED CENTRUM					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.7	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	5.3	0.0	0.0

Statistical key: f=Chi2/Fisher Exact test
OBSERVATION CODE: M-MALFORMATION V-VARIATION A-ANOMALY

9.3 Pre- and Postnatal Development

Study title: YKP3089: Pre- and post-natal development (Segment III) study in rats via oral gavage

Study no.:	13-4398
Study report location:	4.2.3.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/30/13
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	AA-022087-Batch-04-2009, 99.9%

Key Study Findings

When CBM (0, 11, 22, or 44 mg/kg/day) was orally administered to female rats throughout pregnancy and lactation, neurobehavioral impairment (learning and memory deficit and increased auditory startle response) was observed in the offspring at all doses and decreased preweaning body weight gain and adverse effects on reproductive function (decreased numbers of corpora lutea, implantations, and live fetuses) were seen in the offspring at the high dose. Maternal plasma exposures (AUC) at the lowest effect dose (11 mg/kg/day) for adverse effects on pre- and postnatal development were 124, 140, and 101 ug.h/mL on GD 6, GD 18, and PND 20, respectively.

Methods

Doses:	0, 11, 22, 44 mg/kg/day
Frequency of dosing:	QD
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% w/v aqueous methylcellulose
Species/Strain:	Sprague-Dawley rat
Number/Sex/Group:	22/group
Satellite groups:	6/sex/group
Study design:	PPND design, dosing GD6-PND20
Deviation from study protocol:	None

S-D rats (22 time-mated females/group) received CBM (0, 11, 22, or 44 mg/kg/day) once daily via oral gavage from GD 6 through PND 20. TK animals (6/sex/group) were dosed and samples collected on GDs 6 and 18. Blood samples were also collected from dams on PND 20 for TK analysis. Endpoints evaluated in dams included mortality, clinical observations, BW, littering data, and necropsy findings. Pups were evaluated for pre-weaning functional development (surface- and air-righting, auditory and pupillary reflexes). At weaning, 1/sex/litter were retained and evaluated for viability, clinical observations, BW, and post-weaning functional development (auditory startle, open field evaluations, locomotor activity, learning and memory), sexual maturation, reproductive performance, gross necropsy findings, and selected organ weights.

Group	Daily Dose[a] (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Number of animals		
				F_0 females (for LD 20 TK[c] and F_1 generation)	F_0 females (for GD 6 and 18 TK[c] and GD 19 placental transfer)	F_1 weanlings[b]
1	0	0	5	22	6	1/sex/litter
2	11	2.2	5	22	6	1/sex/litter
3	22	4.4	5	22	6	1/sex/litter
4	44	8.8	5	22	6	1/sex/litter

[a]Doses represent active ingredient (correction factor = 1.0).

[b]One male and one female F_1 offspring from each litter (to constitute nominally 20 of each sex per group) were selected and evaluated for post-weaning physical and functional development, including reproductive function. Presumed pregnant F_1 females were sacrificed at GD 14 and the F_1 males were sacrificed shortly after most F_1 females had been sacrificed.

[c]Toxicokinetic (TK) samples were collected on Gestation Days 6 and 18, and Lactation Day 20.

Doses were based on the results of the 28-day and 26-week rat toxicity studies and the rat FEED (conducted by (b) (4)) and EFD studies.

Observations and Results

F0 Dams

Mortality

All F0 females were pregnant and survived until scheduled termination.

Clinical signs

There were no drug-related clinical observations during either the gestation or lactation phases.

Body weight

A transient decrease in BW gain was seen in HD females from GDs 6 to 9, but these weights recovered and were similar to controls thereafter (through the lactation period).

Delivery data

There were no effects on the length of gestation length, number of implantation sites, gestation Index, or delivery Index.

Necropsy

There were no drug-related macroscopic findings.

Toxicokinetics

Maternal plasma TK parameters are shown in Table 1.

Table 1.

Dose level (mg/kg/day)	C_{max} (ng/mL) GD 6	GD 18	LD 20	AUC_{0-24} (ng.h/mL) GD 6	GD 18	LD 20
11	7570	8540	7580	124000	140000	101000
22	18200	23600	11700	335000	401000	151000
44	22300	30400	20600	436000	544000	221000

Offspring

Survival

There was no drug-related mortality throughout the preweaning period. Live Birth, Viability and Lactation Indices were comparable among groups. There was no drug-related mortality post-weaning.

Clinical signs

There were no drug-related clinical observations noted in the pre- or postweaning phases.

Body weight

Drug-related decreases (SS) in BW gain and BW were observed in HD males and females from PND 10 to 21 (Table 2). Postweaning BWs continued to be decreased somewhat at the HD (SS in HD females), but gradually recovered (Table 3).

Table 2.

F_1 All Pups	Mean Litter Weights (Pre-Weaning) (grams)							Table 9
	Before Cull		After Cull					
PND:	1	4	4	7	10	14	18	21
Group 1 - 0 mg/kg/day								
Mean	6.7	9.6	9.7	15.7	22.9	32.7	41.7	52.0
SD	0.5	1.1	1.1	1.6	2.5	2.9	4.1	5.5
N	21	21	21	21	21	21	21	21
Group 2 - 11 mg/kg/day								
Mean	7.0	10.0	10.0	16.2	22.9	32.7	41.8	52.4
SD	0.6	1.1	1.1	1.7	2.2	2.8	3.4	4.1
N	22	22	22	22	22	22	22	22
Group 3 - 22 mg/kg/day								
Mean	7.0	9.7	9.8	15.5	22.0	31.3	40.1	50.9
SD	0.5	1.1	1.1	1.6	2.2	2.5	3.1	4.5
N	22	22	22	22	22	22	22	22
Group 4 - 44 mg/kg/day								
Mean	6.8	9.4	9.5	15.3	21.3*	30.1**	38.3**	48.2**
SD	0.5	1.0	0.9	1.4	2.0	2.8	3.1	4.1
N	22	22	22	22	22	22	22	22

* = $p < 0.05$, ** = $p < 0.01$

Table 3.

F_1 Males	Mean Body Weights (Post-Weaning) (grams)											Table 13
Phase	PND											
Day	24	28	31	35	38	42	45	49	52	56	59	63
Group 1 - 0.0 mg/kg/day												
Mean	66.6	90.3	114.3	152.9	183.3	221.9	250.5	289.0	316.5	343.9	369.1	396.0
SD	7.02	9.83	13.28	18.01	21.78	24.95	30.02	32.49	35.58	37.27	39.53	42.75
N	21	21	21	21	21	21	21	21	21	21	21	21
Group 2 - 11.0 mg/kg/day												
Mean	66.8	91.5	114.3	151.4	181.7	222.2	250.3	286.3	317.7	352.9	376.3	404.8
SD	6.58	8.98	11.62	15.46	17.95	21.32	24.13	27.25	29.78	31.31	33.86	36.47
N	22	22	22	22	22	22	22	22	22	22	22	22
Group 3 - 22.0 mg/kg/day												
Mean	66.5	90.8	114.9	153.1	184.6	224.5	252.2	286.7	318.6	352.0	373.8	401.0
SD	5.60	6.83	8.65	12.06	14.19	16.58	19.55	21.71	23.06	24.93	29.89	31.02
N	22	22	22	22	22	22	22	22	22	22	22	22
Group 4 - 44.0 mg/kg/day												
Mean	62.8	87.9	112.1	148.9	181.3	220.1	247.6	284.1	312.3	343.5	368.0	391.0
SD	4.52	6.52	8.92	12.60	15.00	17.99	21.18	23.38	27.43	32.04	32.14	39.79
N	21	21	21	21	21	21	21	21	21	21	21	21

No statistically significant differences from control mean

F_1 Females	Mean Body Weights (Post-Weaning) (grams)											Table 13
Phase	PND											
Day	24	28	31	35	38	42	45	49	52	56	59	63
Group 1 - 0.0 mg/kg/day												
Mean	63.3	83.7	102.8	130.8	150.7	171.5	184.9	199.5	211.7	225.2	232.6	249.3
SD	6.70	7.77	9.33	9.25	10.66	11.19	11.32	11.89	13.19	13.63	14.42	17.00
N	19	19	19	19	19	19	19	19	19	19	19	19
Group 2 - 11.0 mg/kg/day												
Mean	62.4	82.7	100.5	127.2	147.8	169.9	184.0	199.6	212.0	225.3	233.3	247.1
SD	6.04	7.36	8.18	9.15	11.54	12.35	13.63	13.20	14.38	13.73	15.80	17.54
N	21	21	21	21	21	21	21	21	21	21	21	21
Group 3 - 22.0 mg/kg/day												
Mean	61.7	82.6	100.5	128.8	147.8	170.1	183.3	198.1	211.4	223.3	233.8	248.5
SD	5.76	6.84	9.70	12.19	12.34	13.22	14.66	14.38	15.29	16.39	16.62	18.22
N	22	22	22	22	22	22	22	22	22	22	22	22
Group 4 - 44.0 mg/kg/day												
Mean	57.9**	78.1*	97.6	124.6	144.4	165.5	179.8	196.1	208.8	220.2	229.7	243.3
SD	5.60	7.94	9.97	11.56	12.34	13.53	13.96	15.11	15.78	16.28	18.90	19.25
N	21	21	21	21	21	21	21	21	21	21	21	21

* = $p < 0.05$, ** = $p < 0.01$

Physical and neurological development

There were no drug-related effects on auditory and visual function or number of days to acquisition of surface righting and mid-air righting, preweaning, or posture, gait, vocalization, or behavioral observations in an open field assessment conducted postweaning.

Neurobehavioral assessment

When locomotor activity was assessed on PND 28, there were no consistent differences among groups. When auditory startle habituation was evaluated on PND 65, increases (SS) in startle amplitude were observed in treated males in a generally dose-related pattern ranging from +16 to +51% (SS in MD and HD in blocks 3 and 5) and habituation was dose-dependently decreased (Table 4). There were no clear effects in females.

Table 4.

F_1 Males	Summary of Auditory Startle					Table 18
Occasion PND 65±3	Block 1 (newtons)	Block 2 (newtons)	Block 3 (newtons)	Block 4 (newtons)	Block 5 (newtons)	Habituation (%)
Group 1 - 0 mg/kg/day						
Mean	2.845	2.035	1.690	1.824	1.686	-41.658
SD	0.9005	0.7982	0.8779	0.9483	0.8084	15.0446
N	21	21	21	21	21	21
Group 2 - 11 mg/kg/day						
Mean	3.388	2.359	2.135	2.176	1.952	-39.092
SD	1.2328	0.7242	0.7648	0.8736	0.5689	19.3558
N	22	22	22	22	22	22
Group 3 - 22 mg/kg/day						
Mean	3.819	2.686	2.552	2.684	2.509	-35.330
SD	1.9915	1.4020	1.3869	1.3832	1.7180	24.1252
N	22	22	22	22	22	22
Group 4 - 44 mg/kg/day						
Mean	3.555	2.662	2.325	2.344	2.405	-33.538
SD	1.3911	1.4445	1.1645	1.1455	1.2919	23.8023
N	21	21	21	21	21	21

When animals were tested for learning and memory in a simplified Biel maze paradigm (no B path) beginning on PND 55, deficits in memory recall were observed in HD males based on increases in latency (21%) and errors (59%) compared to C (Table 5). Similar effects were seen in LD males, but increases were minimal at the MD.

Table 5.

F_1 Males	Summary of Learning and Memory											
	Day 1		Day 2		Day 3		Day 4		Day 5		Day 8	
	Swimming Ability		Trial 1		Trial 2		Trial 3		Trial 4		Memory Recall	
	Fastest Time sec	Trial Time sec	Time sec	Errors	Time sec	Errors	Time sec	Errors	Time sec	Errors	Time sec	Errors
Group 1 - 0 mg/kg/day												
Mean	6.3	11.0	61.6	12.1	32.4	6.9	26.7	4.7	20.8	2.9	22.4	3.2
SD	1.55	3.45	28.31	6.04	18.86	5.83	17.61	4.12	13.97	3.58	11.77	2.75
N	21	21	21	21	21	21	21	21	21	21	21	21
Group 2 - 11 mg/kg/day												
Mean	6.2	11.0	53.8	11.2	32.6	7.3	30.1	5.4	23.2	3.6	26.8	4.7
SD	1.48	5.69	24.75	5.56	15.18	5.29	17.43	4.00	13.61	3.72	18.91	5.28
N	22	22	22	22	22	22	22	22	22	22	22	22
Group 3 - 22 mg/kg/day												
Mean	6.1	9.6	64.0	13.8	32.1	7.0	23.2	4.0	19.6	2.7	24.5	3.6
SD	2.98	3.57	28.52	6.45	22.37	6.24	9.96	2.56	12.88	4.45	19.62	5.23
N	22	22	22	22	22	22	22	22	22	22	22	22
Group 4 - 44 mg/kg/day												
Mean	6.2	10.1	55.1	11.6	31.8	6.8	22.5	3.6	22.0	3.3	27.2	5.1
SD	2.05	2.69	21.48	5.02	12.09	4.61	8.44	2.38	12.43	3.86	18.86	5.94
N	21	21	21	21	21	21	21	21	21	21	21	21

Reproduction

No drug-related effects were noted on the attainment of preputial separation in males or vaginal patency in females. There were no effects on estrus cyclicity, mating and fertility indices, or days to mating. However, in C-section evaluations, apparent drug-related adverse effects on reproductive outcome (increased number of early resorptions (+100%) and percent pre- (+83%) and postimplantation loss (+71%) and decreased number of corpora lutea (-8.7%), implantations (-13%), and live fetuses (-15%, statistically significant) were observed in the HD females (Table 6).

Table 6.

F_1 Females	Summary of Female Reproduction Data								
	Corpora Lutea	Implant-ations	Resorptions Early	Resorptions Late	Resorptions Total	Dead Fetuses	Live Fetuses	Pre Impl. Loss (%)	Post Impl. Loss (%)
Group 1 - 0.0 mg/kg/day									
Mean	17.2	16.1	0.9	0.1	1.0	0.0	15.1	6.3	6.2
SD	1.95	1.75	0.74	0.46	1.00	0.00	1.7	4.84	5.74
N	19	19	19	19	19	19	19	19	19
Group 2 - 11.0 mg/kg/day									
Mean	16.6	15.7	1.0	0.0	1.0	0.0	14.8	8.1	6.6
SD	2.94	3.10	1.83	0.00	1.83	0.00	3.45	12.97	11.57
N	21	21	21	21	21	21	21	20	21
Group 3 - 22.0 mg/kg/day									
Mean	16.4	15.6	0.6	0.0	0.6	0.0	15.0	4.4	4.1
SD	2.48	2.02	0.90	0.00	0.90	0.00	2.07	4.64	5.66
N	22	22	22	22	22	22	22	22	22
Group 4 - 44.0 mg/kg/day									
Mean	15.7	14.0	1.8	0.0	1.8	0.0	12.8*	11.5	10.6
SD	3.72	3.57	2.02	0.00	2.02	0.00	3.34	14.75	11.18
N	21	21	21	21	21	20	20	21	20

* = $p < 0.05$
Impl. = Implantation

9.4 Juvenile Animal Toxicity Studies

Study title: YKP3089: An Oral (Gavage) Toxicity Study in Neonatal/Juvenile Rats

Study no.:	14-4424
Study report location:	4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	20 May 2015
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	SKF-PD187-14-001N, 100.6%

Key Study Findings

S-D rats were dosed with vehicle (MC) or YKP3089 (escalating doses of 20/20/30/40, 30/40/60/80, or 40/60/80/120 mg/kg/day in males and 15/15/20/20, 25/35/40/50, or 35/50/60/80 mg/kg/day in females) once daily from PND 7 through 70 and followed by a 6-week recovery period from PND 71 to PND 113. Adverse effects included mortality, delayed sexual maturation, neurological (decreased grip strength) and neurobehavioral (learning and memory deficits) impairment, decreased sperm count, decreased brain weight, and ocular histopathology. Recovery from these effects was observed following discontinuation of dosing. Overall, a no-effect dose for adverse effects on postnatal development was not identified. At the lowest doses tested, plasma cenobamate exposures (AUC) were 121000/91600/164000/238000 ng*h/mL in males and 94900/173000/284000/260000 ng*h/mL in females.

Methods

Doses:	20/20/30/40, 30/40/60/80, 40/60/80/120 mg/kg/day in males; 15/15/20/20, 25/35/40/50, 35/50/60/80 mg/kg/day in females
Frequency of dosing:	QD
Dose volume:	5 mL/kg
Route of administration:	PO
Formulation/Vehicle:	0.5% w/v aqueous methylcellulose
Species/Strain:	Crl:CD(SD)BR
Number/Sex/Group:	20/sex/grp general toxicity; 20/sex/grp neurohistopathology; 20/sex/grp reproductive toxicity; 9-54/sex/grp TK
Satellite groups:	TK
Study design:	Protocol reviewed by division
Deviation from study protocol:	None that impacted interpretation of the results

S-D rats were dosed (oral gavage; 5 mL/kg/) with vehicle (0.5% w/v aqueous methylcellulose) or CBD (see dose groups below) once daily from PND 7 through 70 followed by a 6-week recovery period from PND 71 to 113. Additional animals were included for TK analysis (PNDs 7, 22, 38, 54, and 70). Based on previous TK data, doses were escalated on PNDs 23, 39, and 55, as advised by the division, in an effort to increase

exposure margins. Endpoints included standard general toxicity endpoints (mortality, clinical signs, BW, food consumption, clinical pathology, macroscopic and microscopic pathology), bone densitometry, expanded neurohistopathology (C and HD only), and neurobehavioral and reproductive functional assessments. Neurobehavioral assessments consisted of FOB, automated locomotor and auditory startle habituation, and learning and memory (Biel water maze) at the end of the treatment and recovery periods.

		Treatment Regimen			
		PND 7 to 22	PND 23 to 38	PND 39 to 54	PND 55 to 70
Group[b]	Test Article	Dose 1[a] (mg/kg/day)[a] M/F	Dose 2[a] (mg/kg/day)[a] M/F	Dose 3[a] (mg/kg/day)[a] M/F	Dose 4[a] (mg/kg/day)[a] M/F
1	Control	0 / 0	0 / 0	0 / 0	0 / 0
2	YKP3089	20 / 15	20 / 15	30 / 20	40 / 20
3	YKP3089	30 / 25	40 / 35	60 / 40	80 / 50
4	YKP3089	40 / 35	60 / 50	80 / 60	120 / 80

[a]Doses represent active ingredient (correction factor = 1.0).
[b] Each Group consisted of 20/sex for general toxicity; 20/sex for neurohistopathology; 20/sex for reproductive toxicity; 9 to 54/sex for toxicokinetics.

Doses were based on the results of 2 dose range-finding studies in S-D rats. In the first study ((b) (4) Study No. 13-4401), oral administration for up to 15 days (PND 21-35) resulted in slight decreases in food consumption at the HD of 60 mg/kg, as well as slight increases in absolute and relative liver weight in males at 60 mg/kg/day and females at ≥30 mg/kg/day. Additionally, 1 HD female was found dead. Although the cause of death was undetermined, the MTD was considered to be 60 mg/kg/day in males and 30 mg/kg/day in females. In the second study ((b) (4) Study No. 14-4423), rats were administered (oral gavage) doses of up to 80 mg/kg in males and 60 mg/kg in females from PND 7 to 70. Drug-related mortality and/or clinical signs (decreased activity, unresponsive, cold to touch, irregular breathing, prostration, and paleness) were observed during the early portion of the dosing period in males beginning at 60 mg/kg and females at 45 mg/kg. Decreased BWs were observed in males at 20, 40, and 60 mg/kg and females at 45 mg/kg. It was concluded that doses of 60 and 80 mg/kg/day in males and 45 and 60 mg/kg/day in females were not tolerated either as a single dose on PND 7 or as a repeat dose from PNDs 7 to 22, due to mortality and adverse clinical signs. Therefore, initial doses (PND 7 – 22) selected for the definitive juvenile study were 20, 30, 40 and 15, 25, 35 mg/kg/day for males and females, respectively, with dose adjustments at PND 23, 39, and 55 to increase exposures.

The division reviewed the initial protocol (IND 76809, submission dated 5/13/14) and had the following comments:

> There was no clear dose-limiting toxicity in the preliminary 2-week study in juvenile rats at oral (gavage) doses of up to 60 mg/kg QD, which were associated with plasma drug exposures below those expected in humans. This indicates that higher doses and/or an alternative dosing regimen should be explored. A study in which dosing is initiated on PND 21, as proposed, will only support use in pediatric patients 2 years of age and older. The proposed group sizes for neurobehavioral

> assessments (N=10/sex/group) are considered inadequate. It is recommended that neurobehavioral testing be conducted in ~20/sex/group (Cappon et al., Birth Defects Research (Part B), 86:463-469, 2009). Use of the Biel maze for assessment of possible effects on learning and memory is not considered optimal in terms of sensitivity (Vorhees, Neurotoxicol Teratol, 9:235-241, 1987). More sensitive measures such as the Cincinnati or Morris water maze are recommended. Expanded neurohistopathology and bone densitometry should be included in the terminal assessments.

The sponsor justified the use of the Biel maze as follows:

> SKLSI recently conducted a rat pre- and post-natal development (Segment III) study using the Biel maze as an assessment of learning and memory effects; therefore, SKLSI would prefer to continue the use of the Biel maze in the proposed juvenile rat toxicology study in order to be consistent. In the Segment III study (Study No. SK13019; draft report available), slight increases were observed in both time to complete the maze and in number of errors made for F1 males at 44 mg/kg/day when tested in the Biel maze at PND 55. Using the Biel maze again in the juvenile rat toxicology study will enable SKLSI to compare sensitivities in learning and memory across developmental stages.

Observations and Results

Mortality

Mortality was increased in MD and HD groups: 1 C, 3 LD, 9 MD, and 15 HD were either found dead or sacrificed moribund. Most of these (19/28) occurred during the dosing period from PND 8 to PND 26 and in most cases (21/23 examined) the cause of death/moribundity could not be determined and was considered drug-related. For drug-related deaths, mortality/sacrifice was often associated with acute clinical signs.

Clinical signs

Drug-related clinical observations were primarily seen at the HD and included flattened, abnormal, or unsteady gait and decreased activity.

Body weight

There were no group differences in BW gain over the treatment period or in BWs at the end of the treatment or recovery periods.

Physical development

There were no drug-related effects on preweaning reflex ontogeny (mid-air righting, startle, pupil reflex, surface righting). Delayed sexual maturation (preputial separation) was seen in HD males (SS; Table 1). A small (NS) delay was also seen in HD females.

Table 1. Sexual maturation - group mean values

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group /Sex		Day of Preputial Separation	Body Weight (g)
1M	Mean	48.4	270.3
	SD	6.2	31.6
	N	59	57
2M	Mean	48.6	279.8
	SD	4.2	37.8
	N	57	57
3M	Mean	47.5	270.0
	SD	2.9	31.4
	N	56	56
4M	Mean	49.4**	287.1*
	SD	4.1	40.7
	N	58	58

Group /Sex		Day of Vaginal Opening	Body Weight (g)
1F	Mean	33.7	124.4
	SD	1.8	13.7
	N	60	60
2F	Mean	34.1	126.0
	SD	2.0	12.5
	N	59	59
3F	Mean	34.0	126.1
	SD	2.4	16.9
	N	56	56
4F	Mean	34.5	129.6
	SD	2.5	16.6
	N	56	56

Neurobehavioral assessment (end of treatment and after recovery)

FOB

Drug-related decreases in both forelimb and hind limb grip strength were seen at all doses in both sexes (up to -34% in males and -50% in females compared to C; Table 2). These effects were no longer present after the recovery period.

Table 2. Functional observational battery – summary of assessments – PND 68

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group /Sex		Temperature °C	Forelimb Grip Strength (g)	Hindlimb Grip Strength (g)	Landing Foot Splay (cm)
1M	Mean	37.9	997.4	728.2	6.4
	SD	0.67	184.28	170.69	1.17
	N	20	20	20	20
2M	Mean	37.3	704.9	615.8	6.6
	SD	0.52	143.32	121.10	1.38
	N	20	20	20	20
3M	Mean	37.6	657.0	552.7	6.6
	SD	2.12	114.08	101.12	1.58
	N	20	20	20	20
4M	Mean	37.1	667.2	546.3	6.5
	SD	0.45	151.48	101.37	1.53
	N	19	19	19	19
1F	Mean	38.2	983.2	622.7	5.3
	SD	0.43	153.92	130.20	1.09
	N	20	20	20	20
2F	Mean	37.8	776.2	516.5	6.0
	SD	0.63	178.33	162.92	1.40
	N	20	20	20	20
3F	Mean	37.5	629.1	411.2	5.1
	SD	0.60	175.11	88.36	0.87
	N	18	18	18	18
4F	Mean	37.3	488.8	362.6	4.7
	SD	0.66	159.20	86.35	1.05
	N	18	18	18	18

Locomotor activity

When assessed at the end of the treatment period (PND 68), locomotor activity (horizontal beam breaks) appeared to be dose-dependently decreased (NS) in both sexes (Table 3). After the recovery period (PND 108), activity was increased (NS) in HD males (Table 4) but was not consistently different among groups in females.

Table 3. Motor activity on PND 68 – horizontal (number of beam breaks)

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group /Sex		5 min. intervals 1	2	3	4	5	6	7	8	9	10	11	12
1M	Mean	622	369	233	187	126	76	75	90	74	29	18	34
	SD	96.6	92.9	98.7	92.4	99.3	79.2	104.3	112.2	134.7	30.8	18.6	57.5
	N	20	20	20	20	20	20	20	20	20	20	20	20
2M	Mean	583	296	165	125	98	38	16	19	13	8	15	16
	SD	110.1	143.1	128.0	139.1	99.3	69.2	37.9	42.8	15.1	10.9	19.6	17.8
	N	20	20	20	20	20	20	20	20	20	20	20	20
3M	Mean	498	159	141	58	51	43	24	33	20	18	18	19
	SD	89.3	122.1	130.0	90.0	66.8	68.0	35.7	82.1	67.2	59.3	52.3	48.4
	N	20	20	20	20	20	20	20	20	20	20	20	20
4M	Mean	392	153	72	72	37	29	14	16	31	36	16	20
	SD	174.2	140.0	93.0	109.8	73.4	62.1	21.1	24.2	68.0	69.2	19.4	41.0
	N	20	20	20	20	20	20	20	20	20	20	20	20

Group /Sex		5 min. intervals 1	2	3	4	5	6	7	8	9	10	11	12
1F	Mean	795	490	362	204	94	94	43	22	52	59	32	52
	SD	153.5	162.0	206.9	124.3	104.1	144.4	80.7	33.6	99.9	105.2	47.7	90.4
	N	20	20	20	20	20	20	20	20	20	20	20	20
2F	Mean	794	351	170	109	25	29	22	26	52	45	54	47
	SD	174.7	156.5	128.4	114.4	28.0	78.3	36.8	52.7	139.4	111.2	115.0	67.8
	N	20	20	20	20	20	20	20	20	20	20	20	20
3F	Mean	730	243	147	84	18	13	6	18	11	27	12	45
	SD	222.4	141.7	135.5	140.6	46.9	18.4	7.2	34.3	21.0	74.6	11.9	84.9
	N	19	19	19	19	19	19	19	19	19	19	19	19
4F	Mean	535	238	91	64	46	22	8	14	19	27	38	31
	SD	217.6	195.6	89.2	70.3	116.8	68.9	9.8	15.8	51.7	61.6	56.8	98.0
	N	18	18	18	18	18	18	18	18	18	18	18	18

Table 4. Motor activity on PND 108 – horizontal (number of beam breaks)

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group /Sex		5 min. intervals 1	2	3	4	5	6	7	8	9	10	11	12
1M	Mean	529	355	263	168	95	72	74	85	56	37	29	37
	SD	135.4	98.9	108.1	116.2	70.0	72.4	91.6	100.2	87.8	55.3	37.4	64.4
	N	19	19	19	19	19	19	19	19	19	19	19	19
2M	Mean	586	317	233	155	98	72	68	40	47	43	34	31
	SD	140.9	126.2	96.1	85.9	86.8	79.2	87.9	57.5	87.2	59.3	68.2	72.9
	N	20	20	20	20	20	20	20	20	20	20	20	20
3M	Mean	556	303	215	116	96	62	93	53	23	35	22	25
	SD	124.0	110.4	79.6	86.5	98.8	99.8	132.1	80.3	33.7	45.4	51.8	77.6
	N	18	18	18	18	18	18	18	18	18	18	18	18
4M	Mean	755	445	298	250	150	84	71	72	79	48	31	55
	SD	124.8	94.3	138.6	130.8	148.3	71.7	95.6	125.8	120.0	78.9	55.7	113.1
	N	20	20	20	20	20	20	20	20	20	20	20	20

Group /Sex		5 min. intervals 1	2	3	4	5	6	7	8	9	10	11	12
1F	Mean	755	445	298	250	150	84	71	72	79	48	31	55
	SD	124.8	94.3	138.6	130.8	148.3	71.7	95.6	125.8	120.0	78.9	55.7	113.1
	N	20	20	20	20	20	20	20	20	20	20	20	20
2F	Mean	724	403	278	182	112	50	43	43	37	26	41	32
	SD	127.3	136.9	144.5	131.3	147.2	80.1	73.0	90.0	73.0	49.6	63.2	65.5
	N	19	19	19	19	19	19	19	19	19	19	19	19
3F	Mean	647	399	241	168	89	81	21	53	53	42	13	53
	SD	80.8	108.3	110.4	132.1	84.8	92.6	26.8	85.2	102.1	105.8	24.8	85.7
	N	20	20	20	20	20	20	20	20	20	20	20	20
4F	Mean	704	358	240	174	107	22	8	78	53	35	48	51
	SD	111.9	99.4	134.0	126.5	133.9	33.3	7.5	160.6	124.1	68.1	84.0	106.9
	N	18	18	18	18	18	18	18	18	18	18	18	18

Auditory startle habituation

Apparent increases in startle response and decreases in habituation in MD and HD females at the recovery assessment (PND 108) were not clearly dose related, so their relation to treatment was unclear (Table 5). No other clear group differences were seen at the end of treatment or recovery.

Table 5. Auditory startle on PND 108 - group mean values (newtons)

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group /Sex		Occasion PND 108	Block 1	Block 2	Block 3	Block 4	Block 5	Habituation (%)
1F	Mean		3.518	2.054	1.858	2.010	2.150	-36.5
	SD		1.8093	1.0732	1.1046	1.5776	1.3348	26.37
	N		20	20	20	20	20	20
2F	Mean		4.588	3.144	2.753	2.935	2.822	-39.5
	SD		2.1759	1.5357	1.5687	2.2152	2.0044	17.86
	N		19	19	19	19	19	19
3F	Mean		4.840	3.516	3.397*	3.203	3.286	-30.5
	SD		3.1403	2.8261	2.3505	1.8657	2.0876	24.89
	N		20	20	20	20	20	20
4F	Mean		4.194	3.192	2.764*	3.090	2.690	-32.2
	SD		2.0416	2.0480	1.7754	2.0879	1.3577	22.61
	N		18	18	18	18	18	18

Learning and memory (Biel maze without B path)

At the end of treatment (PND 61), learning and memory deficits (increases in latencies and errors) were seen on several trials in both sexes (SS at all doses in males on Days 1 and 2; Table 6). Learning and memory performance was unaffected in treated females at this time. Possible effects (increased latencies and/or errors on learning Days 3 and 4 and memory recall trials) were seen in MD and HD females after the recovery period (PND 99), but the differences were not dose-related, and SS was not reached (Table 7).

Table 6. Learning and memory - group mean values – PND 61

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group /Sex	Occasion PND 61 ± 2	SWIMMING ABILITY		LEARNING TRIALS								MEMORY RECALL	
				Day 1		Day 2		Day 3		Day 4			
		Fastest Time (sec)	Trial Time (sec)	Time (sec)	Errors	Time (sec)	Errors	Time (sec)	Errors	Time (sec)	Errors	Time (sec)	Errors
1M	Mean	5.1	9.1	61.2	11.3	30.2	4.9	25.3	5.2	23.0	3.8	21.6	2.9
	SD	1.91	3.25	30.60	4.62	19.47	5.16	11.73	3.60	17.11	4.93	8.45	2.33
	N	25	25	25	25	25	25	25	25	25	25	25	25
2M	Mean	6.0	9.5	78.5	18.6**	51.5**	7.8*	21.5	4.3	23.9	4.7	19.9	3.1
	SD	2.44	5.03	38.33	9.86	32.48	4.74	10.50	3.41	18.26	5.01	7.89	2.64
	N	23	23	23	23	23	23	23	23	23	23	23	23
3M	Mean	6.2	8.9	86.3	21.0**	57.6**	10.9**	43.0	11.2	26.2	5.8	21.2	3.9
	SD	2.00	2.94	34.51	9.31	34.88	8.63	35.92	12.65	18.12	6.65	10.12	3.71
	N	22	22	22	22	22	22	22	22	22	22	22	22
4M	Mean	6.4*	9.6	75.1	16.5**	74.6**	15.4**	58.6**	15.3**	38.1**	8.2**	31.5	6.7**
	SD	1.97	5.77	35.40	8.51	47.98	12.49	39.55	12.48	35.01	9.06	24.52	5.97
	N	23	23	23	23	23	23	23	23	23	23	23	23
1F	Mean	4.8	9.4	66.3	12.7	39.1	5.7	22.3	4.9	25.8	4.5	31.6	6.1
	SD	1.94	3.54	29.37	6.18	22.95	5.51	11.53	4.34	17.18	4.00	23.88	4.26
	N	25	25	25	25	25	25	25	25	25	25	25	25
2F	Mean	5.1	9.0	69.2	16.2	43.0	6.4	22.2	4.8	23.2	3.4	23.6	4.8
	SD	1.71	3.17	25.99	8.39	25.03	4.04	16.00	4.77	18.91	3.93	8.88	3.01
	N	25	25	25	25	25	25	25	25	25	25	25	25
3F	Mean	5.2	8.6	70.0	16.1	61.2	9.1	36.9*	9.5*	29.0	6.2	20.6**	3.4*
	SD	1.55	3.00	38.30	12.43	55.65	9.52	24.19	7.57	27.28	8.50	15.72	4.49
	N	21	21	21	21	21	21	21	21	21	21	21	21
4F	Mean	7.4*	11.5	80.5	18.7	62.4	11.7*	39.6*	8.6*	22.3	4.5	17.9**	3.4*
	SD	4.42	4.66	48.00	11.73	48.77	10.60	36.19	6.86	16.00	4.51	12.83	4.06
	N	21	21	21	21	21	21	21	21	21	21	21	21

Table 7. Learning and memory - group mean values – PND 99

Group /Sex	Occasion PND 99 ± 3	SWIMMING ABILITY Fastest Time (sec)	Trial Time (sec)	LEARNING TRIALS Day 1 Time (sec)	Day 1 Errors	Day 2 Time (sec)	Day 2 Errors	Day 3 Time (sec)	Day 3 Errors	Day 4 Time (sec)	Day 4 Errors	MEMORY RECALL Time (sec)	Errors
1M	Mean	4.6	7.1	56.7	11.5	27.3	6.1	22.3	4.0	18.7	2.7	18.5	2.2
	SD	2.14	3.36	22.94	4.50	14.21	3.74	25.25	6.42	12.78	3.20	15.61	2.79
	N	19	19	19	19	19	19	19	19	19	19	19	19
2M	Mean	5.2	9.6	63.2	11.1	34.8	6.4	22.0	3.0	21.7	3.7	18.5	2.1
	SD	2.25	6.11	32.12	6.03	30.57	5.07	12.93	2.84	14.71	4.75	12.27	2.69
	N	20	20	20	20	20	20	20	20	20	20	20	20
3M	Mean	4.4	8.8	71.8	14.2	41.0	8.9	18.0	2.4	18.1	2.5	17.8	2.1
	SD	1.36	4.88	39.22	6.36	32.20	7.02	9.31	2.11	8.87	2.44	10.52	2.23
	N	18	18	18	18	18	18	18	18	18	18	18	18
4M	Mean	4.3	7.1	54.0	11.0	23.8	4.4	19.2	2.8	19.9	2.8	16.2	1.2
	SD	1.14	3.15	18.75	3.69	14.47	3.27	11.80	3.36	16.08	3.59	9.26	1.37
	N	19	19	19	19	19	19	19	19	19	19	19	19
1F	Mean	6.2	10.3	69.6	13.1	35.2	7.3	25.9	4.5	25.6	4.1	23.7	3.7
	SD	2.99	5.98	29.93	5.68	22.90	4.52	16.60	3.69	19.91	4.48	13.84	2.91
	N	20	20	20	20	20	20	20	20	20	20	20	20
2F	Mean	4.6	10.3	66.6	11.9	36.6	6.7	25.3	4.0	20.1	2.5	19.3	2.7
	SD	1.40	3.61	35.08	7.42	26.16	4.90	16.79	3.22	10.47	2.16	9.78	2.32
	N	19	19	19	19	19	19	19	19	19	19	19	19
3F	Mean	5.2	10.6	73.7	13.0	35.1	6.3	32.3	4.8	36.2	4.9	37.3	5.8
	SD	2.05	4.24	29.96	6.78	27.76	5.99	21.83	3.53	28.95	4.16	21.82	3.99
	N	20	20	20	20	20	20	20	20	20	20	20	20
4F	Mean	5.8	12.8	61.5	11.3	36.5	7.5	28.9	4.3	34.5	5.4	29.4	4.8
	SD	2.51	7.61	29.67	6.02	22.59	5.97	18.39	3.42	23.44	3.95	19.66	4.10
	N	18	18	18	18	18	18	18	18	18	18	18	18

Reproduction

When animals were mated at PND 91, there was no clearly drug-related effects on fertility indices or C-section parameters (implantations, corpora lutea, early/late resorptions, number of live/dead fetuses, or % pre- or post-implantation loss). A dose-dependent decrease in epididymal sperm count was seen in treated males, SS at the MD and HD (Table 8). Epididymal organ weights and testicular histopathology appeared unaffected; testicular sperm count was somewhat decreased in treated animals, but the differences were not SS or dose-related.

Table 8. Sperm analysis - group mean values - sperm motility

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group /Sex		Motility		Right Testis			Right Cauda epididymis		
		Motile sperm (%)	Progressively motile sperm %	Weight (g)	Sperm count (millions/g)	Total (million)	Weight (g)	Sperm count (millions/g)	Total (million)
1M	Mean	97.1	63.0	1.9583	67.5	132.4	0.3575	230.5	82.7
	SD	1.8	15.4	0.1485	7.5	19.4	0.0361	68.5	25.8
	N	20	20	20	20	20	20	20	20
2M	Mean	96.9	63.8	2.0183	60.7	122.2	0.3678	203.6	75.0
	SD	2.0	16.6	0.1802	12.8	26.7	0.0368	70.7	27.0
	N	18	18	18	18	18	18	18	18
3M	Mean	96.6	61.0	2.0806	58.4	117.6	0.3840	183.5*	69.7
	SD	3.0	14.6	0.4570	12.0	17.7	0.0420	66.4	23.5
	N	17	17	17	17	17	17	17	17
4M	Mean	94.8	60.5	1.9315	63.8	123.5	0.3769	183.1*	68.8
	SD	5.3	18.1	0.1396	6.7	18.1	0.0290	49.6	17.8
	N	17	17	17	17	17	17	17	17

Bone density

There were no drug-related effects on bone densitometry measures (BMC, BA, BMD) or femur lengths on PNDs 71 or 113.

Neurohistopathology

Decreases (SS) in absolute brain weights were seen in MD and HD (neurohistopathology) females and in HD (general toxicity) males at the end of treatment in the absence of BW deficits (Tables 9 and 10). There were no SS differences in brain weights in these groups after the recovery period. However, decreases (less than 10%, NS) in brain to body weight ratios were seen in MD and HD (general toxicity) males and/or females at the terminal and recovery sacrifices.

Table 9. Brain weights - group mean absolute values (g) - neurohistopathology animals - terminal sacrifice

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group/ Sex		Terminal Body weight	Brain
Statistics test		Wi	Wi
1F	Mean	276.4	2.2784
	SD	40.8	0.1130
	N	20	20
2F	Mean	286.6	2.2395
	SD	43.4	0.1162
	N	20	20
3F	Mean	296.1	2.1953*
	SD	52.1	0.1186
	N	19	19
4F	Mean	294.5	2.1955*
	SD	53.4	0.1398
	N	17	17

Table 10. Organ weights - group mean absolute values (g) - general toxicity animals - terminal sacrifice

	Vehicle Control	YKP3089		
Dose Group	1	2	3	4
Male Dose (mg/kg/day)		20/20/30/40	30/40/60/80	40/60/80/120
Female Dose (mg/kg/day)		15/15/20/20	25/35/40/50	35/50/60/80

Group/ Sex		Terminal Body weight	Adrenals	Brain	Epididymides	Heart	Kidneys	Liver	Pituitary	Spleen	Testes
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	419.9	0.0670	2.1726	1.1599	1.4697	2.7442	12.2128	0.0149	0.8583	3.6899
	SD	45.3	0.0072	0.1346	0.1068	0.2517	0.3405	1.7142	0.0024	0.1791	0.1416
	N	10	10	10	10	10	10	10	10	10	10
2M	Mean	400.7	0.0613	2.1176	1.1185	1.4341	2.8869	12.6237	0.0148	0.8227	3.4450
	SD	27.5	0.0090	0.1141	0.0947	0.1402	0.2257	0.9259	0.0012	0.1050	0.2474
	N	10	10	10	10	10	10	10	9	10	10
3M	Mean	417.0	0.0641	2.1404	1.1402	1.5315	3.1103*	14.4402**	0.0160	0.8036	3.6602
	SD	43.9	0.0083	0.0978	0.1324	0.2688	0.4240	1.5408	0.0025	0.1368	0.2189
	N	10	10	10	10	10	10	10	10	10	10
4M	Mean	405.9	0.0652	2.0594*	1.1003	1.4708	3.1722**	16.3507**	0.0136	0.7870	3.5492
	SD	30.1	0.0103	0.0779	0.1287	0.2506	0.3000	1.5279	0.0013	0.1396	0.2598
	N	10	10	10	10	10	10	10	10	10	10

An ocular lesion (dysplasia) was present at a higher frequency in treated rats (only HD examined) compared to C (Table 11). This lesion involved the sclera, choroid, retinal pigmented epithelium, and retina of the eye and was seen 3 HD males (4128, 4131 and 4179) that were terminated on PND 71, 1 HD male (4133) terminated on PND 113, and 1 C female (1521) terminated on PND 113. The lesions were focal or multifocal, generally unilateral (1 bilateral occurrence in C female 1521), and, according to the pathology report, were "characterized by invaginations of cells through the retina that appeared to be hypertrophic and hyperplastic RPE cells with some evidence of vascular components and in some lesions a connective tissue component that was continuous with the sclera. The retina was disrupted with only remnants remaining at the lesion sites; these changes were, however, of limited extent and the remaining retina was intact and free of lesions. These changes were present in the posterior aspects of the retina and were considered to be of mild severity." According to the pathologist, the nature and characteristics of the changes were consistent with a developmental origin and, since they were seen in the C group, were not considered to be drug-related. However, the lesion was only seen in the HD group after the recovery period (Table 12).

Table 11. Neurohistopathology lesions (PND 71 sacrifice, male)

		[1]Group 1 Vehicle Control	Group 4 [2]High Dose
Number of Animals With Treatment-Related Lesions		0	0
Number Examined		10	10
Organ/tissue	**Diagnoses**		
Eye	Fold, retina, unilateral, present	(1)	(2)
	Dysplasia, unilateral, mild	(0)	(3)
Brain, pineal gland	Ectasia, vascular, moderate	(0)	(1)
Sciatic nerve	Degeneration, unilateral, minimal	(0)	(1)

1 - Numbers in ( ) are total number of occurrences for each group
2 - Progressive dose levels used (per protocol) are shown in Table 2 below.

Table 12. Neurohistopathology lesions (PND 113 sacrifice, male)

		[1]Group 1 Vehicle Control	Group 4 [2]High Dose
Number of Animals With Treatment-Related Lesions		0	0
Number Examined		10	10
Organ/tissue	**Diagnoses**		
Eye	Fold, retina, unilateral, present	(2)	(1)
	Degeneration, retina, unilateral, moderate	(0)	(1)
	Dysplasia, bilateral, mild	(0)	(1)
Spinal nerve	Degeneration, fiber, minimal	(0)	(1)

1 - Numbers in ( ) are total number of occurrences for each group
2 - Progressive dose levels used (per protocol) are shown in Table 2 below.

Toxicokinetics

TK parameters (Cmax and AUC) are shown in Table 13.

Table 13.

Dose level (mg/kg/day)	C_{max} (ng/mL) PND 7		PND 22		PND 38		PND 54		PND 70	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
15	-	9110	-	9020	-	12600	-	-	-	-
20	13100	-	9490	-	9360	-	-	19800	-	18600
25	-	16700	-	13000	-	-	-			-
30	21800	-	14100	-	-	-	12200	-	-	-
35	-	26300	-	18900	-	18800	-	-	-	-
40	32400	-	17500	-	17900	-	-	29600	17400	-
50	-	-	-	-	-	25600	-	-	-	42100
60	-	-	-	-	21500	-	22700	39000	-	-
80	-	-	-	-	-	-	26800	-	33300	45700
120	-	-	-	-	-	-	-	-	45400	-

Dose level (mg/kg/day)	AUC_{0-24} (ng*h/mL) PND 7		PND 22		PND 38		PND 54		PND 70	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
15	-	158000	-	94900	-	173000	-	-	-	-
20	253000	-	121000	-	91600	-	-	284000	-	260000
25		285000	-	163000	-	-	-	-	-	-
30	378000	-	174000	-	-	-	164000	-	-	-
35	-	468000	-	206000	-	250000	-	-	-	-
40	543000	-	209000	-	161000	-	-	431000	238000	-
50	-	-	-	-	-	311000	-	-	-	634000
60	-	-	-	-	283000	-	327000	534000	-	-
80	-	-	-	-	-	-	317000	-	486000	673000
120	-	-	-	-	-	-	-	-	658000	-

10 Integrated Summary and Safety Evaluation

The molecular mechanism by which cenobamate (CBM) produces its anticonvulsant actions appears to primarily involve blockade of voltage-gated sodium channels. In whole-cell patch clamp recording, CBM produced concentration-, voltage- and use-dependent inhibition of rat Nav1.2, with an IC50 value of 68 μM at −67 mV (Liu et al, Epilepsy Res, 83(1):66-72, 2009). CBM blocked rNav1.2 channels only weakly at hyperpolarized voltages, but when the membrane was depolarized, such that substantial channel inactivation occurred, there was a marked increase in the ability of the drug to induce channel block. Channel inhibition by CBM was further enhanced by repeated, high-frequency channel activity. These characteristics of CBM are similar to those of lamotrigine and phenytoin and are thought to account for their ability to selectively inhibit high-frequency repetitive firing without affecting normal, ongoing neuronal firing (Liu et al, Epilepsy Res, 83(1):66-72, 2009). However, CBM demonstrated a broader profile of activity in animal seizure models than older conventional sodium channel-blocking AEDs, suggesting that the mechanism is not fully understood. For example, in contrast to phenytoin and carbamazepine, CBM was reportedly active against spike–wave seizures in the GAERS model of absence seizures and did not aggravate spike-and-wave discharges in the GAERS model (Nehlig et al., Epilepsia, 46(212368):215, 2005)

The sponsor has emphasized the finding, in electrophysiology studies using rat hippocampal neurons, that CBM was more potent in inhibiting the noninactivating (persistent) component of the tetrodotoxin-sensitive sodium current (INaP) compared to the transient component (INaT). Carbamazepine and lamotrigine showed a similar preference for the INaP and this selectivity has been reported for other approved AEDs (Stafstrom CE, Epilepsy Curr, 7(1):15-22, 2007). Interestingly, 2 drugs effective against absence seizures, ethosuximide and valproate, reportedly had little or no effect on INaP, while phenytoin and riluzole strongly depressed INaP at clinically-relevant concentrations (Niespodziany et al, Neuroreport,15(6):1049-52,2004).

CBM positively modulated the GABA-induced current of 6 receptor subtypes, with EC50 values ranging from 42 μM to 194 μM (equivalent to 11.2 to 51.9 μg/mL). However, CBM had limited agonist activity for these GABA receptor subtypes; only small effects on GABA-mediated currents were observed at 100 μM.

CBM was fairly well-absorbed orally in rodents and monkeys, but bioavailability was very low (F: 11%) in dogs, explaining the choice of non-rodent toxicology species. Exposure generally increased dose proportionally with repeated dosing and exposures were generally somewhat higher in females in both rats and monkeys. Apparent auto-induction was seen in rats, supported by data showing increased CYP and UGT activities. But an effort to counteract this problem was only made in the juvenile rat study in which doses were escalated to increase tolerability and exposure.

CBM was extensively metabolized and the metabolite pattern was qualitatively similar across species. The primary metabolic pathway appeared to be O-glucuronidation and hydrolysis of the carbamate ester followed by oxidation of the aliphatic side chain. No major circulating metabolites were observed in humans. After single oral doses of

radiolabeled drug, most of the radioactivity (≥94.0% of the dose) was excreted within 168 hours for all nonclinical species, with 50-80% of the dose excreted in urine (88% in humans).

A complete toxicology package consisted of repeat-dose toxicity studies of up to 13 weeks in mice, 26 weeks in rats, and 52 weeks in monkeys; in vitro and in vivo genotoxicity; 26-week carcinogenicity study in transgenic rasH2 mice and 104-week study in rats; and reproductive and developmental toxicity studies, including a juvenile rat study. TK data were collected in most of these studies (except the rat FEED and EFD studies). In all toxicology species, acute neurotoxicity was dose-limiting and resulted in minimal to no safety margins to clinical exposures (human Cmax and AUC at MRHD of 400 mg: 45.5 µg/mL and 861 µg·hr/mL). Given this limitation, the studies are considered adequate. No unmonitorable serious toxicity was identified in the general toxicity, and there was no evidence of carcinogenic potential, but a potential for adverse effects on developmental was identified in rat and rabbit studies.

In the 3-month mouse study, oral (gavage) administration of CBM (0 (vehicle), 10, 30, 60, or 120/90 mg/day; 5 mL/kg) to CD1 mice resulted in mortality and clinical signs at ≥ 60 mg/kg/day, and centrilobular hepatocellular hypertrophy (non-adverse) at all but the LD. The HD was terminated on Day 7 due to the excessive toxicity (seen after first dose) at 120 and 90 mg/kg/day. The NOAEL (30 mg/kg/day) was associated with Cmax values of 64100 and 62667 ng/mL and AUC0-24 values of 471211 and 412057 ng·hr/mL in males and females, respectively.

In the chronic (S-D) rat study, oral (gavage) administration of CBM (0 (vehicle), 12, 24, 48 mg/day) produced mortality at the HD (8 animals euthanized due to deteriorating condition attributed to drug) and CNS clinical signs (decreased activity, uncoordinated gait) at doses ≥ 24 mg/kg/day. There were no effects on BW, ophthalmology, hematology, or urinalysis parameters. Microscopic changes observed in the kidney (males) and liver (males and females) were attributed to drug, but renal changes were male rat specific, and centrilobular hepatocellular hypertrophy is considered adaptive. The NOAEL (12 mg/kg/day) was associated with Week 26 Cmax and AUC0-t values of 9.3 ug/mL and 113.9 ug.h/mL in males and 17.6 ug/mL and 213.7 ug.h/mL in females, respectively.

In a 14-day (cynomolgus) monkey study at oral doses up to 60 mg/kg, there was no mortality, but clinical signs observed in both HD animals on Days 1 and 2 included crawling, lying on the cage floor, and clonic convulsions that ranged from slight to moderate in the male and slight to severe in the female. The dose level was subsequently decreased to 40 mg/kg/day starting on Day 3. Slight clonic convulsions in both animals and crawling in the female persisted on Day 3 at the reduced dose. Nystagmus was observed in the HD female on Days 3 and 4 along with severe hyperextension of the neck on Day 3, which had diminished to a slight hyperextension by Day 4. The female displayed slight tremors on Day 4. By Day 5 clinical signs at the HD consisted mostly of decreased activity, uncoordinated gait, and drowsiness in both animals. Clinical signs at the MD (30 mg/kg) included uncoordinated gait, drowsiness, and a slight decrease in activity throughout the treatment period, in both animals. The only other findings were increased

WBC count in the HD male and dose-related increases in liver weights without macroscopic or microscopic changes. The NOAEL was 10 mg/kg.

A neurotoxicity study was carried out to evaluate the convulsions seen in the 14-day monkey toxicity study. When (cynomolgus) monkeys were dosed for 4 days, 2 of 6 animals dosed (orally) with 60 mg/kg presented with abnormal, involuntary series of muscle contractions, which would have been recorded as clonic convulsions according to clinical sign lexicon for the contract lab that conducted the 14-day toxicity study. However, when video recordings were reviewed by a veterinary neurologist these were interpreted as episodes of myoclonus or intentional tremors. Examinations of EEGs showed no epileptic spikes or paroxysmal depolarization shifts during these episodes. The muscle contractions appeared to increase upon stimulation of the animal and upon voluntary movements, and the animals appeared sedated but conscious. An increase in beta waves was noted in 1 of the 2 animals during the first episode of myoclonus. One of the 2 monkeys with myoclonus or intentional tremors was euthanized on Day 4 due to poor and deteriorating conditions. TK analysis showed that this animal had the highest Cmax of all monkeys treated with 60 mg/kg (127 ug/mL vs mean of 96 ug/mL in other monkeys). No spike trains were noted during muscular contractions suggesting that the clinical signs did not result from bursts of uncontrolled electrical activity in the brain and thus are not seizures. Animals dosed with 40 mg/kg presented ataxia, tremors, hypoglycemia, hypothermia, and evidences of sedation but did not have clonic convulsions (as defined in the contract lab's clinical sign lexicon). It was concluded that the HD dose of 60 mg/kg induced sedation, a vestibular syndrome (peripheral or central), and myoclonus or intentional tremors.

In the 28-days study in cynomolgus monkeys, oral (gavage) administration of CBM (0, 4, 12, or 36 mg/kg) resulted severe clinical signs (including uncoordinated gait, drowsiness, decreased activity, hyperextension of the neck, ocular nystagmus, tremors, weakness) leading to pre-terminal sacrifice were seen at 36 mg/kg/day and CNS clinical signs were noted at all doses. Decreases in total bilirubin were noted at all doses and were considered drug-related, but there were no histopathological changes. Based on clinical signs and clinical chemistry changes, the LD (4 mg/kg/day) was a LOAEL. This dose was associated with Cmax values of 5.45 µg/mL and 6.47 µg/mL and AUC24 values of 56.8 µg*h/mL and 84.9 µg*h/mL for males and females, respectively.

In the chronic non-rodent study, oral (gavage) administration of CBM (0, 9, 18, or 27/22 mg/kg/day) to cynomolgus monkeys for 52 weeks resulted in severe clinical signs (decreased activity, hunchback, crouching, and incoordination), sacrifice of 1 HD female (on Day 7), and dose reduction (on Day 11) at the HD. Clinical signs were decreased after dose reduction, but incoordination was still noted at the reduced HD. Lymphoid hyperplasia of bone marrow was seen at the end of the treatment period in the MD and HD groups and was still present after the 3-month recovery period at the HD. The MD (18 mg/kg) was considered the NOAEL and was associated with Cmax values of 61.8 and 36.9 µg/mL and AUC24 values of 1049 and 542 µg*h/mL in males and females, respectively.

CBM was negative for genotoxicity in in vitro (Ames, mouse lymphoma) and in vivo (rat bone marrow micronucleus) assays.

Oral (gavage) administration of CBM (0, 5, 15, or 35 mg/kg/day) to Tg.rasH2 mice for 26 weeks did not increase the incidence of neoplastic lesions. There were no drug-related deaths, clinical signs, or BW effects. Incidences of pulmonary tumors, splenic hemangiosarcomas, and other tumor types were comparable across drug- and vehicle-treated groups (there were no SS group differences in sponsor's or FDA statistical analysis). The expected increases in incidences of pulmonary tumors and splenic hemangiosarcomas were seen in positive controls.

In the (S-D) rat carcinogenicity study (oral gavage doses of 0, 4, 8, or 20 mg/kg/day), dosing was terminated during Weeks 87 and 90 for males and females, respectively, due to survival reaching 20 or fewer in controls. Mortality was not increased by CBM and there were no drug-related effects on clinical signs or BW. No drug-related neoplastic findings were observed. Week 26 exposures (AUC) were 173 and 232 µg·hr/mL in HD males and females, respectively.

In the (S-D) rat fertility study, oral (gavage) administration of CBM (0, 11, 22, or 44 mg/kg/day) for 2 (females) or 4 (males) weeks prior to mating, throughout mating, and continuing until terminal sacrifice (2 days after the end of pairing in males, GD 6 in females) did not result in drug-related mortality, clinical signs, BW changes, male or female mating or fertility indices, estrous cyclicity, C-section parameters, or sperm parameters. At the end of the dosing period all drug-treated males had dose-related centrilobular hepatocellular hypertrophy that correlated with increased liver weights and increases in CYP2B1/2, 2E1, 3A1/2, and CYP2A1. TK data were not collected in this study, but based on other rat studies, female exposures at the HD are estimated to have been approximately 400 µg.h/mL.

Oral (gavage) administration of CBM (0, 10, 30, or 60 mg/kg) to pregnant S-D rats throughout organogenesis (GD 6-17) resulted excessive maternal toxicity at the HD (3 deaths, 2 moribund sacrifices, marked clinical signs, 54% decrease in BW gain from GD 6-18), and there was a decrease in the number of females with viable fetuses at term (n=13). Embryofetal toxicity at the HD included increased postimplantation loss (4X), a reduction in fetal weight (-13%), and incomplete ossification. Malformations (anophthalmia and enlarged ventricle) were seen in 3(2) HD fetuses(litters), but as noted in the report, "Marked maternal toxicity at 60 mg/kg/day (far exceeding the minimal maternal toxicity expected in the high dose) confounded the interpretation of any teratogenic potential at this dose level." The MD produced a 14% reduction (SS) in maternal BW gain over the dosing period, but GD 20 BW was only 3.5% below C (NS). There was no clear evidence of embryofetal toxicity at the MD, which was associated with a maternal plasma exposure of approximately 300 µg.h/mL (again based on other rat study TK data). It is recommended that this study be repeated as a postmarketing requirement. Divided (BID) daily dosing should be investigated as a means to increase exposure without producing excessive maternal toxicity.

In the (NZW) rabbit embryofetal development study, oral (gavage) administration of CBM (0, 4, 12, or 36 mg/kg) throughout organogenesis (GD 6-19) resulted in embryofetal mortality (postimplantation loss 2X C) at the HD, which was also associated with decreased maternal BW gain. Similar skeletal malformations (thoracic or lumber vertebral abnormalities) were seen in 0, 1(1), 3(3), and 2(2) fetuses(litters) from the C, LD, MD, and HD groups, but these are not considered drug-related due to the lack of dose response or associated developmental effects. Maternal plasma exposures (AUC) at the NOAEL for developmental toxicity (12 mg/kg) were 63 and 51 ug.h/mL on GD 6 and 19, respectively.

When CBM (0, 11, 22, or 44 mg/kg/day) was orally administered to female rats throughout pregnancy and lactation, neurobehavioral impairment (learning and memory deficit and increased auditory startle response) was observed in the offspring at all doses and decreased preweaning body weight gain and adverse effects on reproductive function (decreased numbers of corpora lutea, implantations, and live fetuses) were seen in the offspring at the high dose. Maternal plasma exposures (AUC) at the lowest effect dose (11 mg/kg/day) for adverse effects on pre- and postnatal development were 124, 140, and 101 ug.h/mL on GD 6, GD 18, and PND 20, respectively.

Although the current indication is for adults only, the sponsor conducted a juvenile toxicity study in rats in which oral doses of up to 120 and 80 mg/kg/day were administered to males and females, respectively, from postnatal day 7 to 70 (escalating doses of 20/20/30/40, 30/40/60/80, or 40/60/80/120 mg/kg/day in males and 15/15/20/20, 25/35/40/50, or 35/50/60/80 mg/kg/day in females). Adverse effects included mortality, delayed sexual maturation, neurological (decreased grip strength) and neurobehavioral (learning and memory deficits) impairment, decreased sperm count, decreased brain weight, and ocular histopathology. Recovery from these effects was observed following discontinuation of dosing. Overall, a no-effect dose for adverse effects on postnatal development was not identified. At the LD, plasma exposures ranged between approximately 100 and 250 µg*h/mL (sexes combined).

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

J EDWARD FISHER
11/20/2019 02:45:32 PM

LOIS M FREED
11/20/2019 04:29:26 PM

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: November 20, 2019

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 212-839 (Xcopri, cenobamate, YKP3089)

NDA 212-839 was submitted by SK Life Science, Inc. on November 21, 2018, to support marketing of cenobamate for the treatment of partial onset seizures in adults. Clinical development of cenobamate for this indication was conducted by the sponsor under IND 76809. A full battery of nonclinical studies was conducted to support the NDA, consistent with the Division's feedback and recommendations provided under the IND.

The nonclinical studies were reviewed by Dr. Fisher (Pharmacology/Toxicology NDA Review and Evaluation, NDA 212839, Ed Fisher, Ph.D., November 20, 2019). Based on that review, Dr. Fisher has concluded the nonclinical data support approval of the NDA for the proposed indication, with a post-marketing requirement for an embryofetal development study in rat.

The following is a brief summary of the nonclinical studies.

Pharmacology: Based on data from in vitro binding and functional assays, cenobamate was characterized as an inhibitor of voltage-gated sodium channel current, with greatest affinity for the inactivated state (IC_{50}'s of 15670, 478, and 2.81 µM for tonic, use-dependent, and inactivated state inhibition, respectively). However, according to the sponsor, a specific receptor responsible for cenobamate's anticonvulsant activity has not been identified. In in vivo studies, cenobamate exhibited anticonvulsant activity in multiple animal models of efficacy (chemically and electrically-induced seizures).

In safety pharmacology studies of orally administered cenobamate, the most notable findings were CNS toxicity in male Sprague-Dawley rat (0, 200, 250, and 300 mg/kg; 0, 10, 30, and 100 mg/kg) and in male ICR mouse (0, 80, 90, 100, and 120 mg/kg). In rat, deaths occurred at 250 and 300 mg/kg; CNS signs (e.g., ataxia, reduced muscle tone and locomotor activity) were evident at ≥100 mg/kg. The peak TD_{50} for rotarod failure in rat was 195.7 mg/kg at 4 hrs post-dose. In mouse, the TD_{50} for rotarod failure was 85.6 mg/kg, at 2 hrs post-dose.

No cardiovascular effects were identified in the in vitro hERG assay (IC_{50} >100 µM) or in vivo in telemetered male cynomolgus monkey.

PK/ADME: The PK/ADME of cenobamate administered orally was assessed in CF-1 mouse, Sprague Dawley rat, cynomolgus monkey, and beagle dog. The half-life was relatively short in mouse, rat, and dog (2.1, 1.98, and 0.46 hrs, respectively) but substantially longer in monkey (13.4-22.7 hrs); oral bioavailable was>50% in mouse, rat, and monkey (59.6, 119, and 83-9-110%, respectively) but only 10.8% in dog. Tissue distribution of radioactivity was extensive following a single oral dose (15 mg/kg) of ^{14}C-cenobamate to male Sprague Dawley rat, with highest peak levels in GI, kidney, and liver; C_{max} in brain was similar to that in plasma at T_{max}. The in vivo metabolic profile following a single oral dose varied among species. In humans, the only major circulating metabolite in vivo was M1, an N-glucuronide; therefore, there were no metabolite issues of concern.

Serum protein binding ranged from 35.4% in rabbit to ~70% in monkey (61% in human).

Toxicology: The pivotal oral toxicity studies were conducted in CD-1 mouse (13-week; 0, 10, 30, 60, and 120/90 mg/kg/day), Sprague Dawley rat (28-day: 0, 10, 30, and 100/60 mg/kg/day; 26-week + 8-week recovery [with 13-week interim sacrifice + 4-week recovery]: 0, 12, 24, and 48 mg/kg/day), and cynomolgus monkey (28-day: 0, 4, 12, and 36/24 mg/kg/day; 52-week + 3-month recovery [with 13- (+ 4-week recovery) and 26-week interim sacrifices]: 0, 3, 9, and 18 mg/kg/day for 13 and 26 weeks; 0, 9, 10, and 27(Day 1-10 only)/22 mg/kg/day for 52 weeks).

In all species, the primary toxicities were death (primarily premature sacrifice following severe clinical signs) and CNS signs. Clinical signs (included ataxia, decreased activity, uncoordinated gait, recumbency, tremors, labored respiration) were dose-related in incidence and severity. Additional clinical signs observed in monkey included clonic convulsions (later determined in special investigative studies in monkey to be myoclonic jerks, which were also observed in mouse), nystagmus, severe hyperextension of the neck, and abnormal cranial nerve function and spinal segmental reflexes. In the chronic toxicity studies, NOAELs were determined to be 12 mg/kg/day in rat and 18 mg/kg/day in monkey. Plasma exposures at these doses were as follows:

- Rat: C_{max} of 9.3-17.6 µg/mL and $AUC_{(0\text{-}24\ h)}$ of 113.9-213.7 µg*hr/mL
- Monkey: C_{max} of 61.8-36.9 µg/mL and $AUC_{(0\text{-}24\ h)}$ of 1049-542 µg*hr/mL

Therefore, rat was the more sensitive species.

Genetic Toxicology: Cenobamate was negative in in vitro (Ames, mouse lymphoma *tk*) and in vivo (rat bone marrow micronucleus) assays.

Carcinogenicity: The carcinogenic potential of cenobamate was assessed in a 26-week oral study in T.rasH2 mouse (0, 5, 15, and 35 mg/kg/day and in a 87/90-week oral toxicity study in Sprague Dawley rat (0, 4, 8, and 20 mg/kg/day). (Early termination, prior to Week 104, in male and female rats occurred when the survival rate in controls reached ~20.) Both studies were negative for drug-induced tumors. Plasma exposure ($AUC_{(0\text{-}24h)}$) at the highest dose tested in rat was 173-243 µg*hr/mL (Week 26).

Reproductive and Developmental Toxicology: Cenobamate was assessed in a standard battery of reproductive and development toxicology studies: fertility and early embryonic development (to implantation), embryofetal development, and pre- and postnatal development in Sprague Dawley rat and an embryofetal development study in New Zealand White rabbit.

In the fertility study, cenobamate (0, 11, 22, and 44 mg/kg) was administered orally to male and female rats prior to and during mating and continuing in females to gestation day (GD) 6. No adverse reproductive or early developmental effects were observed. Toxicokinetic data were not collected; however, the plasma AUC is estimated to be less than that in humans at the maximum recommended human dose (400 mg/day, 861 µg*hr/mL) based on a plasma $AUC_{(0\text{-}24\ h)}$ of 556-628 µg*hr/mL in females at 48 mg/kg/day in the 26-week oral toxicity study.

In the embryofetal development study in rat, cenobamate was administered orally to pregnant rats at doses of 0, 10, 30, and 60 mg/kg/day on GDs 6-17. At the high dose, 5 dams died (3) or were sacrificed prematurely (2), 6 dams aborted or resorbed the entire litter, and one female was not pregnant, which resulted in only 13 evaluable litters, which, as the sponsor notes, is lower than the 16 litters considered necessary to adequately evaluate fetal effects. Other findings at the high dose included reduced fetal body weight, incomplete ossification, and a possible increase in fetal visceral malformations (anophthalmia, enlarged ventricular chambers). No adverse cesarean or fetal findings were observed at the lower doses. Toxicokinetic analysis was not conducted; however, based on data from the 26-week oral toxicity study in rat, plasma AUC at the higher NOAEL (30 mg/kg/day) is estimated to be lower than that in humans at the maximum recommended human dose.

In the embryofetal development study in rabbit, cenobamate was administered orally to pregnant rabbits at doses of 0, 4, 12, and 36 mg/kg/day on GDs 6-19. At the high dose, there were increases in early and late resorptions and post-implantation loss, and evidence of maternal toxicity (reduced body weight, food consumption). Plasma exposure (AUC) at the higher NOAEL (12 mg/kg/day) was 63.1-51.3 µg*hr/mL, based on toxicokinetic data collected in a separate study in pregnant rabbit.

In the pre- and postnatal development study in rat, cenobamate was administered orally to female rat throughout gestation and lactation at doses of 0, 11, 22, and 44 mg/kg. Pup body weight was reduced at the HD, primarily during the lactation period. Neurobehavioral impairment (reduced auditory startle habituation, memory recall deficits [increased latency and errors] in Biel maze) was observed in males at all doses, and reproductive function was impaired (reduced corpora lutea and implantations, increased early resorptions, pre- and post-implantation loss, decreased no. of live fetuses) at the high dose. Cenobamate was detected in fetal plasma (1.58, 4.34, and 5.88 µg/mL at maternal doses of 11, 22, and 44 mg/kg/day, respectively). No NOAEL was identified for adverse effects on pre- and postnatal development. Plasma exposures (AUC) at the lowest dose tested were 124, 140, and 101 µg*hr/mL on GD 6, GD 18, and lactation day 20, respectively.

The effects of cenobamate on postnatal development were assessed in juvenile Sprague Dawley rat, with dosing initiated on postnatal day (PND) 7 and continuing through PND 70, followed by a 6-week recovery period. Doses (males: 20/40, 30/80, and 40/120 mg/kg/day; females: 15/20,

25/50, and 35/80 mg/kg/day) were increased throughout the dosing period in all dose groups (PND 7-22, PND 23-38, PND 39-54, and PND 55-70) in order to compensate for enzyme induction to achieve higher plasma exposures. Premature death or moribund sacrifice and clinical signs (including hindlimb splay and uncoordinated) were observed at the mid and high doses, and sexual maturation was delayed in males at the high dose. Reduced sperm count, impaired learning and memory, and reduced grip strength (fore- and hindlimb) were observed at all doses. The incidence of retinal dysplasia was increased in males at the high dose (the only dose evaluated). (The study pathologist considered the finding to be spontaneous in origin but acknowledged the increased incidence in high-dose males.) An NOAEL for postnatal developmental toxicity was not identified. At the low dose, plasma exposures were 91.6-238 ng/mL for C_{max} and 94.9-284 µg*hr/mL for $AUC_{(0\text{-}24\ h)}$.

Conclusions and Recommendations

Although doses tested in the nonclinical studies were limited because of adverse CNS effects, resulting in plasma exposures generally similar to or less those in humans at the maximum recommended human dose, the nonclinical studies of cenobamate are adequate to support approval of the NDA, with appropriate labeling. I concur with Dr. Fisher's recommendation for an embryofetal development study in rat as a post-marketing requirement, in order to further assess the teratogenic potential of cenobamate.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

LOIS M FREED
11/20/2019 05:21:23 PM